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Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.01/A1

Topic: A.05. Axon and Dendrite Development

Support: K08 NS091531
Young Investigator Research Grant Award
Stanford McCormick Faculty Award
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Title: A microRNA cluster downstream of the selector gene Fezf2 coordinates cortical neuron fate with subtype-specific dendritic branching and synaptic connectivity

Authors: A. IYER[1], V. SITHTHANANDAN[1], V. LU[1], R. NAIR[2], L. O. VAASJO[3], ***C. NNEBE**[1], M. GALAZO[3], S. THARIN[1];
[1]Neurosurg. Dept., Stanford Med. Sch., Stanford, CA; [2]Ctr. for Genomics and Personalized Med., Stanford Univ., Stanford, CA; [3]Neurobio., Tulane Univ., New Orleans, LA

Abstract: In the cerebral cortex, projection neurons comprise distinct classes of neurons that project to distant regions of the central nervous system. While these classes of neurons develop from the same progenitor pool, they acquire strikingly different inputs and outputs to underpin strikingly different functions. The question of how corticospinal projection neurons -involved in motor function and implicated in paralysis - and callosal projection neurons - involved in cognitive function and implicated in autism - develop represents a fundamental and clinically important question in neurodevelopment. A network of transcription factors, including the selector gene *Fezf2*, is central to specifying cortical projection neuron fates. However, gene regulation up- and down-stream of these transcription factors is not well understood, particularly as it relates to the development of the major inputs to cortical projection neurons. Here we show that the corticospinal-enriched miR-193b~365 microRNA cluster is downstream of *Fezf2* and cooperatively represses the signaling gene *Mapk8* to differentially regulate dendritic morphology and synaptic connectivity in callosal and corticospinal projection neurons. *In vivo* overexpression of miR-193b and miR-365 in callosal projection neurons alters their dendritic branching pattern, dendritic spine density, and spine morphology during postnatal development. These findings indicate that the miR-193b~365 microRNA cluster downstream of Fezf2 plays a significant role in establishing neuron subtype-specific synaptic connectivity and likely contributes to distinct synaptic plasticity properties across cortical projection neuron classes.

Disclosures: **A. Iyer:** None. **V. Siththanandan:** None. **V. Lu:** None. **R. Nair:** None. **L.O. Vaasjo:** None. **C. Nnebe:** None. **M. Galazo:** None. **S. Tharin:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.02/A2

Topic: A.05. Axon and Dendrite Development

Support: NSERC RGPIN-2016-06128
Canada Research Chair
Sloan Research Fellowship FG-2015-65234

Title: The clustered Protocadherins regulate filopodia self-recognition and dynamics to drive dendrite self-avoidance and morphogenesis.

Authors: S. ING-ESTEVES, ***J. L. LEFEBVRE**;
Neurosci. and Mental Hlth., Hosp. for Sick Children/ Univ. of Toronto, Toronto, ON, Canada

Abstract: The spatial organization of dendrites is a core feature of neuron type-specific morphology and information processing. Growing dendrites require molecular cues to shape branch numbers and distribution. One growth rule is neurite self-avoidance, where dendrites arising from the same cell avoid each other to minimize branch overlap and maximize dendritic field coverage. In mammalian neurons, dendrite self-avoidance requires the clustered Protocadherins, a large family of recognition molecules. The cPcdhs comprise ~60 cadherin-related isoforms that are encoded by three linked gene clusters, *Pcdh-alpha, Pcdh-beta,* and *Pcdh-gamma.* We have shown previously that deletion of *Pcdhg* or both *Pcdhg* and *Pcdhg* in mice cause loss of self-avoidance in retinal starburst amacrine cells (SACs) and cerebellar Purkinje cells, resulting in altered arbor territories. The cPcdhs also regulate self-avoidance of olfactory sensory axons, and other aspects of neurite patterning in the CNS. Genetic and molecular studies suggest that the cPcdhs mediate homophilic recognition and repulsion between self-dendrites. However, this model has not been tested through direct investigation of self-avoidance during development. Here we performed live imaging and 4D quantifications of dendrite morphogenesis to define the cPcdh-dependent self-avoidance. We imaged the mouse retinal SACs, which require the *Pcdhgs* to establish a stereotypic radial morphology while overlapping and synapsing with neighboring SACs. Through morphogenesis, SACs extend a transient population of dynamic filopodia that fill the growing arbor and contact nearby self-dendrites. Compared to non-self-contacting filopodia, self-contacting events have longer lifetimes and a subset persists as filopodia bridges. In the *Pcdhg* SACs, non-self-contacting filopodia dynamics are unaffected but self-contact-induced retractions are significantly diminished. Filopodia bridges accumulate, leading to the bundling of dendritic processes and disruption to the arbor shape. By tracking dendrite self-avoidance in real-time, our findings demonstrate that the Pcdhgs selectively mediate contact-induced retractions upon filopodia self-

recognition. Our results also illustrate how self-avoidance shapes the stochastic and space-filling behaviors of filopodia for robust dendritic pattern formation in mammalian neurons. We will present ongoing work linking cPcdh isoform expression to neuronal self-avoidance, bearing insights into how cPcdh diversity specifies self-recognition and neuronal patterning in space and time.

Disclosures: S. Ing-Esteves: None. **J.L. Lefebvre:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.03/A3

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant EY EY031690

Title: Role of gamma-Protocadherins in retinal ganglion cell morphology

Authors: S. SON[1], C. M. MCLEOD[2], M. S. AKPO[3], J. W. KENNEY[4], **A. M. GARRETT**[2]; [1]Pharmaco, [2]Pharmacol., [3]Ophthalmology, Visual, and Anatom. Sci., [4]Biol. Sci., Wayne State Univ., Detroit, MI

Abstract: Clustered protocadherins (cPcdhs) generate a vast array of unique homophilic recognition complexes, contributing to the formation of distinct neural circuits. Specifically, γ-Pcdhs, derived from the *Pcdhg* cluster, play a critical role in regulating both cell survival and neuronal self-avoidance in the mouse retina. However, Morphological phenotypes in most cell types have been difficult to disentangle from widespread cell death in the absence of γ-Pcdhs. To address this issue, we employed a novel mutant with reduced *Pcdhg* isoform diversity, which we found to largely preserve neuronal survival while lacking normal self-avoidance in starburst amacrine cells. Based on this, we hypothesize that the impaired self-avoidance may lead to morphological changes in retinal ganglion cells (RGCs). We first focused on investigating the impact of this mutation on the eye specific segregation of retinal projections to the dorsal lateral geniculate nucleus (dLGN). Our findings indicate that γ-Pcdhs are not essential for this segregation process. Subsequently, we used adeno-associated viruses (AAVs) to deliver specific synthetic promoters (ProD1, ProA13, and ProA27) targeting distinct subsets of ganglion cells. Immunohistochemistry was employed to characterize these subsets. As previously described, ProD1 targeted a group of bistratified RGCs that align with SACs in the inner plexiform layer (IPL) sublaminae 2 and 4 of the retina, which were labeled with markers for ON-OFF direction-selective RGCs (CART). ProA13 selectively targeted a subset of RGCs with processes in IPL sublaminae 3 and 5, and co-labeled with markers for specific RGC subtypes (Brn3a and synaptotagmin-6). ProA27 targeted a separate subset of RGCs and exhibited labeling with markers for alpha-type RGCs (SMI32 and Parvalbumin). Furthermore, the axons targeted by ProA13 and ProA27 projected to various brain regions, including the thalamic target (LGN),

midbrain nuclei (Anterior pretectal nucleus and Superior colliculus), and hypothalamic nuclei (suprachiasmatic nucleus). Ongoing work aims to define morphological and targeting defects in these neurons within *Pcdhg* mutants.

Disclosures: S. Son: None. **C.M. McLeod:** None. **M.S. Akpo:** None. **J.W. Kenney:** None. **A.M. Garrett:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.04/A4

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS055272
NIH Grant NS090030

Title: Delineating neurodevelopmental roles of the shared gamma-protocadherin C-terminus in vivo

Authors: C. M. HANES[1], K. MAH[1], L. C. FULLER[1], C. G. MARCUCCI[1], D. M. STEFFEN[1], R. W. BURGESS[2], A. M. GARRETT[3], *J. A. WEINER[1];
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Abstract: Neurodevelopment relies on interactions mediated by cell adhesion molecules (CAMs). Mutations in many CAMs are associated with neurodevelopmental disorders. The clustered protocadherins (Pcdhs) are cadherin superfamily CAMs encoded by three tandem gene clusters: *Pcdha*, *Pcdhb*, and *Pcdhg*. The *Pcdhg* cluster encodes 22 unique γ-Pcdh protein isoforms, each of which shares the same intracellular C-terminal constant domain, suggesting that shared signaling pathways may mediate critical γ-Pcdh functions. In our prior work, we showed that γ-Pcdhs interact homophilically in *trans* and promiscuously in *cis* and are required for postnatal survival. We identified multiple neurodevelopmental roles for these CAMs, including the regulation of neuronal survival, dendritic arborization, and synapse and dendritic spine development. The extent to which shared γ-Pcdh C-terminal signaling, as opposed to homophilic *trans*-interaction, regulates these processes is not entirely understood. Identification of Focal Adhesion Kinase (FAK) and its homologue PYK2 as γ-Pcdh cytoplasmic interactors aided in the discovery of a FAK/PKC/MARCKS signaling pathway that mediates dendritic arborization downstream of γ-Pcdhs. More recent *in vitro* studies indicate that the γ-Pcdh C-terminal constant domain can be phosphorylated by PKC, and that this reduces these CAMs' ability to inhibit FAK. To assess the importance of this pathway *in vivo*, we created two new lines of mice in which the γ-Pcdh constant domain is altered so that the 22 γ-Pcdh isoforms cannot be phosphorylated by PKC due to: 1) a point mutation in the target serine; or 2) deletion of a 15-amino acid C-terminal motif that harbors this target serine. We found that both of these

mutants have significantly *increased* cortical dendritic arborization, and more active MARCKS. Interestingly, these mice do not have altered dendritic spine density, consistent with our prior results showing that extracellular *cis*-interaction of the γ-Pcdhs with neuroligins regulates spines. We further discovered that total γ-Pcdh protein levels are decreased in the 15-amino acid deletion mutant; further experiments indicate that this is not a result of increased protein degradation, but rather is accompanied by a decrease in γ-Pcdh RNA levels. Ongoing experiments are aimed at determining whether the reported cleavage and nuclear localization of the γ-Pcdh C-terminal domain might play a role in regulating the *Pcdhg* gene locus. Together, our results confirm the importance of the shared γ-Pcdh C-terminus to the function of these neuronal CAMs and suggest new functions for this domain in controlling γ-Pcdh expression.

Disclosures: C.M. Hanes: None. **K. Mah:** None. **L.C. Fuller:** None. **C.G. Marcucci:** None. **D.M. Steffen:** None. **R.W. Burgess:** None. **A.M. Garrett:** None. **J.A. Weiner:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.05/A5

Topic: A.05. Axon and Dendrite Development

Support: NIH R01AA029114
Clark Pediatric Pilot Award
Hendricks Foundation Pilot Grant

Title: Calcium signaling during cortical apical dendrite initiation: a role for Cajal-Retzius neurons.

Authors: *J. R. ENCK, E. C. OLSON;
SUNY Upstate Med. Univ., Syracuse, NY

Abstract: The apical dendrite of a cortical projection neuron (CPN) is generated from the leading process of the migrating neuron as the neuron completes migration. This transformation occurs in the cortical marginal zone (MZ), a layer that contains the Cajal-Retzius neurons and their axonal projections. Cajal-Retzius neurons (CRNs) are well known for their critical role in secreting Reelin, a glycoprotein that controls dendritogenesis and cell positioning in many regions of the developing brain. In this study, we examine the possibility that CRNs in the MZ may provide additional signals to arriving CPNs, that may promote the maturation of CPNs and thus shape the development of the cortex. We use whole embryonic hemisphere explants and multiphoton microscopy to confirm that CRNs display intracellular calcium transients of <1-minute duration and high amplitude during early corticogenesis. In contrast, developing CPNs do not show high-amplitude calcium transients but instead show a steady increase in intracellular calcium that begins at the time of dendritic initiation, when the leading process of the migrating CPN is encountering the MZ. The possible existence of CRN to CPN communication was

revealed by the application of veratridine, a sodium channel activator, which at an early developmental time point has been shown to preferentially stimulate more mature cells in the MZ. Surprisingly, veratridine application also triggers large calcium transients in CPNs which can be partially blocked by a cocktail of antagonists that block glutamate and glycine receptor activation. These findings outline a model in which CRN spontaneous activity triggers the release of glutamate and glycine, neurotransmitters that can trigger intracellular calcium elevations in CPNs. These elevations begin as CPNs initiate dendritogenesis and continue as waves in the post-migratory cells. Moreover, we show that the pharmacological blockade of glutamatergic signaling disrupts migration while the forced expression of a bacterial voltage-gated calcium channel (CavMr) in the migrating neurons promotes dendritic growth and migration arrest. The identification of this CRN to CPN communication during early development provides insight into the observation that many autism-linked genes encode synaptic proteins that, paradoxically, are expressed in the developing cortex well before the appearance of synapses and the establishment of functional circuits.

Disclosures: **J.R. Enck:** None. **E.C. Olson:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.06/A6

Topic: A.05. Axon and Dendrite Development

Support: CRC 1080

Title: Cell-specific VEGF function during hippocampal development

Authors: ***M. KRACHT**[1], M. SEGARRA[1,2], A. ACKER-PALMER[1,2,3];
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Abstract: Vascular endothelial growth factor (VEGF) is an essential angiogenic factor that also plays important roles in the nervous system. Previously, we have shown that the major angiogenic receptor VEGFR2 is expressed by pyramidal neurons in the CA3 region during hippocampal development and its neuronal deletion resulted in defects in dendritic tree and spine morphogenesis in CA3 pyramidal neurons. However, it still remains unclear which cell type secreting VEGF is activating VEGFR2 in neurons *in vivo*. In addition to neurons, endothelial and glial cells secrete VEGF in the hippocampus. We hypothesized that signals originating from the different cell types might have a distinguished local influence to maintain neuronal dendritic arbors and regulate spine morphogenesis. To distinguish between autocrine and paracrine VEGF signaling contributions, we generated mouse lines by employing the Cre-LoxP system where VEGF is conditionally deleted in excitatory neurons (CamKII-Cre), glia (GLAST-CreER) or

blood vessels (Cdh5-CreER). By visualizing recombination efficiency of CamKII-Cre with the help of a fluorescent LoxP-reporter we assessed that CamKII-Cre recombination starts at P5. Therefore, to better compare the contribution of VEGF source from the different cell types, we injected tamoxifen for 3 consecutive days as from P5 to the inducible mouse lines. Mice lacking VEGF in endothelial cells showed no significant differences in dendritic arborization and spine density, indicating that the role of vascular derived VEGF is negligible in the second postnatal week. Next, we examined mice lacking VEGF in astrocytes. Interestingly, we observed significant defects in dendritic arborization and spine density, reminiscent to the defects observed in neuronal VEGFR2 mutants. These results indicate that astrocytes are necessary to maintain dendritic arbors and regulate spine morphogenesis through VEGF/VEGFR2 signaling. Finally, we examined mice lacking VEGF in excitatory neurons. We observed significant defects in dendritic arborization exclusively in the apical dendritic tree but not in the basal dendritic tree. Since presynaptic excitatory neurons, such as the granule cells from dentate gyrus or neighboring CA3 pyramidal neurons are only projecting towards the apical dendritic tree, we postulated that VEGF is secreted locally in the axons of neurons. Thus, we performed *in situ* hybridization in primary mouse hippocampal neurons and confirmed VEGF mRNA expression along the axons. These results indicate that neuronal VEGF signaling is integrated in the hippocampal circuitry and plays an essential role in dendritic arborization in a regional-specific manner.

Disclosures: **M. Kracht:** None. **M. Segarra:** None. **A. Acker-Palmer:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.07/A7

Topic: A.05. Axon and Dendrite Development

Support: CRC1080 Neural Homeostasis

Title: Endothelial FLRT2 supports Purkinje cell development

Authors: *J. VOGENSTAHL[1], J. JIN[1], A. ACKER-PALMER[2,3,4];
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Abstract: Recent research has unveiled the contribution of endothelial signalling to crucial neurodevelopmental processes such as neurogenesis and neuronal migration. Neuronal-expressed Fibronectin leucine-rich transmembrane protein 2 (FLRT2) mediates cell-cell interactions involved in several developmental processes, such as neuronal migration. While the angiogenic effects of vascular FLRT2 are slowly emerging, the role of endothelial-specific FLRT2 on neuronal function remains unexplored. By combining transcriptomics and histology, we

demonstrated that FLRT2 is widely expressed in the cerebellar vasculature, particularly in the pia, white matter and Purkinje cell layer (PCL). Therefore, in this study, we investigated the role of endothelial-specific FLRT2 in cerebellar development. To this end, we used a transgenic tamoxifen-inducible mouse line to specifically suppresses FLRT2 expression *in vivo* in endothelial cells (*Flrt2*$^{i\Delta EC}$) from P1. When we examined vascularization at postnatal stages, we found that the number of vascular sprouts (tip cells) in the PCL of our *Flrt2*$^{i\Delta EC}$ mutants was halved in the first postnatal week. Interestingly, expansion microscopy and 3D-reconstructions revealed a physical interaction between vascular tip cells and developing Purkinje cells and their dendrites. Subsequently, we found that postnatal maturation of Purkinje cells was impaired in FLRT2 vascular mutants, suggesting that endothelial FLRT2 is important for Purkinje cell development. Finally, we used spatial transcriptomics to explore which signaling pathway(s) endothelial FLRT2 employs to modulate Purkinje cell development. All in all, our study has unveiled a signalling crosstalk between endothelial cells and Purkinje cells that regulates cerebellar development.

Disclosures: **J. Vogenstahl:** None. **J. Jin:** None. **A. Acker-Palmer:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.08/A8

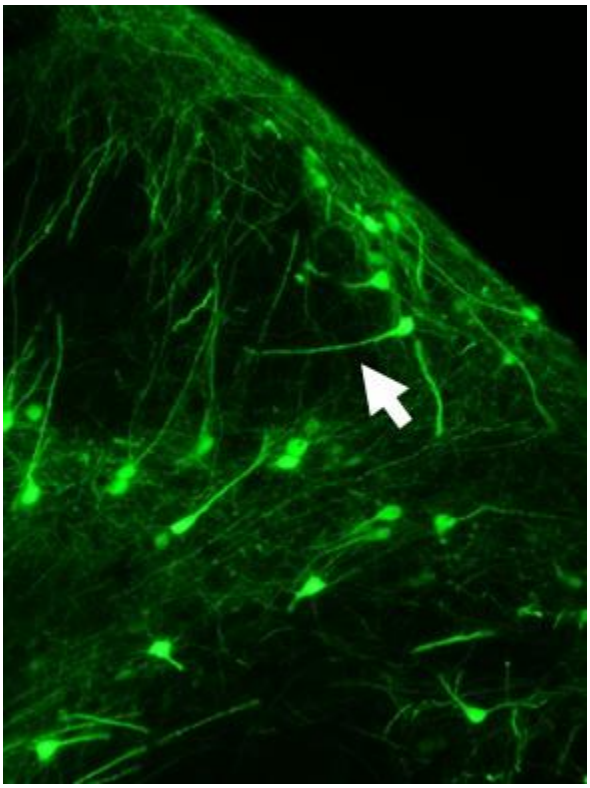
Topic: A.05. Axon and Dendrite Development

Title: Loss of dendritic polarity in layer V neurons in mouse models of neocortical malformations

Authors: *R. L. RAMOS[1], J. T. POPP[2], S. TRINGALI[2], Y. LI[2], R. F. STOUT, JR[3], G. H. OTAZU[4];
[1]New York Inst. of Technol. Col. of Osteo. Med., Old Westbury, NY; [3]Biomed. Sci., [2]NYIT Col. of Osteo. Med., Old Westbury, NY; [4]Biomed. Sci., NYIT-COM, Old Westbury, NY

Abstract: The laminated cytoarchitecture of the neocortex emerges from the precisely timed proliferation and migration of neurons. Deficits in neuronal migration result in phenotypes where lamination is disrupted with neurons having migrated too far or not far enough. Mouse models of neocortical malformations are important tools towards understanding anatomical and physiological changes in the malformed brain. Here we present data from mouse models of two different malformations, each crossed to YFP reporter mice in order to visualize the morphology of layer V neurons. Mice with molecular layer heterotopia as well as those with subcortical band heterotopia displayed large malformations containing YFP+ neurons normally found only in layer V. YFP+ neurons in both models exhibited apical dendrites with a loss of polarity relative to the pial surface and could be found entirely upside-down (arrow in figure; molecular layer heterotopia). Quantification of dendritic morphology is presented. Our results have implications

for understanding the malformed human brain and suggests that loss of polarity and altered dendrite development is a general feature of neuronal migration disorders.



Disclosures: **R.L. Ramos:** None. **J.T. Popp:** None. **S. Tringali:** None. **Y. Li:** None. **R.F. Stout:** None. **G.H. Otazu:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.09/A9

Topic: A.05. Axon and Dendrite Development

Title: Dendritic Filopodia Establish Nanotubular Network for Inter-neuronal Material Exchange

Authors: ***M. CHANG**[1], J. KIM[2], L. K. PARAJULI[3], A. MERODIO[1], S. OKABE[4], H.-B. KWON[1];
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Abstract: Successive studies in recent years have highlighted the novel biology of filopodia, a highly dynamic cellular protrusion. Under certain conditions, filopodia can bind to another

filopodium or cell body, forming a bridge-like structure known as a tunneling nanotube or nanotubular bridge (NB). These nanotubes, only a few hundred micrometers thick, create an additional dimension of cellular networks that various cell types utilize for intercellular material exchange. However, the presence of NB connections in neurons has been largely overlooked in canonical connectomes, despite the extensive presence of filopodia in neuronal dendrites. Furthermore, the minuscule size of filopodia has posed technical challenges in studying the filopodial network in depth. To address these limitations, we developed a specialized super-resolution microscopy technique that enables the visualization of dendritic filopodial bridges *in vitro* and *in situ* in the mouse brain, accompanied by tissue clearing techniques. This approach successfully characterized filopodial contacts, distinguishing them from neurites based on morphology, molecular composition, and plasticity in dissociated neurons. Intriguingly, we observed that an optically induced increase in Ca^{2+} concentration in a single neuron propagated to distant neurons over several tens of micrometers. The propagation pattern was disrupted by inhibitors of NB formation, suggesting an NB-mediated propagation mechanism *in vitro*. Moreover, we validated the existence of NBs connecting dendrites to other dendrites or somas among cortical layer 5/6 pyramidal neurons in the mouse brain using SRRF and EM imaging. By infusing fluorophores via whole-cell patch-clamping in a single neuron, we demonstrated specific transfer from dendrites to neighboring cells, confirming the expected role of the NB network in intercellular propagation. Further investigation using super-resolution imaging of dissociated neurons and the brain of an AD mouse model (APP/PS1XGFP) revealed that NB alterations are associated with Aß spreading and accumulation within individual neurons. Impaired NB formation and intraneuronal accumulation were observed in amyloid-beta-exposed neurons, suggesting a novel mechanism of NB-associated neurodegeneration.

Disclosures: **M. Chang:** None. **J. Kim:** None. **L.K. Parajuli:** None. **A. Merodio:** None. **S. Okabe:** None. **H. Kwon:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

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Program #/Poster #: PSTR182.10/A10

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS084111
NIH Grant NS114914
NIH Grant NS119512

Title: Nir2 localization at ER-PM contact sites regulates dendrite development via PIP2 signaling

Authors: *C.-T. CHIEN[1], A. GUO[1], J. BAUER[1], M. SHELLY[2];
[2]Stony Brook Univ., [1]Stony Brook Univ., Stony Brook, NY

Abstract: Phosphatidylinositol 4, 5-bisphosphate ($PI(4,5)P_2$) is a key phosphoinositide at the plasma membrane and is required for activation of various signaling pathways during early neuronal development. In particular, activation of phosphatidylinositol 3, ,4, 5-triphosphate ($PI(3,4,5)P_3$)-dependent Akt signaling and phospholipase C (PLC)-mediated calcium signaling are critical during early neuronal development and more specifically, neuronal migration and dendrite development. In the developing brain, neurons are perpetually exposed to extracellular growth factors that trigger the use of $PI(4,5)P_2$, resulting in its rapid depletion. Currently, little is known about how neurons regulate PM $PI(4,5)P_2$ homeostasis to enable sustained signaling during early neuronal development. A group of ER-PM non-vesicular lipid transport proteins present at sites of direct contact between the two membranes have been identified to regulate PM $PI(4,5)P_2$ homeostasis. Studies using cell lines have revealed that the lipid transport protein, Nir2, is a regulator of PM $PI(4,5)P_2$replenishment and Akt signaling upon growth factor stimulation by transporting phosphatidylinositol (PI) from the ER to the PM to generate $PI(4,5)P_2$ and phosphatidic acid (PA) from the PM to the ER to synthesize PI. Currently, the function and impact of Nir2 during early neuronal development is unknown. We propose that localized lipid transport via Nir2 is critical for PM $PI(4,5)P_2$ homeostasis to ultimately regulate dendrite development.

Disclosures: **C. Chien:** None. **A. Guo:** None. **J. Bauer:** None. **M. Shelly:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.11/A11

Topic: A.05. Axon and Dendrite Development

Title: Taurine-induced neuronal differentiation via opposing effects of $GABA_A$ and $GABA_B$ receptors in SVZ-derived progenitors cells

Authors: *N. E. GUTIÉRREZ[1], L. D. OCHOA-DE LA PAZ[2], E. GRIEGO[3], E. J. GALVAN[4]; [1]Neruofarmacología, CINVESTAV, Coyoacan, Mexico; [2]Natl. Univ. of Mexico|910004307|0, Natl. Univ. of Mexico|910004307|0, Queretaro, Mexico; [3]Ctr. de Investigación y Estudios Avanzados, Mexico City, Mexico; [4]CINVESTAV SUR, CINVESTAV SUR, Mexico City, Mexico

Abstract: Neuronal differentiation is a complex process characterized by the initial formation of immature neurites, commonly called “neurite outgrowth.” During neurite outgrowth, a diverse array of ligands, including neurotrophins, cytokines, hormones, and neurotransmitters, can stimulate neurite outgrowth upon binding to their cognate receptors. Taurine, a neuroactive factor, plays a central role in the proliferation, differentiation, and migration of neural progenitor cells. However, the mechanism of action of taurine is not well understood. We explored the effect of taurine on the differentiation process of SVZ neural progenitor cells through interaction with GABA receptors. Immunofluorescence assays were performed on cells isolated from SVZ

of CD1 mice (P6) in the absence or presence of taurine. Our results show that the number of DCX+ cells is increased in cultures exposed to differentiation conditions with taurine. Morphometric analysis revealed a significant difference in cell morphology. Compared to the control condition, taurine-treated cells exhibited a significant number of secondary and tertiary neurites, increased dendritic branching, and marked complexity in dendritic arborization. Taurine actions were sensitive to picrotoxin, indicating active participation of $GABA_A$ receptors. Also, the treatment with CGP55485 antagonist of $GABA_B$ receptors increased dendritic complexity and branching. Additionally, we measured the electrophysiologic properties of the control and taurine-treated cells with patch-clamp whole-cell recordings. Taurine-treated cells developed an electrophysiologic behavior that resembles passive and active properties of actual neurons. Our results provide information regarding the role of taurine as a morphogen in the neurogenic processes throughout the interaction with both GABA receptors and their role as a central player in the maturation processes of NPCs into functional neurons. This work suggests that the GABA receptor could act as a modulator that allows for a flexible regulation of neurogenesis through their receptors. It represents an advance in the morphometric effect and suggests a functional effect of taurine in the neuronal differentiation process.

Disclosures: **N.E. Gutiérrez:** None. **L.D. Ochoa-De La Paz:** None. **E. Griego:** None. **E.J. Galvan:** None.

Poster

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Program #/Poster #: PSTR182.12/A12

Topic: A.05. Axon and Dendrite Development

Support: ANPCYT PICT-2016
CONICET PIP 2021-2023
ANPCYT PICT 2020 Serie A-00136

Title: Suppression of spontaneous electrical activity changes the dynamics and maturation of axonal arborization in Zebrafish lateral line afferent neurons

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Abstract: Spontaneous electrical activity (SEA) is required for the proper assembly of sensory circuits during early stages of development, although the underlying mechanisms are still unknown. SEA is generated in sensory cells and propagated to the central nervous system. In order to understand the mechanisms by which SEA affects the assembly of developing sensory circuits, we studied the Zebrafish (Danio rerio) lateral line system (LL). The LL allows fishes and amphibians to detect water motion and pressure changes and consists of clusters of

neuromasts, which contain mechanosensory hair cells (HC) that are innervated by afferent (Aff) and efferent neurons. These HC share structural, functional and molecular similarities with those found in the vertebrate inner ear. Zebrafish LL Aff neurons exhibit SEA between 5 and 7 days post-fertilization. To investigate the effects of SEA on developing sensory circuits, we over-expressed inward rectifier K+ channels in order to silence SEA in single LL Aff neurons, and analyzed the phenotype and dynamics of axonal arbor growth and maturation. Our results indicate that suppression of SEA in single LL afferent neurons led to a decrease in innervation area in the hindbrain, as well as differences in axonal complexity. Moreover, silenced neurites displayed higher motility, formation and elimination rates, and a lower number of varicosities than wild-type neurons, which are features of immature neurons. Our study provides in vivo evidence that SEA regulates axonal arbor maturation, growth, and territory in the hindbrain of developing LL Aff neurons. These findings shed light on the key role that SEA plays in the proper assembly of sensory circuits, and may have implications for understanding the origin of sensory disorders.

Disclosures: **L. Salatino:** None. **A. Elgoyhen:** None. **P. Plazas:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.13/A13

Topic: A.05. Axon and Dendrite Development

Support: NIH-NS108189
NIH T32 Epilepsy training grant 5NT32NS045540
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Graduate Dean's Dissertation Fellowship from the University of California, Irvine School of Medicine

Title: Pten deletion-induced growth of mature granule cells in the adult dentate gyrus is dependent on sustained mTOR activation

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Abstract: Overactivation of the mechanistic target of rapamycin (mTOR) pathway during development is associated with reduced mental capacity and seizures in humans. Phosphatase and tensin homolog (PTEN) is an important negative regulator of this pathway and several studies have reported that PTEN hypofunction in early development leads to neuronal hypertrophy, formation of aberrant circuitry, and network hyperexcitability. We have recently reported that vector-mediated PTEN deletion in mature granule cells of the adult dentate gyrus re-initiates a state of robust neuronal growth characterized by increases in cell body size, increases in dendritic length and diameter, increases in spine density suggesting induction of

synaptic connections by input pathways with intact PTEN expression, and expansion of axonal projections into PTEN expressing target regions. Increases in granule cell body size are evident by 2 months after AAV-Cre injection, with growth of granule cell dendrites and axons by 4 and 6 months. Here, we assess whether the initiation and maintenance of this new growth in adulthood is dependent on mTOR activity. Adult PTEN-floxed, Rosa-reporter mice received unilateral injections of AAV-Cre into the dentate gyrus, resulting in focal PTEN deletion in mature granule cells. One group of mice received the mTOR inhibitor, rapamycin (6mg/kg i.p., 1x/day, 5 days/week), during the acute period after AAV-Cre injection (0-2 months) and a second cohort of mice received rapamycin from 2-4 months after PTEN deletion using the same dosing paradigm. Timing of treatment allowed us to determine whether the initial growth of granule cells depended on mTOR activation and whether the maintenance of that growth relied on continued mTOR activity. At each time period, rapamycin treatment effectively reduced the phosphorylation of ribosomal protein S6 in PTEN deleted granule cells that occurs with PTEN deletion, confirming inhibition of mTOR activity. Quantitative assessment of granule cell morphology revealed that rapamycin prevented the growth of granule cell somata and processes when given prior to their respective growth periods and delayed rapamycin administration reversed neuronal hypertrophy after substantial growth had been achieved. These findings suggest that mTOR activation is responsible for the morphological changes that occur as a result of PTEN deletion in mature neurons, and importantly suggest that down-regulation of mTOR activity could ameliorate the negative network and behavioral consequences in mTOR-related disorders.

Disclosures: J. Yonan: None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OS is a co-founder, scientific advisor, and has economic interests in the company Axonis Inc, which is developing novel therapies for spinal cord injury and other neurological disorders..

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.14/A14

Topic: A.05. Axon and Dendrite Development

Title: The 3'UTR of kpc-1/furin promotes dendritic mRNA transport and local protein synthesis to regulate dendrite branching and self-avoidance of a nociceptive neuron

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Abstract: A recently reported Schizophrenia-associated genetic variant in the 3’UTR of the human furin gene, a *kpc-1* homolog, highlights the important role of its 3’UTR in neuronal

development (Schrode, et al., 2019 *Nature Genetics*). We isolate three *kpc-1* mutants that display dendrite branching and self-avoidance defects in PVD neurons and defective male mating behaviors. We show that the *kpc-1* 3'UTR is required for dendrite branching and self-avoidance. The *kpc-1* 3'UTR facilitates mRNA localization to branching points and contact points between sibling dendrites and promotes local protein synthesis. We identify a secondary structural motif in the *kpc-1* 3'UTR required for dendrite self-avoidance. Animals with *dma-1* receptor over-expression exhibit similar dendrite branching and self-avoidance defects that are suppressed with *kpc-1* over-expression. Our results support a model where KPC-1 proteins are synthesized at branching points and contact points to locally down-regulate DMA-1 receptors to promote dendrite branching and self-avoidance of mechanosensory neurons required for male courtship behaviors.

Disclosures: M. Shih: None. **Y. Zou:** None. **T. Ferreira:** None. **N. Suzuki:** None. **K. Eichel:** None. **C. Chuang:** None. **C. Chang:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.15/A15

Topic: A.05. Axon and Dendrite Development

Support: 1F32MH131420-01A1

Title: Kif11 mutations that cause intellectual disability impact the dendritic arbor and microtubule dynamics

Authors: *J. WINGFIELD[1], S. V. PUTHANVEETTIL[2], N. KAMASAWA[3];
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Abstract: During mitosis, the cell relies on many microtubule motors to dynamically manipulate the microtubule architecture for proper cell division. Interestingly, several of these motors are expressed in postmitotic neurons, including KIF11 (kinesin-5) which walks on antiparallel microtubules to separate the spindle poles. Previous work from our lab found that KIF11 indeed functions in mature mouse primary hippocampal cultures to limit dendritic arborization, the number of synapses, and synaptophysin and piccolo expression (Swarnkar et al. 2018). Mutations in KIF11 cause Microcephaly with or without chorioretinopathy, lymphoedema, or mental retardation (MCLMR) yet little attention has focused on how impaired KIF11 function results in these phenotypes. We sought to understand how KIF11 mutations associated with MCLMR impact neuronal function. Specifically, we identified two mutations that cause minor microcephaly but severe intellectual disabilities, KIF11-Y81F and KIF11-2304_2304del. Expression of KIF11-Y81F in mouse primary hippocampal neurons led to a rapid and significant

decrease in dendritic arborization similar to overexpression of wildtype KIF11, while KIF11-2304_2304del expression had no effect on arborization. We next sought to investigate the mechanism by which KIF11 regulates dendritic arborization by first examining how KIF11 modulates microtubule dynamics in dendrites. We expressed Dendra-EB1 in primary hippocampal cultures and utilized two different KIF11 inhibitors: one that prevents KIF11 from binding to microtubules (Ispinesib) and one that locks KIF11 in the rigor state, bound to microtubules (BRD9876). Ispinesib treatment significantly increased the frequency and growth rate of retrograde EB1 comets compared to both the vehicle and BRD9876. However, BRD9876 had no significant effect on the frequency nor growth rate of EB1 comets in either the anterograde or retrograde direction. Additionally, Ispinesib shifted the distribution of EB1 comets from being primarily in the distal dendrite to being more evenly distributed throughout the dendritic arbor, while BRD9876 did not affect the distribution of EB1 comets. We reason that the observed increase in dynamic minus-end-out microtubules upon KIF11 inhibition is what ultimately leads to an increase in arborization and synapse number seen with KIF11 knockdown and a decrease in arborization with functional KIF11 overexpression. We propose a new model for dendritic arborization in which dynamic minus-end-out microtubules initiate the formation of new dendritic branches.

Disclosures: **J. Wingfield:** None. **S.V. Puthanveettil:** None. **N. Kamasawa:** None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.16/A16

Topic: A.05. Axon and Dendrite Development

Title: Spatial genome organization during neural circuit formation

Authors: *Y. FUJITA;
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Abstract: Chromatin is organized into multiscale three-dimensional structures, including chromosome territories, A/B compartments, topologically associating domains, and chromatin loops. This hierarchically organized genomic architecture regulates gene transcription, which, in turn, is essential for various biological processes during brain development. Here we focus on the role of the cohesin complex, which is chromosome-associated multi-subunit protein, in brain development.The cohesin complex is composed of four subunits, Smc1, Smc3, Scc1, and Scc3. It has been shown that cohesin is involved in chromatin organization by forming chromatin loops at particular gene loci and regulates gene expression in post-mitotic cells. The formation of chromatin loops facilitates enhancer-promoter interaction and alters a repressive chromatin structure. Mutations that disrupt the function of cohesin or the proteins that regulate cohesin cause Cornelia de Lange syndrome (CdLS), a rare malformation syndrome characterized by mental retardation, limb abnormalities, and distinctive facial features. To investigate the potential

role of cohesin in terminally differentiated cells in vivo, we generated conditional Smc3-knockout mice. We observed craniofacial abnormalities and decreased spine density in cortical neurons of heterozygous Smc3-knockout mice. Heterozygous Smc3-knockout mice exhibited increased anxiety-related behavior, a symptom of Cornelia de Lange syndrome. Thus, neuronal cohesin contributes to neural network formation, presumably by modulating gene expression, and cohesin deficiency leads to higher brain dysfunction.

Disclosures: Y. Fujita: None.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.17/A17

Topic: A.05. Axon and Dendrite Development

Title: In vitro screening tools to assess effects of psychedelic substances on structural and functional neuroplasticity in rat primary neurons

Authors: *S. KRASSNIG, T. LOEFFLER, I. SCHILCHER, M. DAURER, S. PEINKIHER, S. FLUNKERT, M. PROKESCH;
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Abstract: Some psychedelic drugs have shown promise as therapies for treatment-resistant depression and post-traumatic stress disorders, while underlying mechanisms are not fully understood. Beneficial effects of psychedelic substances, like psilocin or ketamine, on neurons, especially neurite outgrowth, were recently described in independent publications. In most of these publications Scholl analysis or other semi-high-throughput analysis approaches were used to determine structural and functional plasticity of in vitro neurite networks. To efficiently screen for therapeutic effects of similar substances or developmental compounds, high-throughput platforms need to be optimized for this specific group of substances. For that purpose, primary cortical and hippocampal neurons derived from E18 Sprague Dawley rat embryos were isolated and treated with different psychedelics, including psilocin, 2,5-Dimethoxy-4-iodoamphetamine (DOI) and ketamine. Different treatment schedules, treatment durations, time points of treatment, and cell densities were tested for their effectiveness. Analysis of time-resolved neurite outgrowth, synaptogenesis, as well as spontaneous activity in different automated high-throughput applications was performed. Contrary to Scholl analyses, where the focus lies on individual cells, automated assessment of neurite outgrowth, branching and synaptogenesis of the entire cell population within one well was performed. Automated assessment of all treated neurons led to only small effective windows when compared to the respective vehicle controls. Focusing on activity-based read-outs, including indirect analysis of spontaneous activity by measuring calcium oscillation, revealed that significant beneficial effects on network activity and synchronization can be observed, but only within a short time frame. The effect of psychedelic substances on neurons in vitro depends on various factors, especially timing of treatment and

analysis. High-throughput platforms thus need to be optimized for this specific group of substances to obtain significant treatment windows.

Disclosures: **S. Krassnig:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **T. Loeffler:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **I. Schilcher:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **M. Daurer:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Peinkiher:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Flunkert:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **M. Prokesch:** A. Employment/Salary (full or part-time):; QPS Austria GmbH.

Poster

PSTR182. Mechanisms Underlying Dendrite Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.18/A18

Topic: A.05. Axon and Dendrite Development

Support: MSU seed grant

Title: Effect of human infant microbiome on dendritic morphology and neurobehavioral outcomes in mice

Authors: *H. DUBEY, *H. DUBEY, A. WHITE, S. LIU, R. ROYCHOUDHURY, A. ALEX, R. KNICKMEYER;
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Abstract: Background: Infancy is an important period in the assembly and maturation of the gut microbiome and colonization patterns in human infants have been linked to neurodevelopmental process. However, it is unclear if these associations are causal. To address this question, we performed fecal matter transplant from human infants to germ free (GF) pregnant mice and analyzed brain structure and neurobehavioral outcomes in offspring. **Method:** Pregnant Swiss Webster mice of age 6-8 weeks were divided into 4 groups (n=2-3/group). Group 1 (HUM1) was inoculated with infant fecal slurry characterized by high levels of *Bifidobacterium;* Group 2 (HUM2), was inoculated with infant fecal slurry characterized by high levels of *Bacteroides*, Group 3 (SPF) was inoculated with fecal slurry from specific pathogen free (SPF) mice, and Group 4 (GF), received autoclaved fecal slurry from SPF mice. All pregnant mice received a single 200µL dose of fecal slurry by oral gavage on day 10 of pregnancy. Offspring were evaluated between 6-9 weeks of age using the elevated plus maze (EPM), open field, light dark, social interaction, and novel object recognition tests. Also, spine morphology analysis was performed in prefrontal cortex (PFC), nucleus accumbens (NAc), amygdala, and hippocampus. **Results:** In the EPM, microbiome groups differed significantly in time spent in the closed arm ($F_{3,38} = 11.613$, $p = 0.0000152$) and number of entries into the closed arm ($F_{3,38} = 5.816$, $p = 0.00225$). GF mice spent less time in and made fewer entries into the closed arm than other groups. Microbiome group did not influence the other tasks ($p>0.003$),

though exploratory sex-stratified analyses suggest microbiome group may influence social behavior in males (p=0.0002). Gut microbiome groups differed significantly in dendritic length in PFC ($F_{3,7}$ = 13.934, p = 0.00245) with HUM1 and SPF mice having longer dendrites than GF and HUM2 mice. For dendritic volume, microbiome groups differed significantly in the NAc ($F_{3,8}$ = 8.594, p = 0.006965), with HUM1 and SPF mice having more volume than HUM2 mice. For spine density, microbiome groups differed in hippocampus ($F_{3,3}$ = 70.86, p = 0.00278) and NAc ($F_{3,8}$ = 11.008, p = 0.00327). HUM2 mice have reduced spine density compared to other groups in hippocampus while in the NAc, HUM2 mice have reduced density compared to HUM1 and SPF mice, and HUM1 mice have greater density than GF mice. **Conclusion:** This study provides new information about the impact of gut microbiomes on behavior and dendritic morphology in mouse models. While humanized mouse models have limitations, they also hold great potential for testing the impact of therapeutic strategies on neurodevelopmental and behavioral outcomes.

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Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.01/A19

Topic: A.07. Developmental Disorders

Support: MEXT/JSPS KAKENHI Grant Number JP21K20705
Shinkei Kenkyujo
Meiji Yasuda Kokoro no Kenkou Zaidan

Title: Transient inactivation of developing Purkinje neurons causes male-specific social deficits

Authors: *S. KAMIJO, H. MIWA;
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Abstract: Background: Autism spectrum disorder (ASD) is a common neurodevelopmental disease with an estimated prevalence of 3% and approximately 80% of the patients are male. Although its etiology remained elusive, recent epidemiological data showed that perinatal cerebellar injury raised the risk of developing ASD by 36-fold. Previous studies show that transgenic mice losing their Purkinje neurons in early adolescence express ASD-like traits, suggesting that developmental cerebellar impairment is sufficient to cause the disorder. However, previous studies had three limitations. First, most studied mice had irreversible dysfunction of Purkinje neurons. Second, the tested animals were mostly male. Third, their sensory abnormalities were not investigated. To study the critical period and the treatability of ASD, temporally precise control of cerebellar activity is needed. In addition, if cerebellar dysfunction is the primary cause of ASD, it is important to know how it accounts for the biased

sex ratio of ASD patients and the common accompanying symptoms such as sensory hypersensitivity. Methods: We used the mice expressing inhibitory DREADD (designer receptors exclusively activated by designer drugs) specifically in Purkinje neurons. Clozapine N-oxide was orally administered to their pups at a dose of 5 mg/kg from postnatal day 11 to 15. After 8 weeks old, they were subjected to behavioral and histological experiments. Results: Developmental inactivation of Purkinje neurons did not affect their density and the molecular layer thickness in lobule IX. However, in a three-chambered test, the social preference index of male mice was specifically decreased by CNO administration, while their grooming time was unchanged. The performance in the rotarod test was mildly impaired only in male mice. The acoustic startle response test showed no signs of sensory abnormalities in both sexes. Conclusions: Our results indicate that the transient inactivation of Purkinje neurons during development was sufficient to elicit several ASD-like phenotypes, including social preference and motor coordination, in adult male mice. Interestingly, most of the behavioral phenotypes were observed only in males, which may reflect sex difference in susceptibility to cerebellar functional disruption. Thus, this sex-specific vulnerability may be an underlying cause of a strong male bias in the prevalence of ASD patients.

Disclosures: **S. Kamijo:** None. **H. Miwa:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.02/A20

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 MH097949-01
Autism Speaks Pilot Grant 7359

Title: The Role of PTEN Subcellular Localization in the Regulation of Neuronal Development

Authors: *N. DESMET;
Geisel Sch. of Med. at Dartmouth, Hanover, NH

Abstract: Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental disorder affecting 1 in 36 children in the United States. Symptoms of ASD include repetitive behaviors, social difficulties, and trouble communicating, negatively impacting quality of life, and, in severe cases, financially burdening the healthcare system. While most cases are idiopathic, a growing number of cases have been linked to genetic mutations. Recent genomic sequencing reports indicate that Phosphatase and Tensin Homolog (PTEN) is the 6th most commonly mutated gene in patients with ASD. When *PTEN* is mutated, unregulated activation of the AKT/mTOR pathway drives neuronal hypertrophy. Some mutations found in ASD patients result in nuclear exclusion of PTEN, but we do not fully understand how the localization of PTEN regulates its function.

PTEN appears broadly localized throughout the cell and may have distinct functional impacts depending on where it is localized. For example, nuclear PTEN has been shown to govern chromosome stability, cell cycle regulation, DNA repair, and may downregulate the mTOR pathway by dephosphorylating PIP_3 in the nuclear matrix. PTEN has also been shown to modulate synapse formation and plasticity, but the PTEN localization requirements for this remain unknown. Using $PTEN^{flx/flx}$/Ai14 mice and retroviral-mediated genetic manipulation, endogenous *Pten* can be knocked out and simultaneously reconstituted with *PTEN* fused to localization motifs. By localizing PTEN to the post-synaptic density, to the nucleus, or excluding it from the nucleus, we have determined the importance of subcellular location in regulating neuronal hypertrophy and spine formation.
Loss of *Pten* in developing hippocampal neurons results in an increased soma area and increased dendritic spine density, length, and head area when compared to same tissue, birth-dated wildtype controls. PTEN overexpression, PTEN localized to the post-synaptic density, and nuclear-excluded PTEN rescue each of the phenotypes observed in *Pten* KO neurons. These results suggest that PTEN is needed at the membrane to control neuronal growth and spine density. However, nuclear-localized PTEN can rescue the increase in dendritic spine head area. This may imply a mechanism through which PTEN-regulated transcription controls spine head area, a crucial characteristic of synapse strength and function.
Understanding how subcellular localization regulates dendritic growth, filopodial motility, and synaptic physiology are important future directions.

Disclosures: **N. Desmet:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.03/A21

Topic: A.07. Developmental Disorders

Title: Neural Mechanisms of Visuomotor Processing in Children with Autism Spectrum Disorders: A Magnetoencephalography Study

Authors: *K.-M. AN;
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Abstract: Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by impaired social communication and interaction, restricted interests, and repetitive behaviours. In addition to these core symptoms, sensory and motor difficulties are prevalent in a majority of children with ASD, affecting over 80% of individuals. However, our understanding of the underlying neural mechanisms associated with sensorimotor processing in children with ASD remains limited. To address this gap in knowledge, we conducted a study involving 18 children diagnosed with ASD (mean age = 6.00 years, SD = 0.59, 5 females, 13 males) and 19 typically developing children who were matched based on age and IQ (mean age = 5.71 years, SD = 0.46,

4 females, 15 males). We designed a child-friendly motor task that resembled a video game, consisting of 100 trials where participants had to press a button in response to a visual target while we record child-customized magnetoencephalography (MEG) system. We observed significant power increases in motor gamma oscillations ranging from 70 to 90 Hz during the 0 to 100 ms period following the button response onset. Additionally, we found power increases in visual gamma oscillations ranging from 50 to 60 Hz during the 150 to 450 ms period following the visual target onset. Both the typically developing (TD) and ASD groups demonstrated increased power in visual gamma oscillations within the bilateral cuneus and motor gamma oscillations in the primary motor cortex (M1). Consistent with our previous study, we identified statistically significant differences in motor-related gamma power in the right M1 ($t = 2.412$, $p = 0.021$), but not in the left M1, between the TD and ASD groups. Moreover, in this study, we did not find any statistically significant difference in visual gamma power within the bilateral cuneus between the two groups. Furthermore, to investigate the relationship between visual and motor processing, we conducted correlation analyses to examine the associations between changes in visual- and motor-related gamma power. Within the TD group, we discovered significant negative correlations between visual and motor gamma power specifically within the left hemisphere ($\rho = -0.553$, $p = 0.014$). However, such correlations were not observed within the ASD group. These findings provide compelling evidence for distinct neural mechanisms underlying varied patterns of visuomotor processing in individuals with ASD.

Disclosures: **K. An:** A. Employment/Salary (full or part-time):; Centre for Human Brain Health, School of Psychology, University of Birmingham. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Research Center for Child Mental Development, Kanazawa University.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.04/A22

Topic: A.07. Developmental Disorders

Support: Academia Sinica
Ministry of Science and Technology
Bened Biomedical Co., Ltd

Title: Mechanisms underlying Lactobacillus plantarum-mediated improvement of social behavior in VPA-induced ASD mouse model

Authors: C.-M. CHEN[1,2], C.-C. WU[2], Y. KIM[3], Y.-M. HUANG[3], C.-C. CHUNG[3], W.-Y. HSU[3], Y.-C. TSAI, M[1], *S.-L. CHIU[3];
[1]Inst. of Biochem. and Mol. Biol., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; [2]Bened Biomed. Co., Ltd, Taipei, Taiwan; [3]Academia Sinica, Taipei, Taiwan

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects more than 1% of the world's population. Patients with ASD are often diagnosed with complex behavioral conditions including significant social difficulties and repetitive behaviors with high comorbidity of intellectual disability and anxiety. Due to complex genetic variations and environmental risks, the etiology of ASD is heterogeneous and remains largely unknown. This poses a fundamental challenge to the development of treatments for ASD. We have previously shown that the daily supplementation with the probiotic Lactobacillus plantarum PS128 (PS128) alleviates ASD symptoms in affected children. The underlying mechanism by which it improves ASD-related behaviors remains elusive. Using a well-established ASD mouse model induced by prenatal exposure to valproic acid (VPA), we found that PS128 selectively ameliorated behavioral abnormalities in social and spatial memory, but not repetitive behaviors in VPA mice. Morphological examination of dendritic architectures further revealed that PS128 restored the reduced dendritic arborization and spine density in the hippocampus and prefrontal cortex of VPA mice. Most importantly, PS128 restores oxytocin expression in the PVN and oxytocin receptor signaling in the hippocampus. In the gut, PS128 alters the composition of the microbiota and increases the abundance of Bifidobacterium. Furthermore, PS128-induced changes in Bifidobacterium abundance correlate well with PS128-induced behavioral improvements. Together, our results demonstrate that daily PS128 supplementation ameliorates ASD-associated behaviors and restores oxytocin receptor signaling in VPA mice, providing an effective treatment strategy for the development of ASD therapeutics

Disclosures: **C. Chen:** A. Employment/Salary (full or part-time):; Bened Biomedical Co., Ltd. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Bened Biomedical Co., Ltd. **C. Wu:** A. Employment/Salary (full or part-time):; Bened Biomedical Co., Ltd. **Y. Kim:** None. **Y. Huang:** None. **C. Chung:** None. **W. Hsu:** None. **Y. Tsai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bened Biomedical Co., Ltd. **S. Chiu:** 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.; Bened Biomedical Co., Ltd. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Bened Biomedical Co., Ltd.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.05/A23

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH100028; PI: K.A.P.

Title: Anxiety problems and structural amygdala volume differences in autistic and non-autistic youth

Authors: *J. P. BOYLE[1], G. A. MCQUAID[1], A. JOB SAID[2], G. WALLACE[2], K. A. PELPHREY[3], A. JACK[1];
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Abstract: Background The amygdala is involved in anxiety and has been highlighted in theoretical and practical frameworks of autism. Autistic youth demonstrate elevated anxiety relative to non-autistic youth. To date, however, relatively little work has examined amygdalar involvement in anxiety in autistic versus non-autistic youth. Existing work has also linked female sex assigned at birth to anxiety in autism, yet few to no neuroimaging studies have recruited sex-balanced samples. Thus, understanding how amygdala structure relates to anxiety and sex assigned at birth is an important understudied area.
Methods 122 autistic (48% female) and 109 non-autistic (51% female) youth aged 8-17y (autistic M[SD]=12.9[2.8]y; non-autistic M[SD]=13.3[3.0]y) underwent a T1-weighted structural magnetic resonance imaging (MRI) scan. MRI data were processed via FreeSurfer, producing both cortical and subcortical volumes. Parent-report on the Childhood Behavioral Checklist (CBCL) anxiety problems subscale was collected to characterize anxiety severity in youth. We tested whether there were left or right amygdala volume (normalized by intracranial volume) differences between autistic and non-autistic groups using independent-samples t-tests; we also examined whether autistic individuals differed from their non-autistic same-sex peers in terms of anxiety or social problems. Finally, we examined whether anxiety in autistic female individuals predicted normalized left or right amygdala volume using bivariate regression models.
Results Overall, between-group comparisons did not reveal differences in normalized left (*t*[df]=-0.85[227], *p*=0.34) or right amygdala volume (t[df]=-0.85[218.80], *p*= 0.34). Autistic females reported significantly greater anxiety problems relative to non-autistic females (*t*[df]= 18.40 [79.16], $p < 0.001$). Autistic males and autistic females did not differ on anxiety (*t*[df]= -0.35[114.14] $p = 0.70$) or social behavioral problem measures (*t*[df]= -0.13(116.15) $p = 0.90$). Anxiety levels in autistic females as a predictor of normalized left (β[SE]= 0.35[0.02], $p = 0.95$) or right amygdala volume (β[SE]= -0.01[0.03], $p = 0.25$) did not survive statistical significance.
Discussion These findings reveal heightened parent-reported anxiety levels in autistic versus non-autistic female youth. Contrary to our hypotheses, amygdala volumes did not differ between autistic and non-autistic groups, and was not a predictor of anxiety in this unique sex-balanced sample. We explore areas for future research to determine whether factors such as age or assessment methodology contribute to this lack of correlation.

Disclosures: J.P. Boyle: None. **G.A. McQuaid:** A. Employment/Salary (full or part-time):; Research Assistant Professor. **A. Job Said:** None. **G. Wallace:** A. Employment/Salary (full or part-time):; Associate Professor. **K.A. Pelphrey:** A. Employment/Salary (full or part-time):; Professor. 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.; Principal Investigator. **A. Jack:** A. Employment/Salary (full or part-time):; Assistant Professor. 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.; Co-investigator.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.06/B1

Topic: A.07. Developmental Disorders

Title: Altered white matter microstructure of language networks in autism spectrum disorder: An automated fiber quantification analysis with multi-site datasets

Authors: *L. MIN[1], K. KAGITANI-SHIMONO[1], M. IZUMOTO[1], Y. WANG[1], Y. KATO[1], Y. IWATANI[1], Y. MIZUNO[2], M. TACHIBANA[1], I. MOHRI[1];
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Abstract: Individuals with autism spectrum disorder (ASD) often co-occur apparent language deficits that are related to abnormal connectivity of language networks. The precise association between white matter (WM) microstructure and language profiles in ASD remains unclear. Few studies explored the WM abnormalities of ASD in specific segments. This study aimed to investigate the fine-grained microstructure abnormity of language-related tracts and to elucidate the relationships between WM abnormalities and comprehension ability and severity of ASD traits. ASD subjects (6-18 years) and age-matched typically developing (TD) controls were recruited at Osaka University and Fukui University. Automated fiber quantification(AFQ) was utilized to identify major WM tracts. The group difference in diffusion metrics of three ventral tracts (UF: uncinate fasciculus, ILF: inferior longitudinal fasciculus, IFOF: inferior frontal-occipital fasciculus) at pointwise (100 nodes) and global fiber levels were assessed. ComBat method was applied to correct the multi-site effect on diffusion metrics. The diffusion metrics examined the correlation with the verbal comprehension index (VCI) and autism diagnostic observation schedule-2 (ADOS-2) scores in ASD. Subgroup analyses between the children and adolescents were performed to explore the moderating effects of the developmental stage. A total of 84 ASD and 83 TD subjects were included in this study. In global fiber level, significantly elevated mean diffusivity (MD) in left IFOF ($F=2.214$, $p=0.035$) and left ILF ($F=2.235$, $p=0.027$) in ASD subjects compared to TD controls, especially in the children group. In pointwise analyses, the aberrant microstructure prominently appeared in the anterior portion of left IFOF ($p<0.01$) and the anterior-middle part of left ILF ($p<0.01$). A negative correlation was found between MD in left ILF and VCI in the children with ASD ($r=-0.420$; $p=0.006$), indicating ASD children with lower WM integrity of left ILF have poorer comprehension ability. Increased MD in left IFOF was associated with higher ADOS-2 scores in ASD children ($r=0.443$; $p=0.014$), fractional anisotropy (FA) in left IFOF ($r=-0.512$; $p=0.021$) and left ILF ($r=-0.537$; $p=0.012$) negatively correlated with symptom severity in ASD adolescents. In conclusion, ASD patients have apparent disruption of WM integrity, particularly in the temporal and frontal-temporal parts of left ILF and left IFOF. These altered microstructures accompany poorer comprehension performance and more severe symptoms of ASD. In addition, younger children with ASD tended to show more pronounced abnormalities that indicate a delay in the maturity of WM integration.

Disclosures: **L. Min:** None. **K. Kagitani-Shimono:** None. **M. Izumoto:** None. **Y. Wang:** None. **Y. Kato:** None. **Y. Iwatani:** None. **Y. Mizuno:** None. **M. Tachibana:** None. **I. Mohri:** None.

Poster

PSTR183. Autism: Physiology and Systems

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Topic: A.07. Developmental Disorders

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AMED JP18dm0307009
Moonshot R&D JPMJMS2021

Title: Generalizable neuromarker for autism spectrum disorder across imaging sites and developmental stages: A multi-site study

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Abstract: Autism spectrum disorder (ASD) is a lifelong neurodevelopmental condition, and its underlying biological mechanisms remain elusive. Developing generalizable neuroimaging-based biomarkers for ASD faces several challenges due to confounding factors such as inter-site variability and developmental stages. Bridging the gap between ASD and other psychiatric disorders, such as schizophrenia (SCZ) and major depressive disorder (MDD), has gained significant interest due to overlapping symptoms and cognitive impairments. Despite this, the biological continuity between ASD and these disorders remains enigmatic. In this study, we used a large-scale, multi-site dataset (SRPBS Multi-disorder MRI Dataset; https://bicr.atr.jp/decnefpro/data) from 730 Japanese adults, including 180 adults with ASD and 550 typically developing controls to construct a resting-state functional connectivity-based classifier for ASD. We employed the area under the curve (AUC) and Matthew's correlation coefficient (MCC) for the performance indices. We thoroughly evaluated its generalizability to multiple validation datasets consisting of US and Belgium adults (n=121), Japanese adults (n=60), children (n=321), and adolescents (n=253). Our adult ASD classifier achieved successful generalization for US adults (AUC=0.70 and MCC=0.25) and Japanese adults (AUC=0.81 and MCC=0.52). The classifier also demonstrated acceptable generalization for children (AUC=0.66 and MCC=0.27) and adolescents (AUC=0.71 and MCC=0.32). We identified 141 FCs important for ASD status and found that these FCs spanned multiple networks, including the default mode, somatomotor, and subcortical networks, and encompassed multiple brain regions, such as ventromedial and dorsomedial prefrontal cortices, superior temporal gyri, right amygdala, midbrain, and hippocampus. Furthermore, we constructed SCZ and MDD classifiers to examine the biological relations of ASD and these disorders. By utilizing these classifiers (i.e., ASD, SCZ, and MDD classifiers) as biological axes, we mapped these disorders onto these axes to explore the biological continuity between ASD and the two disorders. We observed the asymmetrical relationship between ASD and SCZ on the biological plane but no such relationship between ASD and MDD. These thorough evaluations ensure the robustness of our ASD neuromarker, and our neuromarker-based analytical framework provides an effective tool for exploring cross-disorder continuity between ASD and other psychiatric disorders.

Disclosures: **T. Itahashi:** None. **A. Yamashita:** None. **Y. Takahara:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **N. Yahata:** None. **M. Nakamura:** None. **Y.Y. Aoki:** None. **R. Aoki:** None. **J. Fujino:** None. **H. Ohta:** None. **Y. Yoshihara:** None. **Y. Sakai:** None. **M. Takamura:** None. **N. Ichikawa:** None. **G. Okada:** None. **G. Okada:** None. **N. Okada:** None. **K. Kasai:** None. **S.C. Tanaka:** None. **H. Imamizu:** None. **N. Kato:** None. **Y. Okamoto:** None. **H. Takahashi:** None. **M. Kawato:** None. **O. Yamashita:** None. **R. Hashimoto:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.08/B3

Topic: A.07. Developmental Disorders

Support: NIMH Grant K01MH116098
NIMH Grant F31MH122107
DoD Grant AR140105
ABRC Grant ADHS16-162413

Title: Verbal fluency in autistic adults: The impact of age on the relationship between cortical thickness and language production

Authors: *S. CORTES CORIA[1], D. OGBEAMA[1], L. C. BAXTER[2], B. BRADEN[1];
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Abstract: Introduction: It is known that as one ages, cognitive function declines. However, cognitive aging research on adults with Autism Spectrum Disorder (ASD) is scant. Verbal fluency is a language-based executive function that is impaired in many with ASD, and negatively affected by normal age-related processes. The brain basis of verbal fluency challenges in autistic adults across the lifespan is largely unknown, but may be related to the cortical integrity of language production and executive function brain regions.
Methods: We investigated cross-sectional verbal fluency behavior, and the relationship between fluency and cortical thickness in right-handed adults with ASD (n=119) versus neurotypical (NT) (n=92) adults, ages 18 to 70 years old. Commonly used behavioral measures of phonemic (i.e. letter) and semantic (i.e. category) word production and cortical thickness of language-related left hemisphere areas (pars opercularis, pars triangularis, superior frontal, rostral anterior cingulate, and caudal anterior cingulate). Cortical thickness correlations with total phonemic and semantic word production were compared between groups.
Results: In the cross-sectional analysis, adults with ASD demonstrated persistent challenges in initiating phonemic ($p=0.026$) and maintaining semantic ($p=0.004$) fluency across the entire adult age range. There were no significant diagnosis group by age effects on behavioral measures of fluency. A significant brain correlation with fluency behavior was found between left hemisphere caudal anterior cingulate thickness and better phonemic fluency in older autistic adults (40-70 years old; $r(64)=0.27$; $p=0.037$), with no other brain regions correlating or any significant correlations in other groups (i.e. young-adult ASD, young-adult NT, or older NT).
Discussion: Behavioral findings from the present study suggest that adults with ASD across a wide age range have persistent difficulties with verbal fluency production, but that these abilities may not change differently from NT adults during aging. Neuroanatomical findings suggest older autistic adults may rely more on the anterior cingulate, which has been previously shown to underly effortful language production, than younger autistic adults or NT adults of any age. More research is needed to determine effective behavioral and/or brain-based intervention approaches to support language abilities in autistic adults across the lifespan.

Disclosures: S. Cortes Coria: None. **D. Ogbeama:** None. **L.C. Baxter:** None. **B. Braden:** None.

Poster

PSTR183. Autism: Physiology and Systems

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Program #/Poster #: PSTR183.09/Web Only

Topic: A.07. Developmental Disorders

Support: NIH Grant R01-MH103494

Title: Brain age index is greater in adults with autism than in age peers without autism

Authors: *G. GARCIA[1], A. C. LINKE[2], J. S. KOHLI[3], I. MARTINDALE[3], I. SHRYOCK[3], M. WILKINSON[3], M. CORDOVA[3], J. HAU[3], K. ALEMU[3], G. TORO[3], S. PEDRAHITA[3], I. FISHMAN[1], R.-A. MÜLLER[3], R. A. CARPER[2];
[2]Psychology, [1]San Diego State Univ., San Diego, CA; [3]Psychology, Brain Develop. Imaging Lab, San Diego State Uni, San Diego, CA

Abstract: Individuals with autism spectrum disorder (ASD), a lifelong neurodevelopmental condition, are at risk for accelerated neurocognitive decline in later adulthood. Neuroimaging research, however, has almost exclusively been conducted in children and young adults with ASD in whom neuroimaging findings have often been interpreted as indicative of maturational mistiming. Little is known about the changes in brain structure and function later in life in middle-aged and older adults with ASD. Estimated "Brain Age" (derived from normative models trained using machine learning) has been increasingly explored as a predictor of various neuropsychological symptoms and neurodegenerative disorders, age-related cognitive decline, and mortality. As such, it is also a promising measure to study differences in brain maturation in adults with ASD. We hypothesized that middle-aged and older adults with ASD would display a "positive" brain age gap (BAG; the difference between estimated brain age and chronological age) as compared to age- and sex-matched typical control (TC) adults. Cross-sectional structural T1-weighted MRI data from an ongoing longitudinal study of middle-age and older adults with ASD were utilized (TR=8.78ms, TE=3.66ms, resolution=0.8mm3, 3T GE MR750). Data from 70 participants aged 40 to 70 years (ASD: n=30, TC: n=40, mean age=52.9 years) were included in analyses. The two groups did not significantly differ on age, sex, ethnicity, body-mass-index, time of the MRI scan, or MRI data quality, nor on self-reported history of hypertension, hypercholesterolemia, or sleep problems, (all $p>.1$). Brain age was estimated using BrainageR (Cole et al. 2017). As expected, estimated brain age correlated highly with chronological age across both groups ($r=.71$, $p<.001$). There were no significant associations between BAG and data quality or time of scan, nor were there significant BAG differences based on sex, ethnicity, history of hypertension or sleep problems. Consistent with the evidence from other adult cohorts, BAG was higher in participants (across groups) with hypercholesterolemia and it had a modest association with BMI. The hypothesized main effect of the ASD diagnosis was supported, with a significantly higher BAG found in the ASD in comparison to the TC group ($t=2.886$, $p=0.003$, $d=0.697$). Although this finding of an increased BAG in adults with ASD is potentially in line with the hypothesis of accelerated aging in people with autism, longitudinal research will be required to determine whether this late-life difference in BAG reflects early neurodevelopmental differences or accelerated aging in ASD.

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Poster

PSTR183. Autism: Physiology and Systems

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Program #/Poster #: PSTR183.10/B4

Topic: A.07. Developmental Disorders

Support: RFS 2021-7H05
GRF 17620520

Title: Temporal Dynamics and Neural Variabilities of Emotion-Cognition Interaction in Autism

Authors: *X. WANG, M. L. ZHANG, H. K. LEE, S. X. TONG;
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Abstract: Increasing evidence shows that autistic individuals exhibit atypical neural variability patterns in a variety of cognitive and emotional tasks. However, the neural dynamics underlying the emotion-cognition interaction in autism remains unexplored. This study examined this question by recording EEG/ERP signals of 50 autistic children and 46 non-autistic children when they performed an emotional Go/Nogo task. Single-trial ERP analyses revealed that at early stage of processing, autistic children exhibited a larger posterior-Nogo-N170 to angry faces and a smaller frontal-N200 to all faces (i.e., neutral, happy, angry, and surprised faces) than non-autistic peers, indicating their stronger early perception to negative emotions but an inadequate conflict monitoring regardless of emotions. At the late stage, a larger posterior-Go-P300 to angry faces and a larger posterior-Nogo-P300 to happy faces were evoked in autistic than non-autistic children, showing that these children required greater effort to resolve the conflicts between emotional processing and cognitive control. Single-trial neural variability analyses showed that autistic children exhibited excessive amplitude variability than non-autistic peers in the Nogo-N170 to happy faces, and insufficient amplitude variability in the Nogo-N200 to angry faces. These results suggest that autistic children are with an over-activation of brain networks during the early perception of emotion-cognition conflicts, and in an inadequate brain connection when viewing negative emotions, particularly during the conflict monitoring stage.

Disclosures: **X. Wang:** None. **M.L. Zhang:** None. **H.K. Lee:** None. **S.X. Tong:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

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Topic: A.07. Developmental Disorders

Support: The Dr Lorus J Milne and Dr Margery J Milne Award, Victoria College, University of Toronto

Title: Neural correlates of biological motion perception in individuals with autism spectrum condition and intellectual impairment

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Abstract: Background
Individuals with autism spectrum condition (ASC) tend to show altered biological motion perception (BMP) processing: the ability to identify human movement and infer socially salient information. However, even though ASC co-occurs at high rates with intellectual impairment (II), individuals with coexisting ASC and II have been largely excluded from studies of task functional MRI during BMP. By including these individuals in our analysis, we aim to contribute towards a more complete understanding of the neuroscience of BMP in ASC.
Methods
We collected multiband multi-echo functional MRI data on 32 intellectually-able individuals with ASC (ASC-IA), 23 individuals with coexisting ASC and II (ASC-II), and 35 typically developing control (TDC) individuals. IA participants were defined as having scores greater than 85 on both the Wechsler Intelligence Scale - 4th edition and Vineland Adaptive Behaviour Scales. Participants were shown six point-light display (PLD) animations of real biological motion and six spatially-scrambled PLD animations in an alternating-block design. Following the multi-echo ICA preprocessing, we constructed whole-brain contrast maps for each participant to identify voxels selectively active while viewing biological motion (vs. scrambled condition). We then used both statistical parametric mapping (SPM) and statistical nonparametric mapping (SnPM) to test between-group differences. Framewise displacement (FD) was controlled as a covariate for each individual in first-level analysis, and age, sex, and mean FD were covaried in second-level analysis.
Results
Using threshold-free cluster enhancement in SPM, we found two clusters in the right intraparietal sulcus (IPS) exhibiting greater BMP-dependent activation in the ASC-II group compared to the TDC group (both cluster-level q=0.040; k=22 and 15; peak coordinates 45, -49, 53 and 51, -39, 48, respectively; both belong to the frontoparietal network). SnPM analysis revealed a cluster of voxels in the right IPS that was activated more during BMP in the ASC-II group compared to the ASC-IA group (cluster-forming threshold=0.001; cluster-level p_{FWE}=0.0138; k=8; peak coordinates 43, -49, 51; frontoparietal network).
Conclusions
ASC-II individuals uniquely recruited the right IPS during BMP. Previous literature suggests that IQ plays a minimal role in BMP performance in individuals with ASC, meaning that IPS activation in the ASC-II group does not confer any obvious advantage or disadvantage to BMP. Therefore, hyperactivation of this area may develop in ASC-II individuals as a compensatory mechanism to allow BMP processing.

Disclosures: **M. Cheng:** None. **H. Lin:** None.

Poster

PSTR183. Autism: Physiology and Systems

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Topic: A.07. Developmental Disorders

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Title: Syngap1 deficiency disrupts synaptic neoteny in human cortical neurons in vivo.

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Abstract: Human brain ontogeny is characterized by a prolonged, neotenic, development of cortical neuronal circuits. Neoteny is thought to be essential for the acquisition of advanced cognitive functions, which are altered in intellectual deficiency (ID) and autism spectrum disorders (ASD). Neuronal neoteny could thus be disrupted in ID/ASD, but this has remained hypothetical because of the difficulties to study human developing cortical circuits. Here we use xenotransplantation of human cortical neurons into the mouse brain to model SYNGAP1 haploinsufficiency, a frequent cause of ID/ASD. We find that SYNGAP1 deficient human neurons display strong acceleration of synaptic formation and maturation. Long-term *in vivo* imaging of transplanted control (CTRL) and mutant (KO and HET) human neurons revealed a doubling of the spine density in KO vs. CTRL at 4 months (0.50 vs. 0.25 spines/µm, $p<0.001$). This difference remained significant between 4 and 7 months. The HET neurons showed a modest increase at 4 months; however, they obtain spine density levels similar to KO at 7 months. We then confirmed whether this initial increase in spines reflects functional synapses. Patch-clamp recordings yielded an increase in frequency of sEPSCs (CTRL 0.28, KO 0.80 Hz, $p<0.001$; CTRL 0.23, HET 1.02 Hz, $p<0.001$). The ratio of AMPA/NMDA amplitudes was increased at 4.5 months (CTRL 0.28, KO 0.85, $p<0.001$; CTRL 0.52, HET 0.72; $p<0.001$). Since we did not find any difference in intrinsic properties (rheobase, membrane potential, f/I curves …), this phenotype is mostly consistent with altered synaptic maturation. We then wondered what the impact on visual function would be. We found during both quiet wakefulness and visual stimulation an increase in calcium transients of HET neurons at all time points tested between

2.5 and 7.5 months. This is consistent with increased synaptic drive. One could hypothesize that precocious synaptic connectivity increases the probability of acquiring visual responsiveness, this is indeed what we found. By pooling data collected between 2.5 and 4.5 months, we found that 20% of HET neurons show robust visual responses while hardly any CTRL neurons are visually responsive (<1%) at this early time point. An increased number of functional synapses might lead to lower selectivity (i.e. broader tuning curves). We find no difference in the distribution of OSI/DSI values between genotypes at any of the time points tested. Our findings demonstrate disrupted neoteny of human cortical neurons in a common form of ID/ASD, providing direct links between human brain developmental mechanisms and neurodevelopmental disorders.

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Poster

PSTR183. Autism: Physiology and Systems

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Topic: A.07. Developmental Disorders

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Title: Corpus callosum microstructural alterations in autism localized with diffusion MRI and along-tract mapping

Authors: *G. S. KIM, S. M. BENAVIDEZ, B. Q. CHANDIO, K. E. LAWRENCE, P. M. THOMPSON;
Imaging Genet. Center, Mark and Mary Stevens Neuroimaging & Informatics Inst., USC, Marina Del Ray, CA

Abstract: Autism is a heterogeneous neurodevelopmental condition. Prior neuroimaging studies of autism have identified subtle and widespread alterations in gray and white matter structure and microstructure. Diffusion tensor imaging (DTI) research in autism has consistently identified microstructural differences in the corpus callosum (CC), although methods employed to date do not provide a detailed mapping of regional microstructure. To address this, we employed the advanced tractography-based approach, BUndle ANalytics (BUAN), to analyze white matter microstructure in autism at a finer anatomical scale. We analyzed diffusion-weighted brain MRI scans from 172 participants (age: 24.3$\pm$15.2 years, 99.4% male, 107 with autism and 65 neurotypicals) from the NIMH Data Archive and the Autism Brain Imaging Data Exchange. Data were preprocessed by denoising and correction for eddy currents, head motion, bias field, and gradient distortions. Standard DTI metrics were calculated at each voxel, including fractional anisotropy (FA), and mean, radial and axial diffusivity. Whole-brain tractograms were generated

using a constrained spherical deconvolution model and local deterministic tractography. We focused on 3 separate CC component tracts: midbody of CC (mid-CC), *forceps minor*, and *forceps major*. Linear mixed models were used to compare microstructural metrics between autism and neurotypical groups, after adjusting for age and sex and accounting for subject and site variability. False discovery rate (FDR) was used for multiple comparisons correction. We found tract segment differences in the mid-CC, with mean FA being lower in autism before FDR (Fig. 1). In sum, we observed localized alterations of the CC tract in autism using BUAN in this pilot study. Future larger sample studies are needed to replicate these findings, and determine microstructural associations with clinical measures and developmental origins. Whole-brain tract analysis will also help to identify subgroups in autism and their neural correlates for interventional studies.

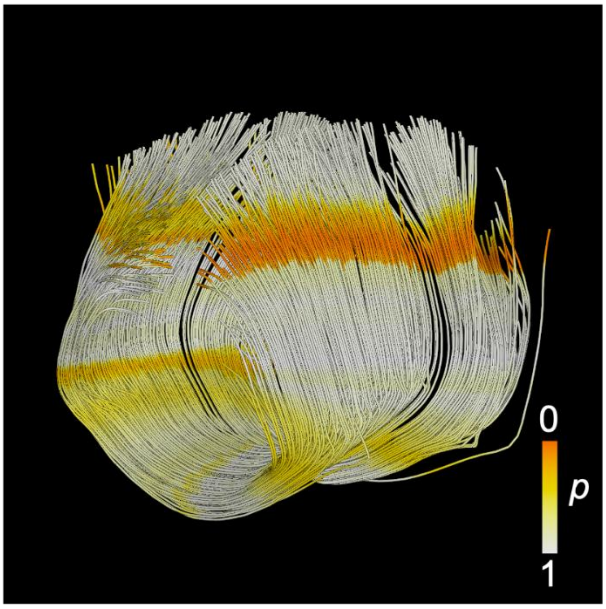

Figure 1. Microstructural alterations of the mid-CC localized in autism.
Autism is associated with localized microstructural differences (in mean FA) in the midbody of the CC (mid-CC) tract, compared to neurotypical controls. Significant p-values are depicted in dark orange.
CC: corpus callosum; FA: fractional anisotropy

Disclosures: G.S. Kim: None. **S.M. Benavidez:** None. **B.Q. Chandio:** None. **K.E. Lawrence:** 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

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.14/B8

Topic: A.07. Developmental Disorders

Support: NA

Title: Neuromelanin Imaging in Children with Neurodevelopmental Disorders: Validation through Ex Vivo Post-mortem Imaging

Authors: *S. AL-SAOUD, E. G. DUERDEN, D. SEGUIN, B. KARAT;
Univ. of Western Ontario, London, ON, Canada

Abstract: Children with autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) can exhibit restricted and repetitive behaviours (RRBs), which are mediated by alterations in dopaminergic processes. Recent advancements in neuromelanin magnetic resonance imaging (NM-MRI), has offered a unique and non-invasive way to examine dopamine concentrations, reflected in neuromelanin signal changes in the substantia nigra (SN) and locus coeruleus. However, current NM-MRI protocols for adults are in the range of ~8 minutes, which may be unsuitable for young children with developmental delay.The present study aimed to validate the utility of a novel NM-MRI protocol in assessing NM concentrations in the SN in relation to RRBs in children with ASD and ADHD through *in vivo* imaging. The sequence was further evaluated through *ex vivo* post-mortem imaging using high-field (3T) and ultrahigh field MRI (7T).Children with ASD (n=5, 2 males, mean age 12.2 years) and ADHD (n=10, 4 males, mean age 11.8 years) were scanned with a modified NM-MRI protocol using a three-dimensional gradient recalled echo sequence with magnetization transfer (MT) contrast (~5 minutes) on a 3T Prisma fit MRI scanner (Siemens, Erlangen, Germany). RRBs were assessed using the Repetitive Behaviors Scale - Revised (RBS-R). Children with ASD had higher RRBs, including restricted interests, compulsive behaviours, stereotyped behaviours, and ritualistic behaviours compared to children with ADHD (all, $p<0.05$). Children with ASD who had increased restricted interests were associated with higher NM-MRI signal changes relative to the crus cerebri. No differences in NM-MRI percent signal change were evidence amongst the original and modified scanning protocols at 3T or 7T ($p=0.072$).Post-mortem imaging at 3T and 7T was of a single brain obtained from a male (65 years) with no known neurological impairment. The specimen was scanned on the modified and original protocols at 3T and 7T. The NM-MRI protocol involved the extraction and quantification of NM signal change in the SN and the crus cerebri (control).The validation of a short NM-MRI sequence through *ex vivo* post-mortem samples provides crucial evidence supporting the use of this non-invasive imaging technique in the diagnosis and characterization of RRBs in children with ASD and ADHD. By identifying neurochemical changes associated with these disorders, NM-MRI has the potential to enhance our understanding of underlying pathophysiological mechanisms and aid in the development of targeted therapeutic interventions for RRBs.

Disclosures: **S. Al-Saoud:** None. **E.G. Duerden:** None. **D. Seguin:** None. **B. Karat:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.15/B9

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH100028; PI: K.A.P.
Autism Speaks, Grant 11808 (G.A.M.)

Title: Examining associations between brain volume and alexithymia in autistic youth and young adults

Authors: *G. MCQUAID[1], A. JACK[1], A. JOB SAID[2], Z. JACOKES[3], K. A. PELPHREY[3], G. L. WALLACE[2];
[1]Psychology, George Mason Univ., Fairfax, VA; [2]Dept. of Speech, Language and Hearing Sci., The George Washington Univ., Washington, DC; [3]Univ. of Virginia, Charlottesville, VA

Abstract: Introduction Alexithymia involves difficulties identifying/labeling emotions, is a trait continuously distributed in the population, and is elevated in autism (~33-63% autistic [AUT] adults vs ~10-17% of the general population). In autism, alexithymia is associated with behavioral and functional brain differences in processing others' emotions, suggesting socio-emotional AUT traits may be attributable not to autism *per se* but to co-occurring alexithymia. Relatively few studies have examined brain structure-alexithymia associations in autism. We examined associations between brain structure in regions implicated in the broader alexithymia literature in AUT and neurotypical [NT] youth and young adults.
Method At Wave [W] 1, 56 AUT (M=13.8 ± 2.5; 44.6% female) and 54 NT (M=14.1 ± 2.5; 53.7% female) participants completed a T1-weighted structural magnetic resonance imaging scan. Volume in eight bilateral FreeSurfer-generated regions of interest [ROIs], normalized by intracranial volume, were examined: amygdala, insula, dorsal anterior cingulate cortex [ACC], rostral ACC. At W2 (5-6y later), the same participants (AUT: M=19.9 ±2.4; NT: M=19.5 ± 2.3) completed the Toronto Alexithymia Scale [TAS-20]. A multivariate analysis of covariance with *W1 brain* ROIs as dependent variables, group, sex, and their interaction as predictors, controlling for W1 age, was conducted. A multiple regression model examined *W2 alexithymia* with TAS-20 as the dependent variable, and group, sex, and their interaction, and W2 age as predictors. Multiple regression modeling explored *W1 brain as a predictor of W2 alexithymia* with TAS-20 as the dependent variable, and ROIs, group, and their interaction, and W2 age as predictors.
Results *W1 brain* showed neither group nor sex differences (*p*s>.05). The *W2 alexithymia* model was significant ($F(3,106)=8.93$, $p=.00003$, Adj. $R^2=0.18$), with AUT persons reporting greater alexithymia (β=0.79, $p=.00002$), and an inverse age-alexithymia association (β=-0.22, $p=.01$). Sex did not reach significance (β=0.31, $p=.07$). The model *predicting W2 alexithymia from W1 brain* was significant ($F(11,98)=3.79$, $p=.0002$, Adj. $R^2=0.22$), with a significant group x right

dorsal [d] ACC volume interaction (β=0.41, *p*=.02).
Discussion AUT participants with larger W1 right dACC volume reported elevated W2 alexithymia. The dACC is associated with socio-emotional processing, and structural imaging studies of alexithymia in non-autistic samples implicate the dACC. While alexithymia is common in autism, it is not universal. Co-occurring alexithymia may thus identify an autism subtype and inform models of brain-based socio-emotional processing in autism.

Disclosures: G. McQuaid: None. **A. Jack:** None. **A. Job Said:** None. **Z. Jacokes:** None. **K.A. Pelphrey:** None. **G.L. Wallace:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.16/B10

Topic: A.07. Developmental Disorders

Support: T.R.I.U.M.P.H. Initiative MU School of Medicine Grant

Title: Dopamine transporter binding performance predict self-injurious behavior in autism spectrum disorder

Authors: *C. APPLING[1,2], M. J. PRENDERGAST[3], N. NURAINI[1], S. GONZALEZ[1], A. M. GUNN, MA[1], A. SINGH[1], D. Q. BEVERSDORF[4];
[2]Intedisciplinary Neurosci., [1]Univ. of Missouri, Columbia, MO; [3]Interdisciplinary Neurosci. Program, Univ. of Missouri, Columbia, Columbia, MO; [4]Univ. of Missouri Columbia, Columbia, MO

Abstract: Background: Autism spectrum disorder (ASD) is characterized by impairments in social communication, and restricted repetitive behaviors. Self-injurious behaviors are often observed in individuals with ASD. Dopamine is critical in reward, memory, and motor control. Some propose the nigrostriatal motor pathway may be altered in ASD, and alterations in dopamine are reported in some rodent models based on specific ASD genes. Additionally, repetitive behaviors may to be related to reward systems. Therefore, we examined the dopaminergic system, using dopamine transporter binding (DaTscan) and a motor task associated with dopaminergic function (Halstead-Reitan finger tapping), to explore their relationship with measures of repetitive behavior in a clinical ASD population.
Objective: Utilizing single-photon emission computed tomography dopamine transporter scans (DaTscan) and the Halstead-Reitan Finger Tapping Test (FTT) we examined whether dopamine markers are related to repetitive behaviors as assessed by the Repetitive Behavior Scale-Revised in ASD.
Methodology: 12 participants (aged 18-27) with ASD were recruited from the Thompson Center for Autism and Neurodevelopment completed a Halstead-Reitan FTT and the Repetitive Behaviors Scale - Revised (RBS-R). During the FTT, participants used alternating index fingers

to tap an apparatus for 10 second trials as seen in related research. Of the 12 participants, 10 underwent a 45-minute DaTscan. One female outlier for both FTT and RBS-R was removed from analysis. ANOVA was used to compare the dopamine related measures (FTT and DaTscan) with the overall total and endorsed RB scores on the RBS-R. As an exploratory study data analysis, other domains of the RBS-R were also investigated.
Results: FTT was not significantly related to the RSB-R scores for the 12 participants with FTT data. Additionally, 5 of the 9 included participants had regional deficits in dopamine transporter binding in the striatum on DaTscan. Individuals with deficits on the DaTscan had a significantly higher Self-Injurious Endorsed Score than those with normal scans.
Conclusion: Over half of the DaTscans obtained were determined abnormal, and abnormal scans were predictive of endorsing self-injurious behavior. FTT was not a significant RB predictor. However, larger samples are needed to confirm this. Findings suggest striatal dopamine binding may a potential biomarker for self-injurious RBs. The elucidation of striatal dopamine as a biomarker for RBs may be important for future interventional outcomes, and its role deserves further exploration for its relationship to other domains.

Disclosures: C. Appling: None. **M.J. Prendergast:** None. **N. Nuraini:** None. **S. Gonzalez:** None. **A.M. Gunn:** A. Employment/Salary (full or part-time):; Thompson Center for Autism and Neurodevelopment. **A. Singh:** None. **D.Q. Beversdorf:** F. Consulting Fees (e.g., advisory boards); consultancy for Quadrant Biosci, YAMO Pharma, Impel Pharma, Scioto Biosci, and Stalicla Biosci.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.17/B11

Topic: A.07. Developmental Disorders

Support: R01MH096847
R01NS110307
R01MH096847

Title: Genetic regulation of cortical sensorimotor processing required for active sensation and learning

Authors: T. VAISSIERE[1], ***S. MICHAELSON**[1], L. FONTOLAN[2], J. GOINS[1], T. CRESON[1], D. FÜRTH[3], D. BALAZSFI[1], C. ROJAS[1], K. MELETIS[4], D. O'CONNOR[5], C. MILLER[1], G. RUMBAUGH[1];
[1]The Herbert Wertheim UF Scripps Inst. for Biomed. Innovation and Technol., Univ. of Florida, Jupiter, FL; [2]Aix-Marseille Univ., Marseille, France; [3]Uppsala Univ., Uppsala, Sweden; [4]Karolinska Inst., Stockholm, Sweden; [5]Johns Hopkins Univ., Baltimore, MD

Abstract: Active sensation promotes adaptive decision-making by facilitating perceptual learning. How and where gene expression acts in the brain to shape active sensation is unclear. Here, we demonstrate in mice that expression of the major autism risk gene, *Syngap1*, in forebrain glutamatergic neurons, regulates perception that emerges from active touch. *Syngap1* regulated dynamics of whisker motion, a prerequisite for active touch, only when expressed in forebrain excitatory neurons and when tactile feedback was present. Long-range synaptic connectivity and communication subspace between cortical sensorimotor areas known to promote active whisker exploration and touch processing were altered in *Syngap1* deficient animals. Measurements of distributed cortical activity during active touch mirrored changes in regional connectivity. Activity representing pure whisker motion was elevated in cortical sensorimotor areas, while activity in response to object touch was depressed. Thus, *Syngap1* promotes efficient active touch by balancing temporally overlapping sensory and motor representations within neocortical circuits.

Disclosures: T. Vaissiere: None. **S. Michaelson:** None. **L. Fontolan:** None. **J. Goins:** A. Employment/Salary (full or part-time):; Cambridge Neurotech. **T. Creson:** None. **D. Fürth:** None. **D. Balazsfi:** None. **C. Rojas:** None. **K. Meletis:** None. **D. O'Connor:** None. **C. Miller:** None. **G. Rumbaugh:** None.

Poster

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.18/B12

Topic: A.07. Developmental Disorders

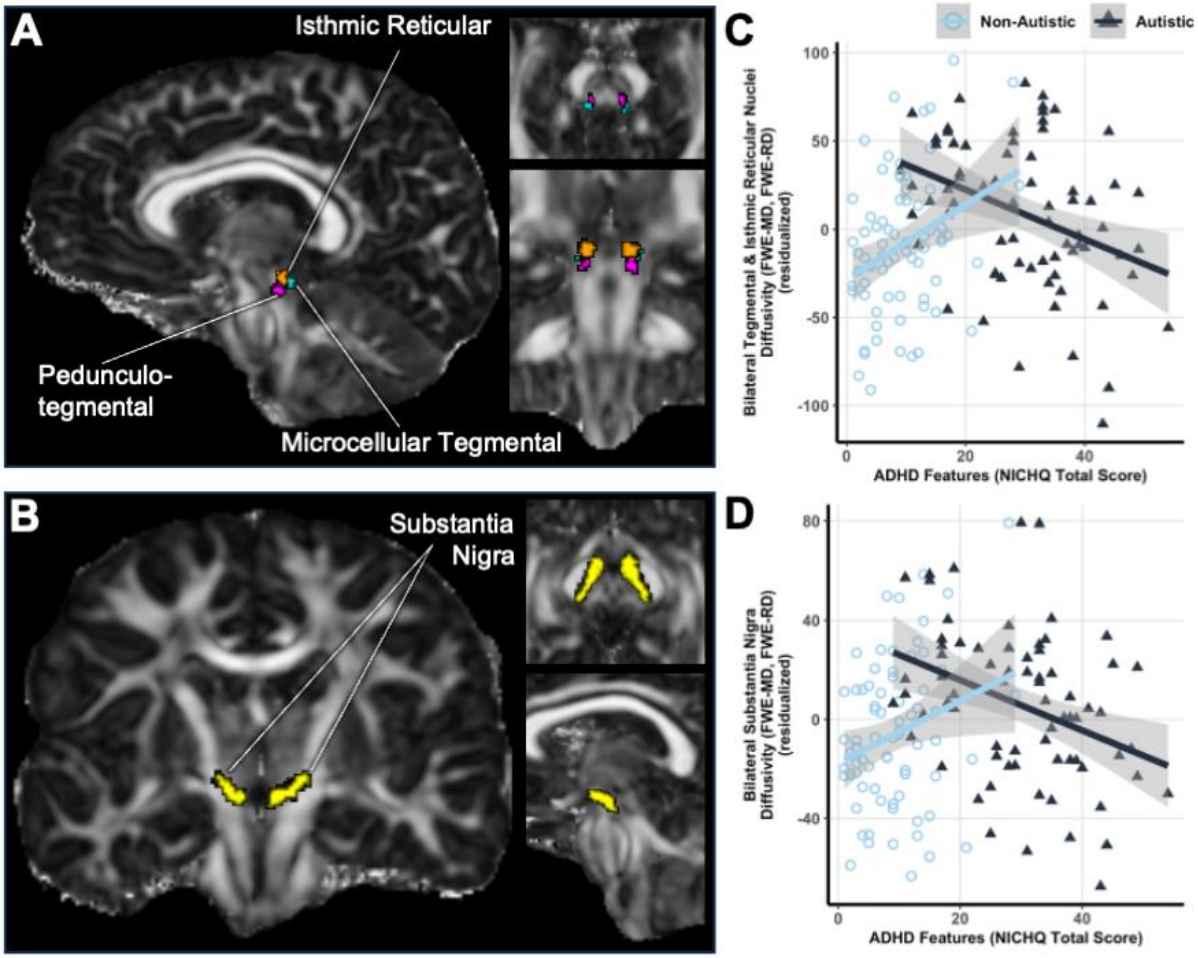
Support: Hartwell Foundation’s Individual Biomedical Award
NIH P50 HD105353
NIH U54 HD090256
NIH R01 HD094715

Title: Brainstem nucleus microstructure is uniquely related to ADHD features in autistic compared to non-autistic children

Authors: *O. SURGENT, M. I. DURAN, J. GUERRERO-GONZALEZ, N. ADLURU, G. R. KIRK, D. C. DEAN, III, A. L. ALEXANDER, J. LI, B. TRAVERS;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: 30-70% of autistic children have co-occurring ADHD (Lai et al., 2019). While the neural basis of co-occurring autism and ADHD is unknown, the brainstem has been linked to the emergence of neurodevelopmental conditions, such as autism spectrum disorder (Rimland et al., 1964; Dadalko & Travers, 2018). In autistic children, brainstem microstructure is uniquely linked to heightened sensory (Surgent et al., 2022) and core autism features (Travers et al., *in prep*) but it is unclear if the brainstem also uniquely contributes to ADHD. Therefore, we

analyzed the relationship between microstructure of 76 brainstem nuclei (Singh et al., 2022) and ADHD features (NICHQ; AAP, 2002) in 160 children (73 autistic; 48 female; 6.0 – 10.9 years). T1 and multi-shell diffusion weighted imaging data were collected with a 3T GE scanner and TiDi-Fused processed (Guerrero-Gonzalez et al., 2022) to optimize brainstem visualization. Microstructural features derived from relaxometry, free water elimination (FWE) diffusion tensor imaging, and neurite orientation dispersion and density imaging models were used in a principal component analysis for brainstem feature data reduction. Partial correlations accounting for age, sex, and head motion revealed that among autistic children (45 with ADHD diagnosis), more prominent ADHD features were associated with decreased diffusivity of bilateral tegmental and reticular nuclei (A) (r=-.38, p=.001), and decreased diffusivity of the substantia nigra (B) (r=-.37, p=.001), after FDR correction. Follow-up general linear models revealed significant ADHD feature-by-diagnostic group (autistic vs non-autistic) interaction effects for both nuclei groups (F(1, 144)=15.8, p<.001 [C]; F(1, 144)=11.7, p<.001 [D]). These results suggest that the brainstem plays a role in ADHD features of both autistic and non-autistic children. However, this role may be distinct depending upon diagnosis, signaling that behaviorally observed ADHD features may have neurobiologically distinct underpinnings in autistic children compared in non-autistic children.



Disclosures: **O. Surgent:** None. **M.I. Duran:** None. **J. Guerrero-Gonzalez:** None. **N. Adluru:** None. **G.R. Kirk:** None. **D.C. Dean:** None. **A.L. Alexander:** Other; ImgGyd, LLC, inseRT MRI, Inc. **J. Li:** None. **B. Travers:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.19/B13

Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative (SFARI)
Institut National de la Santé et de la Recherche Médicale (INSERM)
Fondation FondaMental

Title: Alterations in neuronal excitability and sensory information processing in the somatosensory cortex of Shank3-deletion mouse models of Autism

Authors: *Y. VYAS, A. ABREU, M. GINGER, A. FRICK;
INSERM U1215, BORDEAUX CEDEX, France

Abstract: Autism Spectrum Disorder (ASD) is a multifactorial neurodevelopmental disorder affecting an estimated 1 in 36 children. Genetic factors play an important role in the pathogenesis of ASD, and disruptions in the *SHANK* gene family are related to ASD. Altered sensory experience is one of the core features of the disorder, especially in the tactile domain. However, how tactile sensory information processing is altered in the somatosensory cortex of *Shank3*-knockout (KO) mice has not yet been well understood at the neuronal level. Therefore, we examined the intrinsic excitability properties, spontaneous activity, and stimulus-evoked responses of layer (L) 2/3 and L5 pyramidal neurons (PNs) of 4-6 week-old *Shank3-KO* mice and wildtype littermates using *in vivo* and *in vitro* whole-cell patch-clamp electrophysiology. In *Shank3b*$^{-/-}$ mice, preliminary results highlight a neuronal hypo-responsiveness with a reduced amplitude, shorter duration, and delayed onset latency of tactile stimuli-evoked responses in L2/3 PNs, as well as a higher failure rate to respond to tactile sensory stimuli. In addition, these neurons were hypo-excitable, requiring a greater current input to fire an action potential in comparison to wildtype neurons. In contrast, L5 PNs of *Shank3*$^{ex4\text{-}22\text{-}/-}$ mice demonstrated heightened somatic excitability. Interestingly, we observed a decoupling of neuronal excitability and dendritic excitability in these neurons, as well as a greater intra-genotypic variability in *Shank3*$^{ex4\text{-}22\text{-}/-}$ mice in comparison to wildtype controls. Collectively, our data highlight that two different mouse models of *Shank3-KO* and ASD had significant alterations in their L2/3 and L5 somatosensory cortical pyramidal neurons, which could contribute to the somatosensory abnormalities observed in ASD.

Disclosures: **Y. Vyas:** None. **A. Abreu:** None. **M. Ginger:** None. **A. Frick:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.20/B14

Topic: A.07. Developmental Disorders

Support: Finanziamento Ricerca di Ateneo 2022 – tipologia A

Title: Impairment of synaptosomal protein synthesis in Autism Spectrum Disorders mice model: rescuing effect of serotonin receptor 7 stimulation

Authors: *N. ABATE[1], A. PIZZELLA[1], K. FILIZ[2], F. VOLPICELLI[2], E. LACIVITA[3], M. LEOPOLDO[3], C. PERRONE-CAPANO[2], R. DI GIAIMO[1], M. CRISPINO[1];
[1]Dept. of Biol., [2]Dept. of Pharm., Univ. of Naples "Federico II", Naples, Italy; [3]Dept. of Pharm., Univ. of Bari, Bari, Italy

Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental disease characterized by deficits in social communication and restricted, repetitive behaviors or interests. The etiology of ASD remains poorly understood, but emerging studies linked defects of brain serotonergic signaling with this disorder. Serotonin receptor 7 (5-HT7R) plays a crucial role in modulating synaptic plasticity, neuronal connectivity of brain circuitry and social behavior. The inbred mouse strain BTBR T+tf/J (BTBR), displaying numerous features of autism, is a widely used animal model of ASD. This work aimed to investigate the possible involvement of 5-HT7R in ASD. We focused our attention on synaptosomes isolated from the brain cortex of BTBR and WT mice, as an *in vitro* model of synaptic areas. In BTBR synaptosomes compared to WT, the expression levels of 5-HT7R were significantly reduced, suggesting that the serotonergic signaling is altered in the pathology. Interestingly, in synaptosomes of BTBR mice compared to WT, we detected an increased expression level of synaptophysin, a marker of synaptic vesicles, even though the expression levels of syntaxin, a SNARE protein involved in exocytosis, did not change. These data support the hypothesis that in the brains of BTBR mice some of the impaired mechanisms are related to the synaptic areas. In line with this hypothesis, we investigated the local protein system synthesis in synaptosomes isolated from the brain cortex of BTBR and WT mice. We demonstrated that the synaptic protein synthesis is reduced in BTBR mice compared to WT, indicating for the first time that the local translation system is one of the cellular machinery altered in animal models of ASD. Intriguingly, we demonstrated that the diminished synaptic protein synthesis in the brain cortex of BTBR mice is completely rescued by acute stimulation with LP-211, a selective agonist for 5-HT7R. Overall, our results indicate that the machinery of protein synthesis located in the synaptic territories is altered in the BTBR model of ASD, and that the stimulation of 5-HT7R is able to reverse this deficit. Thus, our data confirm the involvement of 5-HT7R signaling in autism spectrum disorders and open the way to further investigations focusing on the receptor as a possible target for a novel therapeutic approach.

Disclosures: N. Abate: None. **A. Pizzella:** None. **K. Filiz:** None. **F. Volpicelli:** None. **E. Lacivita:** None. **M. Leopoldo:** None. **C. Perrone-Capano:** None. **R. Di Giaimo:** None. **M. Crispino:** None.

Poster

PSTR183. Autism: Physiology and Systems

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Program #/Poster #: PSTR183.21/B15

Topic: A.07. Developmental Disorders

Support: JSPS Grant 19H03535
JSPS Grant 21H05679
JSPS Grant 23H04217
JST Grant JPMJMS2021

Title: Effects of brain-state-driven neural stimulation on autism

Authors: *T. WATANABE;
Univ. of Tokyo IRCN, Tokyo, Japan

Abstract: Our previous study found atypically rigid neural dynamics in high-functioning autistic adults and identified a correlation between such over-stable brain dynamics and autistic symptoms (Watanabe and Rees, 2017). However, whether the correlation is a manifestation of any brain-behaviour causal relationship remains unknown. Here, we examined this question using brain-state-driven neural stimulation (BDNS) system (Watanabe, 2021), which enables real-time tracking of whole-brain neural dynamics and administration of transcranial magnetic stimulation (TMS) only when the brain is dwelling in a specific brain state. First, we found that excitatory BDNS over a parietal region during a specific brain state temporally enhanced the activity of the fronto-parietal network and destabilised the rigid neural dynamics in high-functioning autistic adults. Behaviourally, such a change in the brain state dynamics resulted in the mitigation of autistic cognitive rigidity, which was assessed in a spontaneous task-switching test (Watanabe et al., 2019), and perceptual rigidity, which was evaluated in a test of bistable perception (Watanabe et al., 2014; Watanabe et al., 2019). Moreover, an autistic social symptom, which was assessed with a friend-or-foe test (Watanabe et al., 2014; 2015), was also alleviated after 6-time continual weekly BDNS. Furthermore, such neural and behavioural effects lasted even after the BDNS session. These findings demonstrate that the atypically rigid neural dynamics have a causal role in autistic core symptoms, and such brain-state-driven neural intervention would become a new treatment for high-functioning autism.

Disclosures: T. Watanabe: None.

Poster

PSTR183. Autism: Physiology and Systems

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.22/B16

Topic: A.07. Developmental Disorders

Support: R01MH126531
P2CHD058486

Title: Maternal SARS CoV-2 infection during pregnancy and autism risk scores at 16 to 30 months of age

Authors: *M. R. FIRESTEIN[1], J. WARMINGHAM[1], A. MANESSIS[3], L. SHUFFREY[1], A. LAVALLEE[1], M. KYLE[1], M. HUSSAIN[1], J. AUSTIN[4], R. MARSH[2], W. FIFER[4], C. MONK[4], D. DUMITRIU[1];
[2]Div. of Child and Adolescent Psychiatry, [1]Columbia Univ. Irving Med. Ctr., New York, NY; [3]Teachers College, Columbia Univ., New York, NY; [4]Columbia Univ. Med. Ctr., New York, NY

Abstract: Background: Maternal immune activation (MIA) is among the potential mechanisms through which prenatal exposure to SARS-CoV-2 has been hypothesized to impact offspring development. Reassuringly, vertical transmission of SARS-CoV-2 across the placenta remains rare and several reports have found no association between maternal SARS-CoV-2 infection and parent-reported and observer-based assessments of key domains of infant neurodevelopment between 6-12 months of age. MIA has been implicated in the etiology of autism and the children who were in-utero during the peak periods of the COVID-19 pandemic are reaching the age at which early indicators of risk for autism may emerge. Therefore, we conducted a follow-up analysis to assess the relationship between prenatal SARS-CoV-2 exposure and scores on the Modified Checklist for Autism in Toddlers (M-CHAT).**Methods:** M-CHAT scores, maternal COVID-19 status, and other medical and demographic data were abstracted from electronic medical records of 1,259 children born at Columbia University affiliated hospitals and who were between 16-30 months of age at the time of the M-CHAT assessment. Mothers' COVID-19 status and children's M-CHAT scores were subsequently manually reviewed. Maternal COVID-19 status was determined based on chart review of PCR testing, serology testing, and patient-reported COVID-19 exposures and symptoms. Of these, 258 children were born to mothers who had had a SARS-CoV-2 infection during or prior to the pregnancy and 1,001 infants were born to mothers with no known history of SARS-CoV-2. M-CHAT scores were categorized as low risk (0-2) or moderate/high risk (>2).**Results:** To assess the association of maternal COVID-19 status with M-CHAT scores, we performed a logistic regression adjusting for the child's age at assessment, sex, maternal race and ethnicity, mode of delivery, gestational age at delivery, and maternal age at delivery. We found no statistically significant association between maternal SARS-CoV-2 during or prior to the pregnancy and M-CHAT scores (β=-024, p=0.18).**Conclusions:** Consistent with previous reports in the literature and across multiple cohorts, we did not find an association between maternal COVID-19 and children's M-CHAT scores. Further analyses will be needed to determine the relationship between M-CHAT scores and the timing and severity of the infection. Continued longitudinal follow-up of the generation of children born during the COVID-19 pandemic is critical to understand the myriad ways that the pandemic may impact their developmental course.

Disclosures: **M.R. Firestein:** None. **J. Warmingham:** None. **A. Manessis:** None. **L. Shuffrey:** None. **A. Lavallee:** None. **M. Kyle:** None. **M. Hussain:** None. **J. Austin:** None. **R. Marsh:** None. **W. Fifer:** None. **C. Monk:** None. **D. Dumitriu:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.23/B17

Topic: A.07. Developmental Disorders

Support: CONACyT CVU 818194

Title: Network science applied to the study of cognitive control networks in autism spectrum disorder

Authors: *L. MOLINA-ARCIA[1], K. TRAD-MATEOS[2], Á. TOVAR[1], E. GARZA-VILLARREAL[3], O. GARCÍA[1];
[1]Facultad de Psicología, [2]Facultad de Ciencias, Univ. Nacional Autonoma De Mexico, Ciudad de Mexico, Mexico; [3]Inst. de Neurobiología, Univ. Nacional Autonoma De Mexico, Querétaro, Mexico

Abstract: Several traits in Autism Spectrum Disorder (ASD) have been associated with cognitive control functions, for example, low sensorial arousal, difficulties in the theory of mind, repetitive movements, and restrictive interests. This study aimed to describe and compare the functional brain connectivity of three brain networks that support cognitive control: the default mode network, the salience network, and the frontoparietal network, in people with ASD and typically developing people. We used MRI and fMRI data from four datasets included on the Autism Brain Imaging Data Exchange (ABIDE) repository. We focus on data from people between the ages of 18 to 25 years old. To make comparable data from different datasets, we employed the COMBAT tool after the preprocessing of the images. We analyzed features of cognitive control networks employing centrality measures from network theory: degree, betweenness centrality, and closeness centrality. The preliminary results showed that there are differences in the centrality measures of the salience network, where the ASD group shows a lower number of connections ($Z = -2.23861$; $p < 0.05$), degree ($Z = -2.23861$; $p < 0.05$), betweenness centrality ($Z = -2.02541$; $p < 0.05$), and lower closeness centrality ($Z = -2.45181$; $p < 0.05$) as compared to the typically developing group. We did not find differences between groups in the number of connections and centrality measures neither for the default mode, nor for the frontoparietal network. These results suggest that differences in the organization of the salience network, which responds to salient stimuli in the environment and prepares the system to solve a task, could explain differences in cognitive control in ASD.

Disclosures: **L. Molina-Arcia:** None. **K. Trad-Mateos:** None. **Á. Tovar:** None. **E. Garza-Villarreal:** None. **O. García:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.24/B18

Topic: A.07. Developmental Disorders

Support: Grant # CAUT20APL001 from the NJDOH Governor's Council for Medical Research and Treatment of Autism

Title: Four-month-old infants with a family history of autism already show differences in spectral power and phase synchrony during rapid auditory processing.

Authors: *S. ORTIZ-MANTILLA, T. REALPE-BONILLA, C. P. ROESLER, A. A. BENASICH;
Rutgers Univ. Newark, Newark, NJ

Abstract: During the first months of life, typically developing infants can discriminate fast, successive changes in auditory sounds, occurring in the tens of milliseconds range. This rapid auditory processing (RAP) skill is essential to decoding ongoing speech and to the establishment of phonetic maps. Compromised language abilities are ubiquitous in individuals with autism, a highly heritable developmental condition. Language delays are already present at 12 months of age in infant siblings of children with autism, suggesting that prelinguistic processing abilities are compromised. However, the neural mechanisms sub-serving language acquisition in autism are poorly understood. This EEG-based study investigated oscillatory mechanisms underlying RAP in infants with a family history of autism (FHA) as compared to age/sex matched controls (CT). At 4-months-of-age, infants were presented with an auditory oddball paradigm while dense-array EEG was recorded. Infants passively listened to tone pairs separated by a short inter-stimulus interval of 70 ms. Source localization of event-related generators identified sources of activity in left (LAC) and right (RAC) auditory cortices. Time-frequency analysis of event-related oscillations was conducted in source space within the 2 to 90 Hz frequency range. Statistical analyses were conducted using permutation testing and cluster identification. Preliminary results from a small sample of 14 infants (5 males and 2 females in each group) show significant differences in spectral power and phase synchrony between the groups. Specifically, the FHA group exhibited less spectral power for the standard representation in low- and high-gamma in LAC, and mid-gamma in RAC compared to the CT group. During deviant discrimination, the FHA group showed less power than CTs in theta in the LAC, low-gamma in the RAC, and high-gamma in both LAC and RAC sources. Compared to the CTs, the FHA group also displayed less phase synchrony in the theta range for the standard in RAC and for the deviant in theta and beta ranges in both LAC and RAC sources. Further, during standard representation, the FHA group showed more spectral power in LAC and more phase coherence in RAC in the beta range than CTs. Our preliminary results indicate that RAP is already altered at 4 months in FHA infants and suggest that some of the linguistic differences observed in autism may arise from variations in the magnitude and lateralization of oscillatory patterns sub-serving

pre-linguistic processing abilities. Detecting the earliest signs of deviation may be critical for implementing targeted interventions oriented to improving atypical developmental trajectories.

Disclosures: S. Ortiz-Mantilla: None. **T. Realpe-Bonilla:** None. **C.P. Roesler:** None. **A.A. Benasich:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.25/B19

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 MH126531

Title: No Difference in Autism Screening Scores for Infants Born Before or During the COVID-19 Pandemic

Authors: *A. GIGLIOTTI MANESSIS[1,2,3], D. DUMITRIU[2], M. H. KYLE[2], A. LAVALLEE[2], J. WARMINGHAM[2], M. HUSSAIN[2], M. R. FIRESTEIN[3], L. C. SHUFFREY[3];
[1]Hlth. and Behavior Studies, Teachers College, Columbia Univ., New York, NY; [2]Pediatrics, Columbia Univ. Vagelos Col. of Physicians and Surgeons and NewYork-Presbyterian, New York, NY; [3]Psychiatry, Columbia Univ. Vagelos Col. of Physicians and Surgeons, New York, NY

Abstract: Background: Disturbances in the prenatal environment on the developing fetus are linked to changes in brain development and increased vulnerability to long-term neurological and psychiatric issues in offspring, including risk for Autism. Research on the neurodevelopmental outcomes of infants born during the COVID-19 pandemic has revealed differences in developmental and neurological sequelae compared to those born pre-pandemic, but there is limited work on how birth during the pandemic is related to Autism risk. *The Modified Checklist for Autism in Toddlers* (M-CHAT) is a widely used and validated screening tool with high sensitivity and specificity to identify children who may be at risk for Autism. The M-CHAT consists of 20 questions (yes/no) about the child's behaviors and social interactions. M-CHAT scores range from 0-20, with scores above 3 indicating elevated risk. The M-CHAT is a widely adopted screening tool in primary pediatric care settings, allowing for comparisons to be made between children born before and during the pandemic.
Aims: The current study compared Autism risk assessment scores between children born before and during the COVID-19 pandemic using M-CHAT scores documented in electronic medical records. **Methods**: Data were drawn from the COVID-19 Mother Baby Outcomes (COMBO) Initiative and includes medical record data from babies born at a large university-based medical center in New York City between January 2018 and September 2021. The TRAC system was used to extract M-CHAT scores and sociodemographic data from the electronic medical record. M-CHATs completed when children were 16-30 months old were included in analyses. Low (0-

2) and moderate/high risk (3+) ranges were then computed based on continuous scores. **Results**: There were n = 431 children born before March 2020 and n = 1194 born during the pandemic included in analyses. We found no difference in M-CHAT ranges for children born before March 2020 compared to those born during the pandemic (X^2 = 0.45, df = 1, p = 0.50). Specifically, among children born before the pandemic, 22.04% (n = 95) scored in the moderate/high range, and 23.79% (n = 284) of children born during the pandemic scored in the moderate/high range. In a model adjusted for demographic variables (maternal age, child age at assessment, child sex, race, ethnicity, and gestational age), we found no association between birth during the COVID-19 pandemic and M-CHAT ranges (B = -.24, p = 0.20.) **Summary:** Children born before and during the pandemic did not differ in Autism risk, as measured by the M-CHAT. We found no evidence of an association between birth during the pandemic on elevated risk of Autism.

Disclosures: A. Gigliotti Manessis: None. **D. Dumitriu:** None. **M.H. Kyle:** None. **A. Lavallee:** None. **J. Warmingham:** None. **M. Hussain:** None. **M.R. Firestein:** None. **L.C. Shuffrey:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.26/B20

Topic: A.07. Developmental Disorders

Support: NIMH 1P0MH094271
JSPS

Title: Reversible cognitive rigidity and energy landscapes in Shank3-deficient mice

Authors: M. ABUELEM[1,2], C. ORAM[2], P. N. AWAD[2], M. FAGIOLINI[2], T. WATANABE[3], ***T. K. HENSCH**[1,2,3];
[1]Harvard Univ., Cambridge, MA; [2]Boston Children's Hosp., Boston, MA; [3]Univ. of Tokyo IRCN, Tokyo, Japan

Abstract: Rigid (inflexible) and repetitive thinking patterns are hallmark symptoms of autism. Their early developmental onset points to a critical period when heightened neural plasticity could be targeted to rescue such cognitive deficits. We explored a role for the prominent autism candidate gene, *Shank3*, in a knock-in mouse model that can activate *Shank3* gene expression during a juvenile critical period (Mei et al, *Nature* 2016). Rule learning on an odor foraging task and subsequent reversal discrimination were assessed for flexible cognitive behavior with automated tracking software, followed by biochemical analysis of cortical and striatal synapse-enriched (synaptosomal) brain fractions to detect levels of associated scaffolding proteins. We found that heterozygous *Shank3*-deficient male mice displayed selective cognitive flexibility impairments that could be rescued by juvenile (P20-40) gene re-expression. Strikingly, this also restored CDKL5 protein levels at synapses, whereas *Shank3* haploinsufficiency retained normal

Homer1b/c protein levels. Whole-brain functional ultrasound (fUS) imaging of awake, resting-state neural activity patterns in both *Shank3*- and *CDKL5*-deficient mice showed limited transitions through unstable brain states (i.e., shallow minor attractors in the energy landscape), consistent with impaired rule learning observed in *CDKL5* mutants as well (Awad et al, *Mol Psych* 2023). Notably, juvenile *Shank3* re-expression rescued indirect transition frequency in the energy landscape, which is closely correlated with autistic core symptoms in humans (Watanabe & Rees, *Nat Comms* 2017). Taken together, these results highlight a role for *Shank3* in cognitive flexibility behavior, present a novel synaptic SHANK3-CDKL5 protein relationship, and elucidate neural activity dynamics reflecting *Shank3* expression levels that may underlie autistic-like cognitive rigidity.

Disclosures: **M. Abuelem:** None. **C. Oram:** None. **P.N. Awad:** None. **M. Fagiolini:** None. **T. Watanabe:** None. **T.K. Hensch:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.27/B21

Topic: A.07. Developmental Disorders

Support: R01NS121120
1R01NS121120

Title: Diffusion magnetic resonance imaging in autistic adults: Linking free-water to clinical severity and cognitive function

Authors: *Y. SHIN[1], S. A. COOMBES[1,3], A.-M. ORLANDO[2], R. A. ROMERO[2], D. SHIRLEY[1], D. E. VAILLANCOURT[1], Z. WANG[1];
[1]Applied Physiol. and Kinesiology, [2]Dept. of Psychiatry, Univ. of Florida, Gainesville, FL; [3]Dept. of Biomed. Engin., Univ. of Florid, Gainesville, FL

Abstract: Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder characterized by communication impairments and repetitive behaviors. Neuroimaging studies of ASD have reported altered cortical anatomy and white matter microstructure. Free-water (FW), a diffusion measure estimating isotropic diffusion in extracellular space, has been implemented to investigate white matter alternations in neurodegenerative diseases such as Alzheimer's and Parkinson’s disease. However, few studies have utilized FW measurements in ASD research. In this study, we assessed FW to examine the white matter microstructure of middle-aged adults with ASD and to determine whether FW relates to well established clinical and cognitive measures. FW was evaluated in 44 ASD and 29 healthy individuals controlling for age, sex, and IQ. Subjects also completed the Autism Diagnostic Observation (ADOS), Repetitive Behavior Scale-Revised (RBS-R), Wisconsin Card Sorting Test (WCST) and reverse learning. We first confirmed that FW positively correlated with age across the whole sample as reported in the

other FW studies. We offer three novel contributions to the literature. First, compared to neurotypicals, autistic adults had higher extracellular FW in brain regions including the basal forebrain, fornix, and sensorimotor cortex. Second, in the regions where the group differences were found, FW was significantly correlated with clinical measures in the ASD group. Specifically, FW correlated with behaviors associated with ASD such as poor communication (ADOS) and repetitive behaviors (RBS-R). Third, we examined the relationship between FW and cognitive metrics and found that FW positively correlated with errors and incorrected responses (WCST and reverse learning) in the ASD group. Overall, these results showed that FW is associated with autism severity and cognitive performance. The current study advances our understanding of brain microstructure in ASD and provides insight into the relationship between white matter microstructure, cognitive impairment, and repetitive behaviors in ASD.

Disclosures: Y. Shin: None. **S.A. Coombes:** None. **A. Orlando:** None. **R.A. Romero:** None. **D. Shirley:** None. **D.E. Vaillancourt:** None. **Z. Wang:** None.

Poster

PSTR183. Autism: Physiology and Systems

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.28/B22

Topic: A.07. Developmental Disorders

Support: Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and has received CONAHCyT fellowship 1046691

Title: Contribution of Intestinal Dysbiosis in the behavioral expression of Autism Spectrum Disorder.

Authors: *A. PANTALEON[1], R. FOSSION[2], G. R. ROLDAN[1], J. ESPINAL[3], E. IBARRA[1]; [1]Fisiología, Univ. Nacional Autonoma de Mexico, Ciudad de México, Mexico; [2]Inst. de Ciencias Nucleares, Univ. Nacional Autónoma de México, Ciudad de México, Mexico; [3]División de Genómica Computacional, Inst. Nacional de Medicina Genómica, Mexico, Mexico

Abstract: Autism Spectrum Disorder (ASD) is a group of severe, pervasive, and heterogeneous neuropsychological disorders. It is one of the most frequent disorders in the pediatric population that affects mainly social cognition development, communication processes, and behavior. The ASD etiology is multifactorial and poorly understood; it is a condition that, although considered a neurodevelopmental disorder, affects various physiological, biochemical, and molecular systems. Individuals with this condition frequently present immunological and gastrointestinal comorbidities, and elevated oxidative stress levels among others. Biomarkers like urinary organic acids are altered, suggesting the presence of intestinal microbiota disorders, nutritional deficiencies, neurotoxicity processes, or metabolic alterations. These acids interact directly with elements of different physiological systems and influence the central nervous system

development, exerting effects on the brain through the neuroendocrine, neuroimmune, autonomic nervous system, and intestine-brain axis.Few studies have examined the presence of these comorbidities as part of the pathophysiology of this condition. This research aimed to study the effect of dysbiosis on different physiological functions and its contribution to the severity of behavioral expression as well as its improvement by modifying aspects of the microbiota through dietary restriction and supplementation. Through correlation analysis and descriptive statistics of a clinical database of a ASD population from the Domus Institute of Autism (Mexico City), we have identified biomarkers that indicate alterations of different physiological systems, as well as the relationship between the degree of severity of different behavioral traits with the presence of intestinal dysbiosis and nutritional deficiencies, finding that dietary changes, both dietary restriction and supplementation impact on behavioral expression, and appear to modulate physiological communication. In conclusion, directly or indirectly, intestinal dysbiosis combined with the presence of nutritional deficiencies may contribute to the degree of severity of behavioral symptoms in ASD.

Disclosures: **A. Pantaleon:** None. **R. Fossion:** None. **G.R. Roldan:** None. **J. Espinal:** None. **E. Ibarra:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.01/B23

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant 122842

Title: Co-release of GABA and glycine in the rostral brainstem

Authors: H. GAO, L. D. DESMOULINS, A. J. R. MOLINAS, A. ZSOMBOK, ***A. DERBENEV**;
Tulane Univ., New Orleans, LA

Abstract: Pre-sympathetic neurons in the ventrolateral and ventromedial medulla (VLM/VMM) control the sympathetic tone, and GABA was identified as a primary mechanism for sympathoinhibition. Evidence suggests that both GABA and glycine inhibit sympathetic tone; however, the mechanism of glycine release is unknown. In this study, we tested the hypothesis that GABA and glycine are co-released in the VLM/VMM. Glycine is recycled by glycine transporter 2 ($GlyT_2$), therefore it is a reliable marker for glycinergic neurons. We crossed heterozygous $GlyT_2$ Cre ($GlyT_2^{Cre}$) mice with floxed channelrhodopsin 2 (ChR2-EYFP) mice to generate $GlyT_2^{ChR2/EYFP}$ mice. These mice were used to determine the mechanism of glycine release and reveal the location of glycinergic neurons. Our findings show that $GlyT_2$-expressing neurons were distributed within the ventral gigantocellular nucleus and medial reticular formation, and glycinergic fibers were abundant throughout the rostral brainstem. Whole-cell

patch-clamp recordings from pre-sympathetic VLM/VMM neurons in $GlyT_2^{ChR2/EYFP}$ mice revealed that GABA mediates most spontaneous inhibitory postsynaptic currents; whereas increased activity of inhibitory inputs enhanced glycine release. Light stimulation of $GlyT_2^{ChR2/EYFP}$ fibers triggered evoked IPSCs (eIPSCs) in pre-sympathetic VLM/VMM neurons, whereas blockade of $GABA_A$ receptors decreased the amplitude of eIPSCs. These data suggest that eIPSCs are generated by glycine and GABA. Moreover, inhibition of $GlyT_2$ decreased the amplitude of the eIPSCs. Immunofluorescence staining was used to determine the expression of glycine receptor subunits in the VLM/VMM. Our data revealed the existence of glycine receptors formed by α_1 and α_3 subunits. In summary, we found that GABA and glycine are co-released in the VLM/VMM and they are likely located in separate vesicles.

Disclosures: H. Gao: None. **L.D. Desmoulins:** None. **A.J.R. Molinas:** None. **A. Zsombok:** None. **A. Derbenev:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.02/B24

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Intramural Research Program (IRP) at the National Institute of Mental Health (NIMH) at the National Institutes of Health

Title: Regulated Trafficking of GABA Transporters, GAT1 and GAT3, in Response to Methamphetamine

Authors: *H. GROTE[1], S. G. AMARA[2], S. M. UNDERHILL[3];
[1]NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD; [2]NIMH, NIMH, Bethesda, MD; [3]Natl. Inst. of Mental Hlth., Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The dysregulation of GABA has been implicated in a variety of conditions, from epilepsy to schizophrenia. The main GABA transporters in the CNS, GAT1 and GAT3, are responsible for the maintenance of inhibition throughout the brain by clearing GABA released during neurotransmission. Previous work from this laboratory has demonstrated that psychostimulants such as methamphetamine induce the internalization of the catecholamine transporters DAT and NET. This process involves methamphetamine stimulation of the G-protein coupled receptor TAAR1 and activation of the small GTPase RhoA that facilitates internalization of the transporters. To examine whether GABA transporters are also internalized through a TAAR1/RhoA-dependent mechanism, we measured the uptake of radiolabeled GABA into mouse primary striatal mixed cultures. In these cultures, which express both GAT1 and GAT3, psychostimulant co-application had no measurable effect on GABA uptake, consistent with their reported lack of affinity for GABA transporters. However, pre-treatment with methamphetamine led to a significant decrease in GABA uptake. Inhibitors of RhoA, RhoA

kinase (ROCK), and G-13-mediated GPCR signaling all prevented the methamphetamine-induced loss of GABA uptake, suggesting that RhoA-mediated internalization of GABA transporters is responsible for the reduction in GABA uptake. Recombinant expression systems for GFP-hGAT1 and GFP-hGAT3 were used to characterize GAT1 and GAT3 activity in HEK293 cells. Parallel radiolabeled uptake time course experiments revealed that GAT1 and GAT3 reach saturation after approximately 2 hours. Further work using biotinylation of surface proteins is being undertaken to confirm whether one or both carriers can be internalized through a RhoA dependent mechanism. Our findings show that methamphetamine can decrease GAT activity through TAAR1-mediated activation of RhoA signaling. Thus, the TAAR1 pathway may be an important therapeutic target for a wide variety of conditions and neurodegenerative diseases. GAT inhibitors are currently used as anticonvulsants and off-label anxiolytic treatments, despite subsequent adverse side effects. These findings suggest alternative methods for modulating GABA transport via TAAR1 which could prove to be vital for reevaluating current treatments.

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

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.03/B25

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NINDS 1SC1NS119056
NIH-NIMHD-5U54MD007592
NIH #T34GM145529

Title: Glycine Transporter 1 expression in the globus pallidus

Authors: *L. P. MONTES[1], R. A. PEREZ[2], M. MIRANDA-ARANGO[2];
[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: Glycine is one of the main inhibitory neurotransmitters. Its levels at the synaptic cleft are regulated by the glycine transporter 1 and 2 (GlyT1 and 2). GlyT2 has been identified as a neuronal marker for glycinergic neurons, whereas GlyT1 is believed to be present in glial cells only and recognized as neuronal in the amacrine layer of the retina. GlyT2 is abundant in caudal regions of the brain, and GlyT1 is widespread in the rodent brain. Despite studies focusing on GlyT1 expression in the rat, a detailed description of its expression in the globus pallidus is not yet available in the mouse. The objective of this study is to identify cells expressing GlyT1 in the mouse globus pallidus. A GlyT1 knock-in mouse line expressing tdTomato was used to identify GlyT1-positive cells in the globus pallidus internal and external segments (GPi and GPe, respectively). Immunohistochemical experiments were performed in coronal sections and stained

with a Glial fibrillary acid protein (GFAP) antibody (Ab) or neuronal nuclear protein (NeuN) Ab to identify GlyT1-positive astrocytes or neurons, respectively. GlyT1 labeling was also included in both experiments to confirm its expression in the plasma membrane. Adjacent Nissl-stained sections were used to identify the GPe and GPi and delineated their boundaries in reference to the *Allen Reference Atlas*. GlyT1 expression patterns and cell identification were performed in the rostrocaudal axis of the mouse GPe and GPi. Preliminary data from experiments using GFAP to identify GlyT1-positive glial cells showed few cells that potentially express GlyT1. GFAP-negative cells expressing tdTomato were also labeled by GlyT1 Ab. Additionally, cells positive to NeuN had GlyT1 Ab at the plasma membrane and were surrounded by fibers positive to tdTomato. These patterns were present in both regions in the rostrocaudal axis. These data suggest that neurons in the GPe and GPi are positive for GlyT1, which means they are potentially glycinergic neurons. Further analysis of the expression patterns is required to understand better whether GlyT1 can be used as an additional glycinergic marker in different regions.

Disclosures: L.P. Montes: None. **R.A. Perez:** None. **M. Miranda-Arango:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.04/B26

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: R01-MH126017

Title: Gephyrin promotes the self-organization and synaptic localization of GABAergic postsynaptic components without presynaptic GABA release

Authors: *S. CHANDA;
Colorado State Univ., Fort Collins, CO

Abstract: Synapses that use γ-Aminobutyric acid (GABA) as a neurotransmitter play a critical role in modulating the cellular excitability of neurons. Despite a general understanding of how GABAergic synapses function, it remains unclear how they form and organize their pre- and postsynaptic compositions. We examined whether presynaptic release of GABA is required for the development and alignment of GABAergic postsynapses. We found that postsynaptic $GABA_A$ receptors ($GABA_ARs$), scaffolding proteins, and major cell-adhesion molecules can reliably co-aggregate and localize apposed to even non-functional presynaptic terminals that virtually lack any vesicular GABA release. Genetic deletions of both Gephyrin and a Gephyrin-associated GDP/GTP-exchange factor Collybistin (hPEM-2) severely disrupted the assembly of these postsynaptic structures and their alignment with synaptic inputs. In the absence of functional GABAergic synapse, activities at adjacent glutamatergic synapses contributed to both the development and maintenance of Gephyrin-$GABA_AR$ clusters, which could be subsequently

activated by delayed supply of vesicular GABA. These results suggest that the molecular organization of GABAergic postsynapse can initiate via a GABA-independent but Gephyrin-dependent intrinsic mechanism.

Disclosures: S. Chanda: None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.05/B27

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Activity-dependent glycine release in the rodent hippocampal region

Authors: *J. A. FERIA PLIEGO[1], P. UNICHENKO[1], V. VONGSOUTHI[2], C. JACKSON[2], C. HENNEBERGER[1];
[1]Inst. of Cell. Neurosciences, Friedrich Wilhelms Univ. Bonn, Bonn, Germany; [2]Res. Sch. of Chem., The Australian Natl. Univ., Canberra, Australia

Abstract: In the hippocampus, plasticity inducing stimuli have been shown to increase extracellular glycine levels. For instance, high frequency stimulation (HFS) of the CA3-CA1 Schaffer collaterals causes a transient increase in extracellular glycine in the CA1 *stratum radiatum*. However, the underlying molecular mechanisms of this activity-dependent glycine release remain elusive. We investigate these using the FRET-based glycine sensor GlyFS to monitor extracellular glycine levels in acute hippocampal slices by two-photon excitation fluorescence microscopy First, we explored the dependence of HFS-induced glycine release on ionotropic glutamatergic signaling. We found that the glutamate receptor inhibitor NBQX and APV prevent HFS-induced extracellular glycine increase. Under these same conditions, local injection of K^+ 10 mM was also sufficient to raise extracellular glycine. These experiments suggest that activity of AMPARs and NMDARs as well as local depolarization are sufficient to elicit increases of extracellular glycine. We next asked what the role of glycine transporters is. Glycine transporters expressed in the hippocampus (GlyT1, GlyT2 and Slc6a20a) are Na^+ symporters which regulate extracellular glycine levels. Especially GlyT1 can reverse its transport direction in response to intracellular sodium increases and depolarization. We hypothesized that activity-dependent glutamate uptake by glutamate transporters, which leads to depolarization and intracellular Na+ increase, could also support GlyT reversal and glycine release. Therefore, we locally injected D-Asp in the tissue because extracellular D-Asp can be transported by glutamate transporters and thereby increase intracellular Na^+. Indeed, we found that D-Asp evokes an increase in extracellular glycine. Next, we employed pharmacology to reveal the possible role of GlyT1. Inhibition of GlyT1 by n-ethyl glycine, a competitive GlyT1 inhibitor, lead to an increase in basal glycine levels, but no further increase was found during HFS. Furthermore, NFPS, a non-completive GlyT1 inhibitor, also increased basal extracellular glycine levels with no further glycine increases detected during HFS. Therefore, GlyT1 lowers the extracellular glycine

concentration in the absence of activity but reverses to release glycine when neuronal activity is increased, which can be reproduced by glutamate transporter activation. It appears likely that cellular depolarizations and glutamate uptake together drive activity-dependent glycine release.

Disclosures: J.A. Feria Pliego: None. **P. Unichenko:** None. **V. Vongsouthi:** None. **C. Jackson:** None. **C. Henneberger:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.06/B28

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NINDS 1SC1NS119056
NIH-NIMHD-5U54MD007592
NIH #T34GM145529

Title: Glycine Transporter 1 Expression in the Mouse Brainstem

Authors: *I. A. GONZALEZ[1], L. P. MONTES[2], R. A. PEREZ[3], M. MIRANDA-ARANGO[3]; [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; [3]Biol. Sci., The Univ. of Texas at El Paso, EL PASO, TX

Abstract: Glycine is the primary inhibitory neurotransmitter of the brain's spinal cord and caudal regions. Glycinergic inhibition is mediated by two transmembrane proteins, glycine transporter 1 (GlyT1) and glycine transporter 2 (GlyT2). GlyT1 and GlyT2 are found in glial cells and neurons, respectively. Yet, preliminary data from our laboratory has demonstrated that GlyT1 is also present in neurons. The aim of this project is to locate the expression GlyT1 in the brainstem, a brain region essential for survival, in mice. A GlyT1-Cre knock-in line mouse was crossed with an Ai75 reporter line to create an animal that expresses a tdTomato nuclear localization signal (NLS) in cells containing GlyT1. Additionally, immunohistochemical assays were done using different antibody (Ab) markers, neuronal nuclei (NeuN), and GlyT1 Ab to identify neuronal nuclei and GlyT1 presence in cells, respectively. Adjacent tissue sections were Nissl-stained and used to delineate boundaries of brain regions based on cytoarchitecture in reference to the Paxinos and Franklin's mouse brain atlas 4th edition. Preliminary data showed abundant colocalization of tdTomato and NeuN in regions such as the Ventrolateral Principal Sensory 5 (Pr5VL), Oral Spinal Trigeminal Nucleus (Sp5O), and the Alpha Parvicellular Reticular Nucleus (PCRtA) regions. In contrast, the Granule Cell Layer of the Cochlear Nuclei (GrC), Gigantocellular Reticular Nucleus (Gi), and Intermediate Reticular Nucleus (IRt) expressed less colocalization. These results suggest that GlyT1 can be used as a neuronal marker in these areas. Therefore, further characterization of these cells is required.

Disclosures: I.A. Gonzalez: None. **L.P. Montes:** None. **R.A. Perez:** None. **M. Miranda-Arango:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.07/B29

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Emil Aaltonen Foundation
Sigrid Jusélius Foundation

Title: Acute neuroinflammation leads to disruption of neuronal chloride regulation by KCC2 and NKCC1 and to consequent hyperexcitability in the dentate gyrus

Authors: S. N. KURKI[1], R. SRINIVASAN[2], J. LAINE[2], T. ALA-KURIKKA[2], J. T. VOIPIO[3], ***K. KAILA**[2];
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Abstract: Neuroinflammation is salient part of diverse acute pathologies which associate with both neuronal hyperexcitability and compromised cognitive functions, but the underlying molecular and cellular mechanisms remain to be identified. Here, we show that peripheral injection of lipopolysaccharide (LPS) decreased the threshold of kainate-provoked seizures beyond the duration of acute sickness behavior (72 hours after LPS) indicating post-acute inflammatory hyperexcitability. This was verified in recordings of local field potentials in dentate gyrus (DG) to perforant pathway stimulation *in vivo*. In experiments on hippocampal slices, we observed an increase in the intrinsic spiking propensity of dentate granule cells (DGCs). In parallel with this, there was a prominent downregulation of chloride extrusion via KCC2 and emergence of NKCC1-mediated chloride uptake in DGCs under experimental conditions optimized to detect specific changes in transporter efficacy. These data point to acute LPS-induced disruption of neuronal chloride regulation, which unequivocally leads to a loss of GABAergic inhibition in the DGCs collapsing the gating function of DG. The present work provides a mechanistic explanation for neuroinflammation-driven hyperexcitability and consequent cognitive disturbance.

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Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.08/B30

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: EU860954

Title: Functional characterization of human GAT-1 using solid supported membrane electrophysiology and automated patch clamp

Authors: R. ZERLOTTI[1,2], A. BAZZONE[1], M. BARTHMES[1], G. OKEYO[3], I. LU[3], N. BECKER[1], **A. OBERGRUSSBERGER**[1], *E. DRAGICEVIC[1], N. FERTIG[1];
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Abstract: Solid supported membrane-based electrophysiology (SSME) is a technique that allows the measurement of transport and other electrogenic events in transporters, pumps, and channels. The sample (i.e., membrane vesicles or proteoliposomes) is adsorbed on an artificial membrane generated on top of a gold-coated sensor and a capacitive-coupled membrane system is generated. A fluidic system allows for a fast solution exchange and transport is triggered by substrate concentration gradients as driving force, while membrane voltage is zero. GAT-1 is a secondary-active transport protein that exploits the Na^+ gradient to energize the uphill re-uptake of the neurotransmitter GABA from the synaptic cleft. Through SSME we detected GABA-induced currents on membrane vesicles overexpressing hGAT-1, with a maximum amplitude of 3-5 nA, that showed a triphasic behavior leading us to identify 3 different electrogenic events. We studied these currents in the presence of high and low Na^+ gradients, noticing that the middle component disappeared when the Na^+ gradient is close to zero. The transport component has been identified, showing a K_M of 15-20 µM and we could also assess the Na^+: GABA stoichiometry, which appears to be 2:1. We also used automated patch clamp to characterize GAT-1 in living cells. Holding the potential at -70 mV we were able to detect inward currents triggered by GABA application. The measured Imax was around -150 pA and the KM was 9 µM, in very good agreement with literature data.

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

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.09/B31

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: IBS Grant IBS-R001-D2

Title: Age-dependent tonic inhibition differentially orchestrates information processing in the cerebellar granule cells

Authors: J. KWON[1], ***S. KIM**[1], J. WOO[2], E. D. SCHUTTER[3], S. HONG[3], C. LEE[1]; [1]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Daejeon, Korea, Republic of; [2]Ctr. for Cell & Gene Therapy, Baylor Col. of Med., Houston, TX; [3]Computat. Neurosci. Unit, Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: Cerebellar granule cells, the most numerous neuronal cell type in the brain, have the tonic GABA-dependent inhibitory conductance, which is as large as the intrinsic membrane conductance. This tonic inhibition has neural activity-dependent and independent components with distinct origins. Still, little is known about the origin of each component and the mechanism of how each component controls the network-level information processing. Here, we show that the main source of tonic inhibition shifts from the activity-dependent synaptic GABA spillover to activity-independent glial sources during the developmental maturation stages of young to adults. This developmental switch can significantly change how the granular layer neural network encodes external inputs. We performed the step-by-step elimination of the GABA spillover, GABA transporters (GATs), Bestrophin1 channel (Best1), and their combinations in both groups. Although overall tonic inhibitory conductance was similar, TTX-sensitive, the neuronal activity-dependent component was significantly different, accounting for 63.5% in young but only 22.6% in adult animals. Furthermore, we found that a combination of the spillover and Best1-mediated tonic GABA release from glia can fully account for the entire tonic inhibition in young animals. In contrast, a small fraction of tonic inhibition in adult animals originated from previously unknown sources that are distinct from GATs, Best1, or the spillover. Based on these experimental findings, we simulated a large-scale computational model of the granular layer network in young animal-like and adult-like conditions. We found a more robust oscillatory network activity in the young animal-like condition, suggesting that the granular layer of young animals is better at transmitting time-sensitive information. In contrast, the network of adult animals more accurately encodes smoothly changing inputs. In summary, our study provides novel insights into how activity-dependent and independent tonic inhibition distinctively tunes network-level oscillation which is critical for the timing of movement.

Disclosures: J. kwon: None. **S. Kim:** None. **J. Woo:** None. **E.D. Schutter:** None. **S. Hong:** None. **C. Lee:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.10/B32

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant 1R01NS127819-01
NIH Diversity Supplement Grant 1R01NS127819-01

Title: Astrocytic Control of GABA Signaling via Kir4.1 in the Healthy Brain

Authors: *R. L. GARIEPY[1], C. DULLA[2], M. ARMBRUSTER[2];
[1]Tufts Univ. Grad. Program In Neurosci., Boston, MA; [2]Tufts Univ. Sch. of Med., Boston, MA

Abstract: Astrocytes are glial cells that closely interact with neuronal synapses and modulate neurotransmission. Astrocytic control of the excitatory neurotransmitter glutamate via excitatory amino acid transporters is well established; however, much less is known about how astrocytes interact with inhibitory synapses and control GABAergic signaling via GABA, the primary inhibitory neurotransmitter in the central nervous system. GABA inhibition is critical to regulate neuronal activity and when compromised leads to numerous neurological disorders such as epilepsy. GABA is cleared from the extracellular space by GABA transporters (GATs) that are localized on astrocytes (GAT3) and neurons (GAT1). Kir4.1, an astrocytic inward rectifying potassium channel that removes K^+ from the extracellular space, has been shown to modulate glutamate clearance kinetics. Unlike glutamate transporters, GATs, are driven by the Na^+/Cl^- gradients and are not directly dependent upon the K^+ gradient. Surprisingly, our data suggests that bi-directionally modulating astrocytic K^+ buffering via Kir4.1 modulates GABA clearance as assayed using the genetically encoded GABA sensor, iGABASnFr, to quantify GABA clearance in the extracellular space. Specifically, the pharmacological blockade of Kir4.1 using Ba^{2+} leads to the slowing of GABA clearance and viral overexpression of Kir4.1 speeds up GABA clearance. Using bumetanide to pharmacologically inhibit the Na+/K+/Cl- co-transporter NKCC1 phenocopies the overexpression of Kir4.1, speeding up GABA clearance. We hypothesize that Kir4.1 helps set the basal extracellular K^+ concentration, which in turn modulatesNKCC1 activity on axons thereby increasing axonal Cl^- and inhibiting GAT1. These findings suggest previously unexplored astrocyte-interneuron interactions and will allow us to promote GABAergic inhibition to treat neurological disorders.

Disclosures: R.L. Gariepy: A. Employment/Salary (full or part-time):; Tufts University School of Medicine. **C. Dulla:** A. Employment/Salary (full or part-time):; Tufts University School of Medicine. **M. Armbruster:** A. Employment/Salary (full or part-time):; Tufts University School of Medicine.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.11/B33

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NINDS 1SC1NS119056
NIH-NIMHD-5U54MD007592
NIH #T34GM145529

Title: Analysis of glycinergic reactivity in the substantia nigra

Authors: *R. A. PEREZ, L. P. MONTES, M. MIRANDA-ARANGO;
Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: Glycine functions as an inhibitory neurotransmitter in the CNS that regulates sensory and motor functions primarily in caudal areas. Levels of glycine in the synaptic cleft are regulated by two membrane transporters, glycine transporter 1 and 2 (GlyT1 and GlyT2). These transporters differ in function and location within the CNS. GlyT1 is recognized as an astroglia marker and is widely expressed across the CNS, which function is the fast transport of glycine. In contrast, GlyT2 is a neuronal marker whose main function is recycling glycine into presynaptic neurons and is found in the brainstem and spinal cord. Due to GlyT1 expression attributed to glial cells, very few studies have focused on this transporter. Still, some studies have recognized GlyT1 as a neuronal marker in some areas, such as in the amacrine layer of the retina and the spinal cord's dorsal horn. Furthermore, our previous findings have suggested neuronal expression of GlyT1 in the substantia nigra (SN). Therefore, this study aims to identify glycinergic projections from the SN. To do so, anterograde adeno-associated viral particles (tdTomato flex AAVs) were delivered in the SN through stereotaxic surgery using a new rat GlyT1-Cre knock-in line. Nissl stained was performed in adjacent coronal sections to identify the forebrain regions that receive glycinergic projections from the SN. Preliminary data shows accurate delivery of anterograde particles in the SN, projections to the subthalamic nucleus (STN), striatum (STR), and globus pallidus (GP). Yet, further characterization is necessary; traditional immunohistochemical assays will be done using specific neuronal and glial markers. Filling the gap of knowledge about the nature of GlyT1 expression across the CNS is essential to better understand inhibition, as the location and function of cells containing GlyT1 remain unknown.

Disclosures: R.A. Perez: None. **L.P. Montes:** None. **M. Miranda-Arango:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.12/B34

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NSERC (RGPIN-217-05510)
FRQS (283473)

Title: Multimodal assessment of the GABAergic system in healthy adults: a study combining TMS, MRS and EEG

Authors: *M. LANGLOIS[1,2], S. REMAHI[2], M. MABIKA[2], S. CÔTÉ[3], S. LIPPE[4], K. WHITTINGSTALL[3], J.-F. LEPAGE[2];
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Abstract: Several neuroimaging techniques allow one to non-invasively probe the GABAergic system in humans, including EEG-gamma power, magnetic resonance spectroscopy (MRS), and transcranial magnetic stimulation (TMS), but the relationship between these metrics remains elusive. While, some pharmacological evidence suggests that these various techniques share a common sensitivity to GABAergic modulation, it remains to be established if these measurements are directly related to one another in healthy individuals. The objective of the current work is to characterize the relationship between electroencephalogram (EEG), TMS, and MRS measurements of the GABAergic system in humans. It is hypothesized that the individuals showing higher induced EEG gamma power would present a higher intracortical inhibition measured with TMS, and a higher GABA level measured with MRS. In this study, 21 healthy adults came for a single visit at the University of Sherbrooke Hospital Research Center to undergo three protocols completed in random order: TMS (60 minutes), MRS imaging (60 minutes), and EEG recording (90 minutes). TMS was performed with a 70 mm figure-of-eight coil on a Magstim Bistim2 equipped with a neuronavigational system. The following paired-pulse measures were collected short (SICI) and long (LICI) intracortical inhibition, as well as short (SICF) and long (LICF) intracortical facilitation (ICF) and corticospinal silent period (CSP). MEGA-PRESS MRS was collected on a 3T Ingenia MRI scanner (Philips Netherlands) a whole-body 32-channel head coil scanner. The spectroscopic voxel of interest (VOI) was placed bilaterally over the occipital cortex, EEG recording was performed using a 64 channel cap While an auditory chirp stimulus was presented to the participants (pure tone, 1000 Hz, whose amplitude was modulated from 0 to 120 Hz over 2000 ms); the gamma band was divided into high (65-100Hz) and low (30-65) power spectrum. Preliminary analysis show that individuals presenting stronger Gaba a+b inhibition, indexed by TMS induced corticospinal silent period, showed less induced low-gamma power ($p=0.0426$). No significant relationship was observed between TMS and MRS metrics, nor between MRS and EEG metrics of GABAergic activity. Taken together, these results suggest that MRS, EEG, and TMS, probe different and non-overlapping elements of the GABAergic system. The significance of these results with regards to pathophysiology is discussed.

Disclosures: M. Langlois: None. **S. Remahi:** None. **M. Mabika:** None. **S. Côté:** None. **S. Lippe:** None. **K. Whittingstall:** None. **J. Lepage:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.13/B35

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NINDS 1SC1NS119056
NIH-NIMHD-5U54MD007592
NIH #T34GM145529

Title: Expression of Glycine Transporter 1 in the mouse striatum

Authors: *M. A. YAGUE[1], L. P. MONTES[1], R. A. PEREZ[2], M. MIRANDA-ARANGO[3]; [1]Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX; [2]Biol. Sci., Univ. of Texas At El Paso, EL PASO, TX; [3]Dept. of Biol. Sci. and Border Biomed. Res. Ctr., Univ. of Texas at El Paso, EL PASO, TX

Abstract: Glycine is a major inhibitory neurotransmitter that facilitates inhibitory neurotransmission. At the synapse, extracellular glycine is cleared by two membrane transporters, glycine transporters 1 (GlyT1) and 2 (GlyT2). It is believed that GlyT1 is expressed solely in the CNS's astroglia cells, except in the retina, in which GlyT1 is expressed in amacrine neurons. However, preliminary studies from our laboratory suggest that GlyT1 may also be expressed in neurons in certain brain regions. The rat striatum is known to express moderate levels of GlyT1 in the caudoputamen (CP), yet the cell type expressing GlyT1 has not been identified. Therefore, this study aims to identify and characterize the type of cells expressing GlyT1 in the mouse CP. To visualize GlyT1 in the CP, a mouse knock-in line that allows the expression of tdTomato under the GlyT1 promoter was used. Additionally, traditional immunohistochemical assays were done using neuronal nuclei (NeuN) to identify neurons. Initial analysis showed high levels of expression of tdTomato in the CP, suggesting the presence of GlyT1-containing cells in this region. Moreover, we observed some co-labeling of NeuN with tdTomato, which suggests the presence of the reporter in neurons. These findings reinforce the idea that GlyT1 is also expressed in neurons and can potentially be used as a neuronal marker. Understanding more about the presence of GlyT1 in the CP might give us insight into how inhibitory neurotransmission in this region is mediated in part by glycine.

Disclosures: M.A. Yague: None. **L.P. Montes:** None. **R.A. Perez:** None. **M. Miranda-Arango:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.14/B36

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: KQTD20200820113040070
JCYJ20200109141433384
NSFC81971062

Title: Neural mechanisms by which the brain regains consciousness from anesthesia

Authors: *J. HU[1], Y. LIU[2], H. YAO[1], P. CHEN[1], X.-J. SONG[1];
[1]Southern Univ. of Sci. and Technol., Shenzhen, China; [2]SUStech, Shenzhen, China

Abstract: Recovery of consciousness from anesthesia was assumed to be passive paralleling elimination of the anesthetics from body. It is recently considered to be an active and controlled process. We aimed to understand neuronal mechanisms by which the brain regains consciousness from anesthesia. We used diverse general anesthetics, propofol, pentobarbital, ketamine, and isoflurane to induce reversible loss of righting reflex (LORR) in adult mice and evaluated levels of consciousness during LORR by our newly established rating scale. We used western blot and immunofluorescence to map the changes of K^+-Cl^- cotransporter 2 (KCC2) expression in the brain tissues, MQAE Cl^- imaging to detect the effect of anesthetics on KCC2 function, two-photon imaging and gramicidin-perforated patent-clamp to examine the effect of anesthetics on GABAergic transmission on brain slices, Proteomics, co-immunoprecipitation and PLA to explore the detailed molecular mechanism of KCC2 downregulation. Our results showed that KCC2 expression was significantly and consistently decreased in both thalamus and hypothalamus in the minimum responsive state (MRS) following exposure to the diverse anesthetics. The capacity of KCC2 to transport Cl^- was impaired in ventral posteromedial nucleus of the thalamus (VPM). The decreased activity of KCC2 in the VPM resulted in weakened anesthesia. Anesthesia-induced depolarized shift of GABAergic transmission depends on the decreased expression of KCC2. Pharmacological and genetical inhibition of the ubiquitin proteasomal degradation pathway prevented anesthesia-induced decrease in KCC2, prolonged the duration of anesthesia, and delays recovery from anesthesia. Fbxl4 was identified as the specific E3 ligase driving KCC2 ubiquitination. When Fbxl4 was specifically altered in the VPM, KCC2 expression and recovery of consciousness were correspondingly altered. The recognition of KCC2 by Fbxl4 was regulated by of KCC2 Thr1007 phosphorylation. The ubiquitinated KCC2 formed a complex with valosin containing protein (VCP) with assistance of FAF1 and was then transported to the proteasome for final degradation. We conclude that, when the level of consciousness approaches MRS, the brain initiates the ubiquitin proteasome system to degrade KCC2. KCC2 degradation leads to $GABA_AR$-mediated disinhibition in VPM neurons, enabling accelerated recovery of the neural excitability and from anesthesia. These findings demonstrate that downregulation of KCC2 in the VPM plays a critical role in the emergence of consciousness from general anesthesia. This recovery of consciousness is an active process and occurs independent of anesthetic choice.

Disclosures: **J. Hu:** None. **Y. Liu:** None. **H. Yao:** None. **P. Chen:** None. **X. Song:** None.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.15/B37

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Medial ganglionic eminence-derived GABAergic neuron transplanted into the frontal cortex improves cognitive function in schizophrenia model mice

Authors: *K. TAKASU[1], S. SUYAMA[1], Y. DEGUCHI[1], K. TAKAHASHI[1], H. HAGIHARA[2], T. TAKAGI[3], T. MIYAKAWA[2], K. OGAWA[1];
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Abstract: Schizophrenia is a psychiatric disorder with positive, negative and cognitive dysfunction. Particularly, amelioration of cognitive dysfunction in schizophrenia is important for improving quality of life in patient. Recent studies have reported a relationship between cognitive dysfunction and hypofunction of frontal GABAergic neurons in schizophrenia clinically and non-clinically. However, there is no approved treatment to improve cognitive dysfunction in schizophrenia by enhancing the function of frontal GABAergic neurons. In the present study, we investigated effects of medial ganglionic eminence (MGE)-derived GABAergic progenitor cells transplanted into the frontal cortex in a schizophrenia model (Shn-2 knockout mice), which is characterized by the dysfunction of GABAergic neuron. The protocol of MGE-derived cells transplantation was optimized and a sufficient number of engrafted mature GABAergic neurons 3 months after transplantation was confirmed with expression of parvalbumin and fast spiking neuronal activity, excitatory postsynaptic current and calcium signal. Behavioral test battery was conducted to assess possible effects on Shn-2 knockout mice transplanted MGE-derived cells, and found significant improvement of cognitive dysfunction in Shn-2 knockout mice. In addition, the improvement of anxiety-like behavior in open field test was correlated with the number of engrafted GABAergic neurons in Shn2 knockout mice. These results demonstrated that for the first time the transplantation of MGE-derived GABAergic neuron into the frontal cortex of Shn-2 KO mice elicited improvement of cognitive function and anxiolytic-like effects.

Disclosures: **K. Takasu:** A. Employment/Salary (full or part-time):; SHIONOGI&Co.,Ltd. **S. Suyama:** A. Employment/Salary (full or part-time):; SHIONOGI&Co.,Ltd. **Y. Deguchi:** A. Employment/Salary (full or part-time):; SHIONOGI&Co.,Ltd. **K. Takahashi:** A. Employment/Salary (full or part-time):; SHIONOGI&Co.,Ltd. **H. Hagihara:** A. Employment/Salary (full or part-time):; Fujita Health University. **T. Takagi:** A. Employment/Salary (full or part-time):; Aichi Developmental Disability Center. **T. Miyakawa:** A. Employment/Salary (full or part-time):; Fujita Health University. **K. Ogawa:** A. Employment/Salary (full or part-time):; SHIONOGI&Co.,Ltd.

Poster

PSTR184. GABA and Glycine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.16/B38

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

Support: NIH HL131403
UW-Madison - WARF discretionary funds

Title: Cotranslational association of mRNA transcript encoding heteromeric $GABA_A$ receptors

Authors: N. TARIKERE, K. DECK, F. LIU, G. ROBERSTON, ***C. CZAJKOWSKI**; Neurosci., Univ. Wisconsin-Madison, Madison, WI

Abstract: Type A γ-aminobutyric receptors (GABAR) are pentameric ligand gated ion channels that mediate fast inhibition in the adult brain and are drug targets for barbiturates, benzodiazepines, intravenous anesthetics, and neurosteroids. Consistent with their essential role in regulating neuronal excitability, dysregulation of GABAR activity contributes to anxiety, autism, depression, epilepsy, substance abuse and schizophrenia. GABARs are heteropentamers that can be assembled from α(1-6), β(1-3), γ(1-3), δ, ε, θ and π subunits. The abundance, type, and function of GABARs at synaptic and extra-synaptic sites directly controls the strength of GABAergic transmission. Surprisingly, our understanding of how different GABAR subtypes, each comprising a unique complement of subunits, are assembled is rudimentary. Moreover, how the appropriate levels of these receptors are maintained to control excitatory/inhibitory balance remains unclear. In preliminary experiments, when expressed in HEK293 cells, we find that mRNA transcripts encoding the GABA-A receptor α1, β2 and γ2 subunits are physically associated and can be co-immunoprecipitated with nascent GABAR protein using an antibody against the GABAR α1 subunit suggesting that hetero-oligomeric assembly of GABARs is mediated by a complex comprising the mRNAs encoding each of the subunits. The transcript association only occurred when GABAR subunits were co-expressed. When the potassium channel, hERG, was co-expressed with GABARs, *hERG* transcript was not co-immunoprecipitated. The GABAR transcript association was also observed when GABARs were immunoprecipitated from mouse brain cortex. Our data suggests a new way in which GABAR heteromeric receptor assembly expression is coordinated.

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Poster

PSTR184. GABA and Glycine

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.17/B39

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

Support: R01NS112534

Title: Astrocyte-derived cholesterol regulates gamma-aminobutyric acid type A ($GABA_A$) receptors in neurons

Authors: *Z. YUAN, M. PAVEL, S. HANSEN;
The Scripps Res. Inst., Jupiter, FL

Abstract: The mammalian central nervous system relies heavily on the neurotransmitter (γ-aminobutyric acid) GABA for inhibition, with GABA type A receptors ($GABA_AR$s) being a critical class of ligand-gated chloride ion channels. $GABA_AR$s are predominantly composed of α, β, and γ subunits, with α1β2γ2 receptors being the most abundant in the brain(Pirker *et al.*, 2000). γ2 subunits undergo palmitoylation(Keller *et al.*, 2004; Rathenberg, Kittler and Moss, 2004), adding a palmitate that favors the ordered lipid environment in monosialotetrahexosylganglioside (GM1) clusters(Levental *et al.*, 2010). GM1 clusters are known to be packed with cholesterol, which has been shown to modulate $GABA_AR$ function. Specifically, changes in membrane cholesterol levels have been shown to alter the effect of neuroactive steroids on potentiating $GABA_AR$, with increased membrane cholesterol reducing the effect and depletion of membrane cholesterol increasing the potentiation(Sooksawate and Simmonds, 2001). A potential reason is that the cholesterol-rich GM1 clusters serve as an entry point for $GABA_AR$ endocytosis in regulating membrane expression. Moreover, α1 subunits have a PIP2 binding site(Laverty *et al.*, 2019), and PIP2 clusters separate from GM1 clusters on the membrane(Pavel *et al.*, 2020; Yuan *et al.*, 2022). These features provide a structural basis for $GABA_AR$ anchoring to GM1 clusters and translocating to PIP2 regions when the integrity of GM1 clusters is changed with altered cholesterol levels. Modulating cholesterol levels in a physiologically relevant way is always a challenge, especially in cellular studies. Previous studies used the chemical Methyl-β-cyclodextrin (MβCD), which is unreliable and not regulated properly by the cell. In the brain, apolipoprotein E (ApoE) binds to receptors that regulate the unloading and uptake of cholesterol. In this study, we develop loading cholesterol with astrocyte conditioned media (ACM), which provides better physiological relevance on neurons since astrocyte is the main resource of cholesterol production in the brain and FBS (the frequently used cholesterol resource in cell culture) is not present in healthy animals with the intact blood-brain barrier. With ACM, we are able to recapitulate the effect of cholesterol on neurons in a brain-like environment.Here, we apply confocal and super-resolution imaging to show that loading cholesterol into neurons increases the endocytosis of $GABA_AR$s, and lowering cholesterol shifts the receptor out of the endocytosis pathway to maintain surface expression. We also use whole-cell patching electrophysiology to prove the regulation of cholesterol on $GABA_AR$ functions.

Disclosures: Z. Yuan: None. **M. Pavel:** None. **S. Hansen:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.01/B40

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Ministry of Science and Technology of China 2021ZD0202500

Title: The molecular mechanism through which chemokine CCL2 rapidly promotes GluA1 surface expression

Authors: *E. JI[1,2,3,4], Z. WU[3], Y. LI[3], T. SONG[3], X. YU[3];
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Abstract: The CC chemokine ligand 2 (CCL2), also known as MCP-1 (Monocyte Chemoattractant Protein-1), has well-characterized roles in chemotaxis. A number of works also suggested important roles of CCL2 in the central nervous system (CNS) under both physiological and pathological conditions. Furthermore, CCL2 has been shown to regulate neuronal activity in different types of neurons, including spinal cord lamina II neurons, primary sensory neurons, hippocampal neurons, and VMPO neurons. In previous work, we showed that lipopolysaccharide (LPS)-induced neuroinflammation upregulated CCL2 expression in the brain; CCL2 in turn, rapidly elevated excitatory synaptic transmission in CA1 hippocampal neurons, as well as a number of other neuronal types. What is the molecular mechanism underlying this process? Using western blots, we showed that phosphorylation of S831 and S845 sites on the AMPA receptor subunit GluA1 were upregulated following i.p. injection of LPS; importantly, this effect was blocked in *Ccr2*$^{-/-}$ mice. Consistently, using cultured hippocampal neurons, we showed that exogeneous CCL2 application rapidly upregulated surface expression of GluA1, using both surface GluA1 antibody staining, as well as live imaging of surface SEP-GluA1. These upregulations were effectively blocked by antagonists of CCR2, the G-protein couple receptor mainly mediating the effects of CCL2. We next assayed the downstream signaling pathway of CCR2 using three reporter assays, including the Gs-dependent luciferase assay, the Gq-dependent and the Gq/i-dependent FLIPR assays. The assays showed that CCL2 signaled via $G\alpha_q$ and $G\alpha_i$, but not $G\alpha_s$. Results of pharmacological experiments in cultured hippocampal neurons indicated that CCL2 mainly signaled via $G\alpha_q$-Ca^{2+}-CaMKII-GluA1 (S831) to facilitate excitatory synaptic transmission, and that cAMP-PKA-GluA1 (S845) likely also contribute to this process. Taken together, our data strongly suggest that CCL2 elevated excitatory synaptic transmission primarily via $G\alpha_q$-Ca^{2+}-CaMKII-GluA1 (S831) signaling. The methods we developed provide a promising strategy for systematically investigating the mechanism through which G-protein coupled receptor-dependent cytokines regulate the strength of excitatory synapses.

Disclosures: **E. Ji:** None. **Z. Wu:** None. **Y. Li:** None. **T. Song:** None. **X. Yu:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.02/B41

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant T32 GM008244
NIH Grant T32 DA007234
NIH Grant R01 DA048946
Department of Defense Grant W81XWH2010509

Title: Characterization of VGF in the nucleus accumbens and its contribution to opioid-evoked behaviors

Authors: *A. P. ADKE[1,2], S. M. MULLOY[3], R. GAO[4], A. M. LEE[4], L. VULCHANOVA[5], P. E. ROTHWELL[5];
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Abstract: Endogenous neuropeptides in reward circuits integrate complex, multivalent information, and altered peptidergic signaling can have dramatic effects on physiology and behavior. Opioid addiction is a manifestation of this imbalance and may involve dysregulated signaling by neuropeptides derived from VGF (non-acronymic), which facilitate synaptic plasticity in the central nervous system. VGF expression is upregulated in the nucleus accumbens (NAc), a brain region critical for processing reward, following exposure to misused substances. Our prior work demonstrated that VGF mRNA levels increased in the NAc when opioid exposure was periodically interrupted by withdrawal. Opioid intake and withdrawal hijack the mechanisms underlying synaptic plasticity in the NAc, toppling the balance of neuropeptides and increasing vulnerability to dependence and addiction. However, the action of VGF-derived peptides on NAc physiology and withdrawal-evoked behaviors has never been examined. Our objective is to interrogate the contribution of VGF to the mechanisms underlying plasticity in the NAc. Preliminary data show that knocking out VGF in the NAc eliminates the behavioral changes typically evoked by opioid exposure and withdrawal in mice. These observations have led us to hypothesize that VGF-derived peptides facilitate the maladaptive plasticity in the NAc and the behavioral adaptations that occur after repeated cycles of opioid withdrawal. We are currently characterizing VGF mRNA expression across the NAc and its colocalization with known markers of different NAc cell types. We are also investigating how the expression of VGF-derived peptides changes following an interrupted pattern of oxycodone exposure, in addition to determining the effect of VGF conditional knockdown in the NAc on behavioral consequences of oxycodone exposure and withdrawal. Uncovering the impact of VGF on NAc plasticity will further our understanding of how the brain is impacted by exogenous opioid exposure and may lead to novel therapeutic targets to treat opioid use disorders.

Disclosures: **A.P. Adke:** None. **S.M. Mulloy:** None. **R. Gao:** None. **A.M. Lee:** None. **L. Vulchanova:** None. **P.E. Rothwell:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.03/B42

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: R01AA209403
R21AA028088
P50AA017823
R01NS078168
R01NS101353
T32NS115667
ZIA NS003168

Title: Unraveling the Power of Somatostatin: Insights into Prefrontal Cortex Function

Authors: *D. BROCKWAY[1], K. R. GRIFFITH[1], C. M. ALOIMONOS[2], T. CLARITY[3], B. MOYER[1], G. SMITH[1], N. DAO[4], M. HOSSAIN[1], P. J. DREW[1], J. A. GORDON[5], D. A. KUPFERSCHMIDT[6], N. A. CROWLEY[1];
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Abstract: Somatostatin (SST) neurons in the prelimbic (PL) cortex mediate a variety of behavioral states. Until recently, little was known about the actions of somatostatin peptide signaling in shaping cortical functioning and behavior. Here, we characterize the unique physiological role of the SST peptide in the PL cortex. We employed a combination of ex vivo pharmacologic and optogenetic electrophysiology and in vivo calcium monitoring, to explore the role of SST neuron and peptide signaling in the mouse PL cortex. Whole-cell slice electrophysiology was conducted in pyramidal and GABAergic neurons in the PL cortex of C57BL/6J and SST-IRES-Cre male and female mice to characterize the pharmacological mechanism of SST signaling. Fiber photometry of GCaMP6f fluorescent calcium signals from SST neurons was conducted to characterize the activity profile of SST neurons during exploration of an elevated plus maze (EPM) and open field test (OFT). SSTR activation broadly hyperpolarized layer 2/3 pyramidal neurons in the PL cortex in both male and female mice ex vivo, an effect that was recapitulated with optogenetic stimulation of SST neurons, through both monosynaptic and polysynaptic GABA neuron-mediated mechanisms of action. This hyperpolarization was blocked by pre-application of the SSTR antagonist cyclo-somatostatin (cyclo-SST) and was non-reversible. SST neurons in PL were activated during EPM and OFT exploration, indicating task-related recruitment of these neurons. Together, this work describes a broad ability for SST peptide to modulate microcircuits within the prefrontal cortex and related behaviors.

Disclosures: D. Brockway: None. **K.R. Griffith:** None. **C.M. Aloimonos:** None. **T. Clarity:** None. **B. Moyer:** None. **G. Smith:** None. **N. Dao:** None. **M. Hossain:** None. **P.J. Drew:** None. **J.A. Gordon:** None. **D.A. Kupferschmidt:** None. **N.A. Crowley:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant R01AA209403
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NIH Grant P50AA017823
NIH Grant U19NS128613
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NIH Grant R01NS101353
NIH Grant T32NS115667

Title: Somatostatin peptide administration to the prefrontal cortex promotes exploratory behavior in mice

Authors: *K. R. GRIFFITH[1,2], D. F. BROCKWAY[2], G. C. SMITH[3], M. HOSSAIN[3,4], P. J. DREW[2,3,4,5,6], N. A. CROWLEY[2,3,4];
[2]Biol., [3]Biomed. Engin., [4]Ctr. for Neural Engin., [5]Engin. Sci. and Mechanics, [6]Neurosurg., [1]Pennsylvania State Univ., University Park, PA

Abstract: Somatostatin (SST) neurons in the prelimbic cortex have been identified as a key population in the control of exploratory behavior and cortical functions. Although SST neurons have long been implicated as a key subset of γ-Aminobutyric acid (GABA)-expressing inhibitory neurons for their role in modulating behavior, the role of SST peptide is largely not understood. Through the use of *in vivo* bilateral peptide administration of both SST receptor agonist and antagonist in the prelimbic cortex of mice, we begin to elucidate the behavioral shifts caused by SST peptide signaling. Administration of SST receptor agonist Octreotide increased open arm exploration in the elevated plus maze. There was no significant effect of SST receptor antagonist, cyclo-somatostatin, on the same behaviors, highlighting the unique role of SST peptide on the listed measures of exploratory behavior. These results indicate the unique role that SST peptide can play in future modulation of cortical circuitry. Future work will continue to expand on the function of SST peptide in the prelimbic cortex and will examine new methods of noninvasive peptide administration for cortical modulation.

Disclosures: K.R. Griffith: None. **D.F. Brockway:** None. **G.C. Smith:** None. **M. Hossain:** None. **P.J. Drew:** None. **N.A. Crowley:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.05/B44

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Altered insulin signaling pathway in the brain of taurine deficient mice

Authors: *F. SIDIME[1,2,4], **P. V. SODHI**[3], V. MOROZOVA[2,5,4], D. D. TARAZONA[2], K. LEVANO[1,4], A. EL IDRISSI[2,4];
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Abstract: In this study, we examined the functional consequences of altered glucose homeostasis on insulin receptor activation and expression in the brain. Previously we found that Cysteine Sulfinic Acid Decarboxylase Knock-Out mice (CSAD KO) have altered response to glucose tolerance. They were hypoglycemic at T0 and showed significantly low glucose plasma levels 30 min post-injection, indicating an altered signal transduction mechanism of insulin that could be due to reduced insulin secretion or resistance to insulin. Insulin receptor (IR) is abundantly expressed in central neurons, and insulin crosses the blood-brain barrier with a high-affinity uptake system. To further investigate the type of alteration in the IR signal transduction pathway in the brain of CSAD KO mice, we measured neuronal excitability in response to glucose or insulin injection. We found that subcutaneous injection of glucose or intracerebral injection of insulin led to a significant increase in the amplitude of field potentials and a significant increase in the firing rate. Consistent with this observation, we found that glucose injection significantly increased the amplitude of the auditory-evoked startle response in the KO mice. Furthermore, biochemical analysis of the IR in the brain using western blot (WB) and immunohistochemistry (IHC) revealed a significant and global increase in the expression levels of IR in the brain of CSAD KO compared to controls.These data indicate that taurine deficiency alters glucose homeostasis and induces several biochemical, electrophysiological, and behavioral alterations in the brain of CSAD KO mice.

Disclosures: F. Sidime: None. **P.V. Sodhi:** None. **V. Morozova:** None. **D.D. Tarazona:** None. **K. Levano:** None. **A. El idrissi:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.06/B45

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: The Role of a R>Q mutation in Anorexia

Authors: *A. C. KOEHLER;
Med. Univ. of South Carolina Neurosci. Grad. Program, Charleston, SC

Abstract: The role of a R>Q mutation in Neurotensin in Anorexia

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Primary Theme: B.01.d: Neural Excitability, Synapses, and Glia: Neuropeptides, cytokines, growth factors, and other signaling molecules

Anorexia Nervosa (AN) is a psychiatric disorder in which people voluntarily refuse food intake due to altered reward processing. It has no approved treatments and the highest rate of mortality among neuropsychiatric disorders. AN emerges from a complex interplay between social, psychological, neural, and genetic factors. In this study we focus on the impact of genetics on neurobiology in AN patients. Many AN patients have nonsynonymous mutations in the genes that encode neurotensin (nts) and neurotensin receptor 1 (ntsr1). Neurotensin is a neuropeptide that binds to G-protein coupled receptors (i.e. ntsr1) in the brain and gut and has many functions including feeding regulation. One of these mutations is a substitution of arginine to glutamine (R>Q) at amino acid position 150, which alters the proteolytic site at which prohormone convertase cleaves proneurotensin to neurotensin. This results in a longer neurotensin neuropeptide than the wildtype protein. Thus far, no studies have analyzed the neural implications of this mutation. In this study we are testing the functional consequence of this clinically relevant mutation to neuronal physiology in mouse brain cells. Using slice electrophysiology, we analyzed both the R150Q mutant and wild-type protein in mouse brain cells from the ventral tegmental area (VTA). The VTA has a high concentration of ntsr1 and is clinically relevant for eating disorder studies due to its role in reward processing. We have assessed baseline activity, firing rate, action potential, rheobase, and NMDA and AMPA ratio of VTA cells, including dopaminergic and non-dopaminergic neurons in the presence of wild-type and mutant neurotensin. Understanding the effect of this specific mutation on the electrophysiological properties of neurons will give us a better understanding of the role it plays in the brain and its implication in AN.

Disclosures: A.C. Koehler: None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.07/B46

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: MH122461

Title: Oxytocin modulation of lateral hypothalamus inputs to dorsal raphe serotonin neurons

Authors: *S. OUBRAIM, K. A. HAUSKNECHT, R.-Y. SHEN, S. HAJ-DAHMANE; Pharmacol. and toxicology, Univ. At Buffalo, Buffalo, NY

Abstract: The lateral hypothalamus (LH) inputs to the dorsal raphe nucleus (DRN) play a key role in the regulation of multiple physiological processes and behaviors including food intake, sleep, social interaction, stress homeostasis, and anxiety. However, the precise organization of the LH inputs to the DRN and its modulation by the prosocial hormone oxytocin (OXT) remains unknown. In this study, using Tph2-Cre knockin rats, combined with pharmacological electrophysiology and optogenetic approaches, we examined the synaptic organization of LH inputs to DRN 5-HT neurons projecting to the medial prefrontal cortex (DRN^{mPFC}) and central nucleus of the amygdala (DRN^{CeA}), and their modulation by OXT. We found that LH sends monosynaptic GABAergic, glutamatergic, or mixed synaptic inputs to DRN 5-HT neurons projecting to the CeA. In contrast, DRN 5-HT neurons projecting to the mPFC (DRN^{mPFC}) receive only monosynaptic GABAergic inputs from the LH. Activation of postsynaptic OXT receptors inhibits LH excitatory synapses of DRN^{CeA} 5-HT neurons, while it induces long-term depression of LH GABA synapses of both DRN^{CeA} and DRN^{mPFC} 5-HT neurons. Thus, these results unravel that OXT exerts target-dependent modulation of LH synaptic inputs to DRN 5-HT neurons.

Disclosures: S. Oubraim: None. **K.A. Hausknecht:** None. **R. Shen:** None. **S. Haj-Dahmane:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.08/B47

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Extrasynaptic NMDA receptors expressed by magnocellular oxytocin neurons in mouse PVN

Authors: *N. ALSHAKHSHIR, S. W. HARDEN, C. J. FRAZIER;
Univ. of Florida, Gainesville, FL

Abstract: Oxytocinergic magnocellular neurons (OT-MCNs) release oxytocin (OT) from multiple compartments, including their axon terminals, soma, and dendrites. Dendritic release contributes to a local paracrine signal and may support activation of distal OT receptors via volume transmission. Existing evidence suggests that there exists a complex relationship between cellular activity (action potential firing initiated in the soma), and calcium-dependent dendritic release of OT into the CNS. Recent work has also established that dendritic conductivity is a dynamic and endogenously regulated feature of OT-MCNs that robustly modulates action potential induced dendritic calcium influx. In the current study, we sought to evaluate other modulatory mechanisms that further contribute to dynamic regulation of activity dependent calcium influx in the dendrites of OT-MCNs. Towards that end we used a combination of electrophysiological, optical, and pharmacological approaches, in acute brain slices extracted from adult male and female C57BL mice, to carefully evaluate NMDA receptor

expression and function in PVN OT-MCNs. We find that the dendrites of these neurons robustly express magnesium sensitive NMDA receptors, a significant subset of which are blocked by an antagonist selective for NR2c/d containing receptors, implying a possible extrasynaptic location. Preliminary data further suggests that these receptors are likely to be activated by physiologically relevant levels of ambient glutamate. Additional work will carefully evaluate the ability of these receptors to directly amplify activity dependent calcium influx in both proximal and distal dendrites when activated by endogenous vs. exogenous agonists. An ability to use a pharmacological approach to amplify activity dependent calcium influx in OT-MCN dendrites might ultimately have utility as a mechanism to increase release of endogenous oxytocin into the CNS.

Disclosures: N. Alshakhshir: None. **S.W. Harden:** None. **C.J. Frazier:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.09/B48

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Brain Research Foundation Scientific Innovations award
T32NS007292

Title: Homeostatic maintenance of neocortical excitation-inhibition balance by ciliary neuropeptidergic signaling

Authors: *N. F. WONG, L. TERESHKO, P. SENGUPTA, G. G. TURRIGIANO;
Biol., Brandeis Univ., Waltham, MA

Abstract: Primary cilia are organelles involved in multiple signaling processes important for cell division, cell migration and metabolism. Much of the research on neuronal primary cilia has focused on ciliary signaling during early development. However, neuronal primary cilia persist in fully differentiated adult neurons, suggesting they play important roles in mature neuronal circuits. Interestingly, human ciliopathies, a set of disease and disorders caused by cilia dysfunction, are associated with cognitive defects as well as epilepsy, raising the possibility that ciliary signaling contributes to the homeostatic maintenance of excitation and inhibition (E/I). In neocortex, primary cilia of pyramidal neurons (PNs) contain a high concentration of somatostatin receptor 3 (SSTR3) G-protein coupled receptor. This is particularly interesting because somatostatin-positive (SST+) interneuron activity correlates well with overall cortical network activity. Thus, SST release from SST+ interneurons and signaling through SSTR3 in PN cilia is an intriguing candidate signaling pathway to mediate activity-dependent homeostatic adjustment of the E/I balance. Consistent with this idea, recent work from the Turrigiano and Sengupta labs has shown that SSTR3-mediated ciliary signaling can modulate excitatory synaptic strength and number onto cultured neocortical PNs, to shift the excitation and inhibition (E/I) balance toward

excitation. Here, we expand on these findings to determine whether SST signaling can homeostatically regulate excitatory synapses *in vivo* during postnatal development, and thus contribute to the dynamic regulation of E/I balance. The primary visual cortex (V1) of P14-P16 rats was infected with an AAV, expressing either a GFP and an shRNA targeted against rat SSTR3, or GFP alone. SSTR3 knockdown was confirmed using immunohistochemistry and reached ~90% loss after 1 week. Whole-cell voltage clamp recordings from GFP-expressing layer 2/3 PNs (L2/3 PNs) from *ex vivo* slices of V1 was then performed to measure postsynaptic strength in control and knockdown neurons. SSTR3 KD significantly increased excitatory postsynaptic strength, whereas inhibitory postsynaptic strength was unaffected. In addition, morphological analysis of L2/3 PNs showed an increase in dendritic spine density after SSTR3 KD. These data suggest that activity-dependent SST release within neocortex can target PN cilia to selectively modulate excitatory synapse number and strength. Further work on the underlying inter- and intracellular signaling mechanisms will greatly expand our understanding of the role of ciliary signaling in neuronal and network function.

Disclosures: **N.F. Wong:** None. **L. Tereshko:** None. **P. Sengupta:** None. **G.G. Turrigiano:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.10/B49

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: MH002386

Title: Molecular modeling of the PAC1 receptor predicts PACAP 6-38 deletants as PACAP antagonists

Authors: *W. XU[1], G. DEGANUTTI[2], P. SEXTON[3], L. EIDEN[1];
[1]NIMH, NIH, Bethesda, MD; [2]Coventry Univ., Coventry, United Kingdom; [3]Monash Inst. of Pharmaceut. Scien Drug Discovery Biol., Parkville, Australia

Abstract: The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is a ligand for the family B G-protein coupled receptor PAC1, and for the VPAC1 and VPAC2 receptors, for which vasoactive intestinal polypeptide (VIP) is also a ligand. PACAP exists in a 27-amino acid (P27) and a 38-amino acid (P38) form, which are essentially equipotent as PAC1 agonists. Considerable attention has been focused on obtaining both peptide-based, and non-peptide agonists and antagonists for PAC1, given the potential role(s) of PACAP, presumably acting at PAC1, in atherosclerogenesis, depression, migraine, neurodegenerative consequences of stroke and ischemia and post-traumatic stress disorder, but with limited success. Peptide-based antagonists include an N-terminally truncated version of PACAP38 itself, PACAP6-38. The activity of PACAP6-38 as an antagonist is consistent with models for PACAP binding to PAC1

in which the N-terminus of PACAP is required for receptor activation leading to signaling through the Gs protein, while the C-terminus is involved in initial binding (affinity-trapping) to PAC1. Recently, cryo-EM structures of a number of family B (secretin family) GPCR-ligand structures have been obtained, and these allow detailed molecular dynamics analysis of ligand-receptor binding, and prediction of residues contributing most strongly to initial binding to, and further engagement with, the receptor. PACAP6-38 inhibits P27 and P38 signaling (cyclic AMP elevation through Gs engagement with adenylyl cyclase) in the PAC1-expressing SH-SY5Y neuroblastoma cell line with an IC_{50} similar to that observed for PACAP6-38 inhibition of PACAP signaling in neurons, and unlike cell lines such as HEK293, in which signaling through exogenously expressed PAC1 is inhibited significantly less potently by PACAP6-38. Molecular dynamics predictions of the relative importance of residues 28-30 of PACAP6-38, compared to residues 31-38, led us to construct a series of deletants of PACAP6-38 and to test their relative inhibitory potency in SH-SY5Y cells. PACAP6-30 (IC50 ~33 nM) was about as potent as PACAP6-38 (IC50 ~21 nM) as an inhibitor of cyclic AMP elevation by 0.2 nM P38, while PACAP6-27 was considerably less potent (IC_{50} >10 μM). A series of lactam modifications that increase the potency of N-terminally truncated versions of secretin-27 for the secretin receptor, SCTR, failed to improve the potency of corresponding PACAP6-27 and PACAP6-38 analogs as PAC1 inhibitors. Thus, the affinity-trap model for PAC1-ligand interaction accommodates the unique features of PACAP27 and PACAP38 initial binding to, and activation of, PAC1.

Disclosures: **W. Xu:** None. **G. Deganutti:** None. **P. Sexton:** None. **L. Eiden:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.11/B50

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant NS094597

Title: Human iN neuronal model of schizophrenia displays dysregulation of chromogranin B and related neuropeptide transmitter signatures assessed by peptidomics

Authors: *S. PODVIN[1,2], P. REED[4], C. B. LIETZ[2], J. THEN[2], K. LEE[2], L. EYLER[2], D. JESTE[2], F. H. GAGE[5], V. HOOK[3];
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Abstract: Schizophrenia (SZ) is a serious mental illness and neuropsychiatric brain disorder with behavioral symptoms that include hallucinations, delusions, disorganized behavior, and cognitive impairment. Regulation of such behaviors requires utilization of neurotransmitters released to mediate cell-cell communication which are essential to brain functions in health and disease. We hypothesized that SZ may involve dysregulation of neurotransmitters secreted from

neurons. To gain an understanding of human SZ, induced neurons (iNs) were derived from SZ patients and healthy control subjects to investigate peptide neurotransmitters, known as neuropeptides, which represent the major class of transmitters. The iNs utilized patient fibroblasts in a direct reprogramming protocol that retains the adult age phenotype of these human neurons. The iNs were subjected to depolarization by high KCl in the culture medium and the secreted neuropeptides were identified and quantitated by nano-LC-MS/MS tandem mass spectrometry in peptidomics studies. Several neuropeptides were identified from schizophrenia patient-derived neurons, including chromogranin B (CHGB), neurotensin, and natriuretic peptide. Focusing on the main secreted CHGB neuropeptides revealed differences in SZ iNs compared to control iN neurons. Lower numbers of distinct CHGB peptides were found in the SZ secretion media compared to controls. Mapping of the peptides to the CHGB precursor revealed peptides unique to either SZ or control, and peptides common to both conditions. Also, the iNs secreted neuropeptides under both KCl and basal (no KCl) conditions. These findings are consistent with reports that chromogranin B levels are reduced in the cerebrospinal fluid and specific brain regions of SZ patients. These findings suggest that iNs derived from SZ patients can model the decreased CHGB neuropeptides observed in human SZ.

Disclosures: S. Podvin: None. **P. Reed:** None. **C.B. Lietz:** None. **J. Then:** None. **K. Lee:** None. **L. Eyler:** None. **D. Jeste:** None. **F.H. Gage:** None. **V. Hook:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.12/B51

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant F31NS124107
NIH Grant R01MH119355
NIH Grant R01DA048822
NIH Grant HD100007

Title: Estrogen Signaling in Hippocampal Astrocytes

Authors: *J. GOENAGA, C. NANCLARES, P. KOFUJI, P. MERMELSTEIN, A. ARAQUE; Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Estrogen Signaling in Hippocampal AstrocytesJulianna Goenaga, Carmen Nanclares, Paulo Kofuji, Paul Mermelstein, Alfonso Araque There is emerging research about the effects of neurotransmitters on astrocytes and their subsequent release of gliotransmitters. However, there is little known about how other signaling molecules such as hormones impact astrocyte signaling. Estradiol (E2). is an important hormone that regulates neuronal activity and brain function. Previous studies have also examined the signaling effects of E2 in astrocyte cell cultures. However, whether E2 specifically signals to astrocytes *in situ* remains unknown. This

study aimed to determine how E2 affects astrocyte calcium activity and then determine the consequences on neuronal signaling. We used hippocampal slices of female mice and monitored the astrocyte calcium activity in the CA1 region of the hippocampus. Calcium imaging of GCaMP6f -expressing astrocytes was used to record astrocyte calcium activity before and after E2 application. We found that E2 significantly increased calcium event probability as well as other calcium dynamic parameters, such as amplitude and event frequency. To determine how this could impact neuronal signaling, we also electrophysiologically recorded CA1 hippocampal neurons to monitor the slow inward currents (SICs), which are known to be mediated by astroglial glutamate release and activation of neuronal NMDARs. We found that E2 increased the frequency of SICs. Taken together, these results indicate that E2 increases astrocyte calcium activity and stimulates the release of astroglial glutamate. Therefore, astrocytes are a cellular target of E2 signaling in the brain.

Disclosures: J. Goenaga: None. **C. Nanclares:** None. **P. Kofuji:** None. **P. Mermelstein:** None. **A. Araque:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.13/B52

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Whitehall Foundation Grant 2017-05-35
Brain & Behavior Research Foundation NARSAD Young Investigator Award (Grant 28549)
National Science Foundation CAREER Award(Award Number (FAIN) 2047700)

Title: The role of Arc turnover in cognitive flexibility

Authors: *W. WEI[1], M. GHANE[1], Z. D. ALLEN[1,2], J. HAMM[1,2], A. MABB[1,2];
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Abstract: Protein degradation is an essential mechanism to suport learning-related behavior. Notably, many forms of neurological disorders are associated with dysfunctional protein turnover. The activity-regulated cytoskeleton-associated protein (Arc), as an immediate early gene, plays a key role in synaptic plasticity, learning and memory. Upon learning, Arc is induced followed by its rapid decline. To test how Arc turnover is involved in learning, we created a transgenic mouse that has disrupted Arc removal (ArcKR). We showed that Arc and its temporally regulated turnover is necessary for spatial reversal learning. Defects in Arc degradation also led to enhanced metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD). However, the precise intracellular and network changes resulting in these synaptic and behavioral phenotypes are unknown. We found that blocking Arc

ubiquitinaton altered DHPG-induced ER Ca^{2+} release, AMPA receptor trafficking, Arc self-association and led to enhanced interactions with the ER membrane protein Calnexin. Here, we used the photoconvertible Ca^{2+} indicator CaMPARI2 to explore IEG expression profiles in active and inactive dorsal CA1 hippocampal neuron populations during a spatial learning task. Varying task demands across epochs of behavior led to the engagement of neural ensembles with discrete IEG profiles. We also found that recapitulated deficiencies in ArcKR spatial reversal learning were paired with altered gross hippocampal activity and differential relationships of the IEGs Arc and c-Fos. To couple these findings to neural ensemble patterning, we monitored changes in the activity of multiple neurons simultaneously in WT and ArcKR mice by expressing the genetic encoded neural reporter, GCaMP6f in the dorsal CA1 hippocampus. Using a miniature fluorescence microscope, we were able to image WT and ArcKR mice in a spatial learning task. Overall, our work illustrates that Arc ubiquitination acts as an essential regulator of hippocampal-dependent behavioral flexibility by tuning Ca^{2+} signaling pathways and regulating ensemble IEG signatures during spatial reversal learning.

Disclosures: W. Wei: None. **M. Ghane:** None. **Z.D. Allen:** None. **J. Hamm:** None. **A. Mabb:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.14/B53

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NRSA Grant 1F31NS127592-01A1
NIH NINDS R01-NS126247
VA I01-BX004938
Department of Defense W81XWH-18-1-0598
NGP 5T32NS007466-24
MSTP 5T32GM141938-02
Lacroute Fellowship

Title: Differential modulation of excitatory and inhibitory synaptic transmission by enkephalin in the mouse hippocampus

Authors: *N. WARIKOO[1,2,3], A. ANDERSON[1,3], E. SCHNELL[1,3];
[1]OHSU, Portland, OR; [2]Neurosci. Grad. Program, Vollum Inst., Portland, OR; [3]Veterans Affairs Portland Healthcare Syst., Portland, OR

Abstract: Opioid drugs potently control neuronal circuitry in the brain through their actions at opioid receptors. Endogenous opioid peptides (EOs) are produced, packaged, and released by neurons, but their contributions to neural network dynamics are less known. In the brain, the hippocampus contains numerous pathways which produce and release the endogenous opioid

peptide met-enkephalin. Given the important role of the hippocampus in memory function as well as its involvement in circuit rearrangements underlying epilepsy, we applied met-enkephalin while performing voltage-clamped single cell recordings from visually identified hippocampal granule cells and CA3 pyramidal cells in acutely prepared mouse brain slices, while electrically or optogenetically stimulating afferent fibers to isolate specific components of the hippocampal circuit. Met-enkephalin inhibited electrically-evoked inhibitory post-synaptic currents (IPSCs) as well as optogenetically evoked IPSCs from Parvalbumin-positive interneurons in the dentate gyrus ($p<0.0005$), and was associated with increased paired-pulse facilitation, suggesting a presynaptic mechanism. In CA3, inhibitory transmission was similarly inhibited ($p<0.0005$); however, glutamatergic transmission at the mossy fiber-CA3 synapse was depressed in the absence of a change in paired pulse facilitation ($p<0.005$). Ongoing studies aim to dissect the underlying signaling mechanisms involved in mediating the circuit effects of enkephalins, as well to further understand the release of endogenous opioid peptides from enkephalin-producing neurons. We look forward to studying evoked opioid peptide release in healthy and translationally modeled epileptic brain slices to understand how EOs modulate circuit excitability in brain disease.

Disclosures: **N. Warikoo:** None. **A. Anderson:** None. **E. Schnell:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.15/B54

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH NINDS R01-NS126247
VA I01-BX004938
Department of Defense W81XWH-18-1-0598
F31 1F31NS127592-01A1
MSTP 5T32GM141938-02
NGP 5T32NS007466-24

Title: Timecourse of activity-dependent endogenous opioid peptide gene transcription after seizures in the mouse hippocampus

Authors: *A. N. ANDERSON[1,2], N. WARIKOO[1,2], K. GUPTA[1], W. HENDRICKS[3], E. SCHNELL[2,1];
[1]Oregon Hlth. and Sci. Univ., Portland, OR; [2]Portland VA Med. Ctr., Portland, OR; [3]Vollum Inst., Portland, OR

Abstract: Endogenous opioid peptides (EOs) may modulate excitability within the hippocampal circuit, particularly in the context of temporal lobe epilepsy. EOs, such as enkephalin and dynorphin, demonstrate notable changes in localization and staining during epileptogenesis, but

little is known about the roles of EOs in hippocampal circuitry in both healthy and epileptic brains. Here, we examine changes in expression of the endogenous opioid genes Prodynorphin (PDyn) and Proenkephalin (PEnk) in hippocampal tissue from wildtype and genetically modified mouse models using immunohistochemistry, quantitative RT-PCR, and in situ hybridization. First, we quantitatively characterized two PEnk-IRES-Cre::Rosa26 reporter mouse lines, and found that the density of PEnk-reported neurons increases with mouse age. We also stained tissue for cFos and PEnk after pentylenetetrazole (PTZ)-induced seizures using immunohistochemistry, and found significant increases in cFos staining at early time points after seizure, with poor detection of enkephalin using commercially available antibodies. To improve enkephalin detection, we used Real-Time PCR and in situ hybridization to characterize hippocampal mRNA changes at different timepoints after PTZ-induced seizures or during kainic acid-induced epileptogenesis. Preliminary RT-PCR data show a 60-fold increase in cFos mRNA transcription peaking 15-30 minutes after PTZ-induced seizure and a more delayed increase in Penk transcripts across different mouse seizure models and at early stages after translational epilepsy models. The effects of seizures on Prodynorphin gene expression at early timepoints after seizures was analyzed and suggested a different pattern of regulation. Together, these data suggest activity-dependent endogenous opioid peptide gene expression may be a component of post-seizure functional circuit reorganization, with potential implications for epileptogenesis and the control of brain hyperexcitability.

Disclosures: A.N. Anderson: None. **N. Warikoo:** None. **K. Gupta:** None. **W. Hendricks:** None. **E. Schnell:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.16/B55

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NS104297-04

Title: Calcitonin Gene Related Peptide activates oligodendrocyte precursor cells, not neurons, in the insula cortex

Authors: *R. LORSUNG[1], J. KOENIG[2], A. KELLER[3];
[1]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Univ. of Maryland, Baltimore, Univ. of Maryland, Baltimore, Baltimore, MD; [3]Univ. of Maryland Sch. of Med., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Elevated Calcitonin Gene Related Peptide (CGRP) signaling has been linked with central sensitization in chronic pain disorders. However, most CGRP studies in the CNS examine direct effect of CGRP on *neuronal* signaling; little is known regarding CGRP's actions on glial CNS intermediaries. We hypothesize that elevated CGRP signaling occurring in chronic pain

conditions activates glial cells, disrupting normal brain homeostasis and contributing to central sensitization. The first aim of this project is to identify these glial populations, and elucidate the function of CGRP in these cells. As one of the main sources of CGRP in the brain is the parabrachial nucleus (PB), we began by investigating PB's efferent projection to the insula cortex, a region linked with affective pain processing in humans. We first tested whether PB CGRP release directly acts on insula neurons. We performed whole-cell, voltage-clamp recordings in insula slices from $CGRP^{Cre}$ mice, in which an excitatory opsin was expressed in PB_{CGRP} terminals. Neither stimulation of endogenous CGRP release from these PB terminals, nor acute application of exogenous CGRP, significantly altered optically-evoked excitatory postsynaptic currents. Additionally, CGRP did not alter frequency or amplitude of spontaneous synaptic inputs to insula neurons. Furthermore, RNAscope confirmed the two subunits required for a functional CGRP receptor (CGRPr), Calcr and Ramp1, are not expressed in NeuN+ insula cells. However, CGRPr was expressed in Olig2+ cells in the insula. As Olig2 is a nonspecific marker for both oligodendrocytes (Oligs) and oligodendrocyte precursor cells (OPCs), we analyzed previously published single cell datasets to identify this subpopulation of CGRPr+ glial cells. In mice, 80% of cortical OPCs express CGRPr, compared to 30% and <1% in committed OPCs and fully differentiated Oligs, respectively. This suggests CGRPr is expressed primarily in OPCs, while expression is gradually downregulated upon differentiation into Oligs. There are similar relative expression levels in human cortical OPCs and Oligs. Taken together, we have identified OPCs as the primary CGRPr+ glial population in the insula. Preliminary immunohistochemistry experiments targeting NG2 (OPC membrane marker), show an increase in OPC complexity in the insula in response to CGRP incubation. This may suggest CGRP acts as a chemotaxic signaling agent in OPCs, inducing process migration toward highly active terminals requiring additional trophic support. Future experiments will further investigate the function of CGRPr activation in OPCs, and how OPC activation influences insula neuronal signaling in chronic pain states.

Disclosures: **R. Lorsung:** None. **J. Koenig:** None. **A. Keller:** None.

Poster

PSTR185. Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.17/Web Only

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: JST SPRING JPMJSP2132

Title: Uncovering the Influence of Long-Term Coffee Exposure on Intestinal Epithelial Cells' Serotonin Uptake

Authors: ***S. KIKKAWA**[1], S. IMAMURA[2], K. HARADA[1], S. TANAKA[1], I. HIDE[1], N. SAKAI[1];

[1]Dept. Mol. Pharmacol. Neuroscience, Grad. Sch. Biomed. Hlth. Sci., [2]Dept. of Dent. Anesthesiology, Grad. Sch. of Biomed. and Hlth. Sci., Hiroshima Univ., Hiroshima, Japan

Abstract: The production of serotonin(5-HT) in the gastrointestinal tract depends on Enterochromaffin cells and Itsconcentration is regulated by the serotonin transporter (SERT) expressed inepithelial cells. Importantly, peripheral 5-HT in the mother is a criticalregulatory factor in the development of fetal 5-HTergic neurons, andinflammation can cause fluctuations in placental-derived 5-HT, potentiallyinterfering with fetal neurodevelopment (Goeden et al., 2016).The 5-HT produced in thegastrointestinal tract may be supplied to the fetus through the placenta(Kliman et al., 2018). Additionally, considering the association between SERTfunction and ASD and ADHD in childhood, exploring lifestyle factors that affectSERT in intestinal epithelial cells is an important study in revealingpotential risk factors for fetal neurodevelopmental disorders.Coffee is the most consumedbeverage worldwide, and its associations with the risk of mortality fromvarious diseases follow J-shaped and U-shaped patterns. High coffee consumptionduring pregnancy may pose a risk for ADHD (Hvolgaard et al., 2017). Previousstudies have mainly focused on caffeine consumption and have not considered theimpact of various chemicals present in coffee. As evidenced by recent cohortstudies, decaffeinated coffee has been found to possess benefits similar tothose of regular coffee. Therefore, in this field as well, research focusing oncaffeine-independent biological pathways is crucial.Here, we report that prolongedexposure of intestinal epithelial cells to coffee, in a concentration-dependentmanner (coffee concentrations of 1%, 5%, and 10%), significantly reduced SERTuptake activity and mRNA expression.The experiments were conductedusing Caco-2 cells derived from colon adenocarcinoma. The uptake assaydemonstrated a concentration-dependent and significant decrease in 5-HT uptakemediated by SERT when exposed to regular coffee and decaffeinated coffee(exposure to 10% each type of coffee for 48 hours resulted in decrease to 51.1%and 63.1%, respectively). Furthermore, using real-time PCR, it was confirmedthat SERT mRNA levels significantly decreased in a similar pattern (exposure to10% each type of coffee for 48 hours resulted in decreases to 44.7% and 55.6%,respectively).The inhibition of SERTexpression was observed even in decaffeinated coffee, suggesting that caffeineis not involved in this phenomenon. Indeed, exposure to caffeine (0.03, 0.3,3mM) did not alter SERT mRNA levels. Additionally, well-known chemicals such aschlorogenic acid and trigonelline did not affect SERT uptake activity or mRNAexpression.

Disclosures: S. Kikkawa: None. **S. Imamura:** None. **K. Harada:** None. **S. Tanaka:** None. **I. Hide:** None. **N. Sakai:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.01/B56

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

Support: NIH grant R01GM128195

Title: Intracellular route of access for membrane to channel inhibition of NMDA receptors by channel blocking drugs

Authors: *E. G. NEUREITER, A. NIGAM, J. W. JOHNSON;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: N-methyl-D-aspartate receptors (NMDARs) are ligand-gated ion channels present at most excitatory synapses in the brain. They have been found to underlie processes such as long-term potentiation and have been implicated in a range of diseases including Alzheimer's disease, cell death following stroke, depression, and schizophrenia. NMDAR hyperactivity can be ameliorated using channel blocking drugs, including memantine, ketamine, and MK-801. NMDAR channel blocking drugs can either enter the NMDAR channel directly from the extracellular space (traditional channel block), or first enter the plasma membrane before transiting into the channel through a fenestration (membrane to channel inhibition, MCI). MCI has recently been described using extracellular application of channel blockers. Whether MCI can also occur following application of intracellular channel blocking drugs is unknown. However, intracellular application of MK-801 (iMK-801) has been used extensively as an experimental method to inhibit NMDARs on specific cells via an unknown mechanism. We hypothesize that NMDAR inhibition by iMK-801 is mediated by MCI. Further exploration of this process is necessary to ensure a more thorough comprehension of how pharmacological agents affect NMDARs, in addition to more completely understanding a common experimental method used for scientific discovery. We used whole-cell patch clamp electrophysiology in tsA201 cells transfected to express NMDARs to characterize inhibition by iMK-801 and developed a protocol to quantify NMDAR inhibition by intracellular drug. iMK-801 did not inhibit the peak NMDAR mediated current activated by the first glutamate application following initiation of whole-cell recording. This peak current can be observed due to the inability of MK-801 to enter the NMDAR channel before channel opening. Great care was taken to protect each cell from agonist prior to the start of an experiment to prevent channel opening and subsequent block by iMK-801 before the recording began. Thus, channel opening is required for inhibition by iMK-801, as is the case in traditional block and MCI. The current then decayed to a steady state, and the fractional inhibition by iMK-801 was measured and normalized to account for endogenous NMDAR desensitization. We also found a difference in iMK-801 affinity for NMDARs based on subtype, mirroring subtype dependence of MCI with extracellular MK-801 but not traditional block by MK-801. Additionally, both MCI and block induced by iMK-801 are relieved by depolarization and have kinetics that depend on drug concentration. These data support our hypothesis that intracellularly induced block occurs through MCI.

Disclosures: E.G. Neureiter: None. **A. Nigam:** None. **J.W. Johnson:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.02/B57

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

Support: NIH R01GM128195

Title: Investigation of NMDAR membrane to channel inhibition by extracellular (+)-MK-801

Authors: *A. NIGAM, E. G. NEUREITER, J. W. JOHNSON;
Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: N-methyl-D-aspartate receptor (NMDAR) antagonism is a research area with broad implications for treatment of pathological conditions such as Alzheimer's disease and epilepsy. NMDARs are highly calcium-permeable tetrameric glutamate receptors that are composed of two GluN1 subunits and two GluN2 (A-D) subunits and/or GluN3 (A-B) subunits. The extensively studied traditional channel block mechanism is exhibited by many compounds such as MK-801 and memantine, and occurs when charged blocker molecules from the extracellular solution enter the open channels of agonist-bound NMDARs, blocking ion flux. Our lab recently investigated another channel block mechanism known as membrane to channel inhibition (MCI). We showed that memantine MCI occurs when uncharged memantine molecules in the extracellular solution enter the membrane, and then transit from membrane to open channels of agonist-bound NMDARs through a fenestration, blocking ion flux at the deep site. Here, we investigate properties of MK-801 MCI. We found that the MK-801 MCI IC_{50}s for diheteromeric GluN1/2A (0.85 ± 0.04 μM) and GluN1/2B (0.67± 0.16 μM) receptors are ~60-fold greater than the IC_{50}s for traditional channel block of GluN1/2A (14.4 ± 1.4 nM) and GluN1/2B (13.4 ± 0.4 nM) receptors. In contrast, GluN1/2C and GluN2D receptors show much weaker MCI than GluN1/2A or GluN1/2B receptors: fractional current during MCI by 1 μM MK-801 was 0.97 ± 0.27 for GluN1/2C and 0.95 ± 0.02 for GluN1/2D receptors, suggesting the MK-801 MCI IC_{50}s for GluN1/2C and GluN1/2D receptors are far above 1 μM. Previous NMDAR structural modeling identified a putative path (fenestration) that memantine could use to transit from the membrane to the deep site when the channel is open. GluN2A(M630) appeared to be a path-lining residue that forms a constriction in the fenestration, an idea supported by our observation that mutating GluN2A(M630) to a tryptophan residue increased memantine MCI IC_{50}. To test the hypothesis that MK-801 uses the same fenestration as memantine, we compared MK-801 MCI of GluN1/2A and GluN1/2A(M630W) receptors. First, we compared traditional MK-801 IC_{50}s of GluN1/2A (14.4 ± 1.4 nM) and GluN1/2A(M630W) (10.5 ± 1.8 nM) and found no significant difference, indicating that the mutation does not modify MK-801 binding to the deep site. We then compared MK-801 MCI IC_{50}s of GluN1/2A (0.85 ± 0.04 μM) and GluN1/2A(M630W) (0.67 ± 0.002 μM) receptors, and to our surprise, found that the mutation did not increase MK-801 MCI IC_{50}. These results suggest MK-801 and memantine may use different fenestrations to transit from the membrane to the deep site during MCI.

Disclosures: A. Nigam: None. **E.G. Neureiter:** None. **J.W. Johnson:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.03/B58

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

Support: NIH Grant R01AG065594
NIH Grant T32N007433-21

Title: Characterizing intracellular calcium dependence of triheteromeric NMDA receptors

Authors: *Q. M. ERICKSON-OBERG[1], M. B. PHILLIPS[2], J. W. JOHNSON[1];
[1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Neurosci., NYU Langone, New York, NY

Abstract: *N*-methyl-D-aspartate receptors (NMDARs) are ionotropicglutamate receptors found at most excitatory synapses. Calcium(Ca^{2+}) influx through NMDARs is essential for development, learning,and memory. Aberrant NMDAR Ca^{2+} flux is linked to nervous systemdisorders, including Alzheimer's disease (AD). Better understanding of NMDARfunction and regulation will yield valuable insights into developing treatmentsfor disorders such as AD. Despite their prevalence and roles in critical brainprocesses, much remains unknown about NMDARs. Triheteromeric NMDARs (trihets)are of particular interest, as most NMDARs in the brain are thought to betrihets. Formed by two GluN1 subunits and two different GluN2 or GluN3 subunits(commonly GluN2A and GluN2B), trihets have proved difficult to study.Heterologous expression of GluN1/2A/2B trihets requires coexpression of GluN1,GluN2A, and GluN2B, creating 3 NMDAR subtypes simultaneously. While approaches for isolating trihets have beendeveloped, they require modification of the C-terminal domain, a domainessential for NMDAR regulatory mechanisms including calcium dependentdesensitization (CDD). CDD is activated by elevated intracellular $Ca^{2+}$and results in decreased NMDAR activity during long exposure to agonists. Wehave shown that CDD is enhanced by the AD drug memantine (Mem) and proposedthat as a result, Mem preferentially inhibits NMDAR populations implicated indisease. We are developing an approach for expressing trihets withunmodified C-terminal domains to electrophysiologically characterize trihet CDDand its role in Mem's mechanism of action. We coexpressGluN1, GluN2B, and mutant GluN2A(T690I) subunits in tsA201 cells. TheGluN2A(T690I) mutation is used because glutamate potency was reported to bemuch lower for GluN1/2A(T690I) diheteromeric NMDARs (dihets) than forGluN1/2A(T690I)/2B trihets, allowing minimization of current "contamination" byGluN1/2A(T690I) dihets. We found that glutamate activated GluN1/2A(T690I)dihets with an EC_{50} of 10.8 ± 0.36 mM (n = 5), a much lower EC_{50}than previously estimated. We therefore will also use MPX-004, whichpreferentially inhibits GluN1/2A dihets, to minimize and quantify contaminationby GluN1/2A(T690I) dihets. We use the GluN2B-specific antagonist CP-101,606 tominimize GluN1/2B dihet activation. We quantify contamination by GluN1/2B dihetcurrent by applying 10 mM Glu, which saturates GluN1/2B dihets withoutactivating trihets or GluN1/2A(T690I) dihets. Understanding CDD and its role inMem inhibition of trihets has broad implications for mechanisms of therapeutic drugaction.

Disclosures: Q.M. Erickson-Oberg: None. **M.B. Phillips:** None. **J.W. Johnson:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.04/B59

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

Title: N-methyl-D-aspartate receptors' proteostasis is modulated by autophagic flux

Authors: *T. M. BENSKE, Y.-J. WANG, T.-W. MU;
Physiol. and Biophysics, Case Western Reserve Univ., CLEVELAND, OH

Abstract: N-methyl-D-aspartate receptor (NMDAR) subunits are encoded by *GRIN* genes that are highly intolerant to genetic variation, as such, mutations are likely to result in various neurological disorders including epilepsies, intellectual disabilities, and neurodegenerative diseases. Variants within NMDARs result in protein misfolding, improper assembly, increased aggregation, defective trafficking, and impaired functionality on the cell surface. However, little is known about the proteostasis network that regulates the functional expression of NMDARs. This study utilizes HEK293T stably expressing heteromeric NMDARs composed of GluN1_GluN2B subunits to investigate the stability and turnover of NMDARs under physiological conditions and perturbations as a result of a variant, R519Q, in the ligand binding domain of the GluN2B subunit. We show that autophagy, rather than the proteasome, is majorly responsible for the degradation of GluN2B variants, as demonstrated by inhibition of either pathway (n=4). Further, immunofluorescence confocal imaging demonstrates significant colocalization of GluN2B_R519Q with the lysosome, formation of aggregates within the cell, and of particular interest, diminished surface expression of NMDARs (n>30). Interestingly, the GluN2B subunit is the only GluN subunit to contain a conserved LC3-interacting region (LIR) motif, found within its extended C-terminal tail. Mutagenesis was performed to change the critical phenylalanine residue, F1307, within the LIR domain, to an alanine in both GluN2B_WT and GluN2B_R519Q subunits, as the variant demonstrates more profound autophagic clearance. Further, siRNA was utilized to knockdown key autophagy proteins in order to determine which are involved in the clearance of NMDARs, and co-immunoprecipitation studies were performed to determine whether direct interactions with such proteins mediated autophagic clearance. Mechanistic studies were performed in order to determine whether the LIR domain played a role in NMDAR-dependent autophagy initiation, and if it was essential in the clearance of NMDARs. Future studies aim to utilize iPSC-derived neurons harboring the GluN2B_R519Q variant to investigate how autophagic flux regulates NMDARs in an endogenous system. Results from these studies in combination with the presented data, will provide great insight into the homeostasis of NMDARs. Indeed, these results present novel therapeutic targets for treatment of disorders in which NMDARs are dysregulated, including GRIN diseases, schizophrenia, and Alzheimer's disease.

Disclosures: **T.M. Benske:** None. **Y. Wang:** None. **T. Mu:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.05/B60

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

Support: DNRF grant 133

Title: Is the NMDA receptor targeted to synapses by liquid-liquid phase separation?

Authors: *E. C. PHEASANT[1,2,3], M. GOMEZ DE SALAZAR[1,2,3], X. L. WARNET[1,2,3], S. NABAVI[1,2,3], M. KJÆRGAARD[1,2,3];
[1]Dept. of Mol. Biol. and Genet., Aarhus Univ., Aarhus, Denmark; [2]Ctr. for Proteins in Memory - PROMEMO, Danish Natl. Res. Fndn., Aarhus, Denmark; [3]The Danish Res. Inst. for Translational Neurosci. (DANDRITE), Nordic EMBL Partnership for Mol. Med., Aarhus, Denmark

Abstract: The postsynaptic density (PSD) regulates synaptic signalling by recruiting and organising the synaptic signalling machinery. An important component of this machinery is the NMDA-type glutamate receptors, which are crucial for maintaining synaptic plasticity, through their induction of long-term potentiation (LTP) and long-term depression (LTD). However, while the trafficking and synaptic targeting of these receptors is known to be controlled by the NMDA receptor's disordered C-terminal tails; the underlying molecular mechanisms remain poorly understood.
We have found that the C-terminal tail of the NMDA receptor subunit GluN2B undergoes liquid-liquid phase separation (LLPS) in vitro when alone and when in complex with PSD-95, the most abundant scaffolding protein of the PSD. Furthermore, GluN2B's propensity to undergo LLPS is partly driven by the presence of aromatic residues within its C-terminal tail, allowing for a way to regulate the occurrence of LLPS experimentally. Additionally, we have identified a hitherto unmapped interaction between the GluN2B C-terminal tail and the GK domain of PSD-95. And we have found that this intermolecular multivalency is involved in driving the LLPS of the GluN2B:PSD-95 complex.
Interestingly, it has been proposed that liquid-liquid phase separation (LLPS) of PSD scaffolding proteins may be an underlying mechanism of PSD formation and synaptic clustering. Thus, to study the significance of the GluN2B:PSD-95 LLPS in vivo, we have designed GluN2B variants in which the tendency to undergo LLPS is either enhanced or disrupted. Subsequently, we are quantifying the synaptic targeting of GluN2B containing NMDA receptors by immunofluorescence microscopy of primary neuronal cultures.
In the end, our results will uncover whether LLPS is an underlying mechanism of synaptic targeting and clustering of NMDA receptors. Such molecular insights will bring us closer to understanding the mechanisms which function in maintaining the molecular architecture of the PSD.

Disclosures: E.C. Pheasant: None. **M. Gomez de Salazar:** None. **X.L. Warnet:** None. **S. Nabavi:** None. **M. Kjærgaard:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.06/B61

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

Support: NIH Grant R01NS123735

Title: Neuropeptide signaling negatively regulates NMDA receptor-mediated currents and behavior -- a possible mechanism for metaplasticity

Authors: *D. WANG, *D. WANG, J. E. MELLEM, D. MADSEN, A. V. MARICQ; Neurobio., Univ. of Utah, Salt Lake City, UT

Abstract: NMDA receptors (NMDARs) are meticulously regulated at synapses to ensure proper excitatory synaptic function and activity-dependent plasticity, yet the regulatory mechanisms remain elusive. In a forward genetic screen based on NMDAR-dependent locomotion behavior in *C. elegans*, we discovered that neuropeptide signaling negatively regulates NMDAR-mediated currents. *In vivo* patch-clamp records from the interneuron AVA reveal that NMDAR-mediated currents were approximately 3-fold increased in proprotein convertase gene *egl-3* mutants compared to wild-type. Significantly, NMDA-gated currents in transgenic worms in which native NMDARs were replaced by mammalian NMDARs (GluN1, GluN2B), were similarly increased when expressed in the *egl-3* mutants background, indicating conservation of the peptide signaling pathway with respect to regulation of NMDA-gated currents. EGL-3 is required for the processing of a large class (~100) of neuropeptides in *C. elegans*; thus, we next attempted to identify the relevant neuropeptide(s) that regulate NMDARs. From a screen of available neuropeptide (NP) and receptor (NPR) mutants, we identified a specific peptide and its receptor. Mutations in these genes phenocopied the increased current measured in *egl-3* mutants, and double mutants with *egl-3* did not show an additive effect. Because the time course of NMDA-gated currents was similar in mutants and wild-type, we hypothesized that the neuropeptide signaling pathway regulates the number of membrane NMDARs. Confocal imaging of fluorescently labeled surface NMDARs supported this hypothesis where the fluorescent signal is increased in neuropeptide signaling pathway mutants compared to control. Neuropeptides are signaling molecules released in an activity-dependent manner. Here, we discovered a novel and conserved mechanism that could allow dynamic regulation of NMDAR signaling. Since NMDARs play key roles in inducing plasticity, neuropeptides might have a role in metaplasticity, and provides a mechanism to ensure plasticity occurs to the right extent at the right time.

Disclosures: D. Wang: None. **D. Wang:** None. **J.E. Mellem:** None. **D. Madsen:** None. **A.V. Maricq:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.07/B62

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

Support: NS11619
HD082373
OD011132
the GRIN2B Foundation
Sage Therapeutics

Title: Potentiation of NMDA receptors by a neuroactive steroid rectifies circuit and behavioral deficits in a mouse harboring the loss-of-function GRIN2B variant p.E413G

Authors: *L. ZHANG[1], R. SONG[1], H. XING[1], T. G. BANKE[1], S. DIAZ[1], A. T. ALLEN[2], J. T. BECKLEY[3], A. J. ROBICHAUD[3], M. C. QUIRK[3], J. J. DOHERTY[3], S. L. GOURLEY[2], S. F. TRAYNELIS[1,4], H. YUAN[1,4];
[1]Dept. of Pharmacol. and Chem. Biol., [2]Dept. of Pediatrics, Emory Univ. Sch. of Med., Atlanta, GA; [3]Sage Therapeut. Inc, Cambridge, MA; [4]Ctr. for Functional Evaluation of Rare Variants (CFERV), Atlanta, GA

Abstract: NMDA receptors mediate a slow calcium permeable component of excitatory synaptic transmission, which is an important trigger for synaptic plasticity that is thought to underlie learning and memory. Genetic variation in the family of genes encoding NMDA receptor subunits has emerged as a significant underlying cause of multiple neurological disorders ranging from intractable epilepsy to intellectual disability. Among NMDA receptor genes, missense variants in the *GRIN2B* gene encoding the GluN2B subunit that produce a loss-of-function are often associated with developmental delay and intellectual disability. We developed a genetic knock-in mouse expressing the human missense variant p.Glu413Gly, which reduces glutamate potency by over 50-fold, accelerates the deactivation, and reduces receptor response amplitude. We have evaluated the behavioral features of the mouse harboring this variant, and also explored effects on circuit function. Voltage clamp recordings from slices harboring this variant show small, faster NMDA receptor-mediated EPSCs, and reduced level of LTP. Golgi staining from hippocampal CA1 pyramidal cells showed a reduced spine density. Mice harboring this variant show reduced habituation on the second day of two consecutive locomotor activity monitoring sessions, compared to wild type mice, which explore less on day two, presumably due to their memory of the previous day. We also found reduced time on an open arm of an elevated zero maze, consistent with an anxiety phenotype. We observed a deficit in pre-pulse inhibition in the mice harboring this variant.Treatment of hippocampal slices with 1 uM of the NMDA receptor potentiator SGE-550, a neuroactive steroid, increases NMDA receptor EPSC response amplitude and prolonged the duration, suggesting it might mitigate some of the effects of the p.Glu413Gly variant. SGE-550 also restored LTP to normal levels in knockin mice. We also tested the effects in vivo of 1 mg/kg SGE-550, and found that it rectified the lack of habituation on day two of consecutive locomotor activity trials. We also found that SGE-550 restored exploration of the open arm on the zero maze to near normal levels. These data suggest

NMDA receptor potentiation may be a viable approach to improve symptoms in patients with loss of function GRIN variants.

Disclosures: **L. Zhang:** None. **R. Song:** None. **H. Xing:** None. **T.G. Banke:** None. **S. Diaz:** None. **A.T. Allen:** None. **J.T. Beckley:** None. **A.J. Robichaud:** None. **M.C. Quirk:** None. **J.J. Doherty:** None. **S.L. Gourley:** None. **S.F. Traynelis:** Other; a member of the SAB for Eumentis Therapeutics, Sage Therapeutics, and Combined Brain, a member of the Medical Advisory Board for the GRIN2B Foundation and the CureGRIN Foundation, an advisor to GRIN Therapeutics, co-founder of NeurOp Inc. and AgriThera Inc., a member of the Board of Directors of NeurOp Inc. **H. Yuan:** Other; on a research grant from Sage Therapeutics to Emory University School of Medicine and received funding from the GRIN2B Foundation.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.08/B63

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

Support: NINDS IRP 1ZIANS002994-21

Title: Characterizing heterozygous GluN2B knockout mice as a model for human GRIN disorders

Authors: ***R. ZACHARIAS**[1], S. WON[1], M. VIEIRA[1], J. VON ENGELHARDT[2], K. ROCHE[1]; [1]NIH, Natl. Inst. of Neurolog. Disorders & Stroke (NINDS), Bethesda, MD; [2]Inst. of Pathophysiology, Univ. Med. Ctr. Johannes Gutenberg-University of Mainz, Duesbergweg 6, Germany

Abstract: N-methyl-D-aspartate (NMDA) receptors are heterotetramer ionotropic receptors consisting of two obligatory GluN1 subunits and two GluN2 (GluN2A-D) or GluN3 (GluN3A-B) subunits. NMDA receptors are involved in synaptic plasticity and long-term potentiation (LTP). Variants within these receptor subunits are associated with neurological disorders, such as autism-spectrum disorders (ASDs), epilepsy, and intellectual disability. *GRIN2B*, the gene encoding NMDAR subunit GluN2B, has been identified in SFARI as a high confidence ASD-associated gene. Previous reports show that when *GRIN2B* is deleted in mice, pups die shortly after birth due to an impairment of the suckling response. These data demonstrate the important role GluN2B plays in NMDA receptor function, as well as in the survival and development of mice. We are using biochemical approaches to characterize the heterozygous GluN2B knockout mouse as a model for studying GRIN disorders. We hypothesize mice lacking one WT copy of *GRIN2B* exhibit GluN2B haploinsufficiency, potentially resulting in a compensatory effect in the GluN1 and GluN2A subunits. To investigate this, we compared protein expression levels of NMDA subunits GluN1, GluN2A, and GluN2B in whole brain homogenate and postsynaptic density fractions of adult GluN2B heterozygous mice. We also assessed the expression levels of

scaffolding proteins, such as PSD-95. Our results show a significant decrease in GluN2B protein expression, but no significant compensatory changes in GluN1, GluN2A, or PSD-95 expression in adult whole brain. The results of this study will allow us to establish the GluN2B heterozygous mouse as a model for human GRIN disorders and set a foundation for future therapeutic research.

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Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.09/C1

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

Support: NICHD; ZIA-HD000711 to A.B.

Title: Pathway-specific differences in NMDA receptor function in adult PFC parvalbumin-positive inhibitory interneurons

Authors: *E. LEWIS[1], H. E. SPENCE[2], R. MURPHY-AVILA[2], D. VULLHORST[2], A. L. BUONANNO[2];
[1]NICHD/NIH, Bethesda, MD; [2]NICHD, NIH, Bethesda, MD

Abstract: Parvalbumin-positive (PV+) inhibitory interneurons are critical to prefrontal cortical (PFC) circuit function and their altered activity is implicated across multiple psychiatric disorders. Therefore, it is a priority to understand the mechanisms of synaptic excitation of PV+ interneurons within PFC. Among glutamate receptors, N-methyl-D-aspartate receptors (NMDARs) in PV+ interneurons have been of particular interest from a clinical perspective because of hypotheses that their dysfunction contributes to psychiatric disease, and that they are a therapeutic target of fast-acting antidepressants like ketamine. However, the significance of PV+ interneuron NMDARs to adult PFC circuit function has been controversial. We recently discovered that within adult PFC, relative NMDAR contribution to glutamatergic excitatory post-synaptic currents (EPSCs) in PV+ interneurons is pathway-specific varying based on synaptic input origin, and that NMDARs in PV+ interneurons support fundamental PFC circuit functions. Here we build on those discoveries by testing two mechanisms that may contribute to differences in NMDAR currents at synapses that arise from distinct anatomical locations. Using a combination of 3D reconstructions of PV+ interneurons and enhanced GFP reconstitution across synaptic partners (eGRASP) we can visualize distinct synaptic contacts from thalamus and contralateral PFC onto individual PV+ interneurons and quantify their subcellular distribution. In parallel experiments, we tested if subunit composition of NMDARs in PV+ interneurons differs based on the source of presynaptic input. Our preliminary analysis suggests that relative differences in NMDAR contribution to EPSCs across synaptic inputs to PV+

interneurons are more likely to be driven by differences in NMDAR composition than subcellular distribution of synaptic contacts on PV+ interneuron dendrites. Our findings indicate that NMDAR channel-blockers like ketamine and memantine that preferentially inhibit NMDARs containing specific subunits may be biased to more strongly inhibit glutamatergic excitation of PV+ interneurons coming from some upstream brain regions than others.

Disclosures: E. Lewis: None. **H.E. Spence:** None. **R. Murphy-Avila:** None. **D. Vullhorst:** None. **A.L. Buonanno:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.10/C2

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

Support: ERC Starting Grant #678250
NARSAD young investigator grant #27653

Title: Excitatory GluN1/GluN3A glycine receptors control interneuron function in cortical microcircuits

Authors: *D. DHANASOBHON, M. DE BRITO VAN VELZE, Y. ZERLAUT, N. REBOLA; Paris Brain Inst. (ICM), Paris, France

Abstract: N-methyl-D-aspartate receptors (NMDARs) are fundamental molecular components of excitatory synaptic transmission, and their activation is critical for brain development and function. The vast majority of the reported actions of NMDARs are mediated by the formation of tetrameric assemblies between mandatory glycine (or D-serine)-binding GluN1 subunits and glutamate-binding GluN2 subunits (GluN2A-D). This conventional view was challenged by the finding that GluN1 subunits could also co-assemble with GluN3 subunits to form atypical GluN1/GluN3 receptors gated uniquely by glycine. Recently, neurons in the juvenile hippocampus (Grand et al., 2018) as well as the adult medial habenula were reported to be equipped with excitatory glycine receptors (eGlyRs) and to modulate modulated animal behaviour (Otsu et al., 2021). In the neocortex, multiple transcriptomic studies reported elevated levels of the mRNA levels coding GluN3A subunits, specifically in somatostatin interneurons (SST-INs). Combining multi-disciplinary approaches, we have recently observed that GluN1/GluN3A receptors represent a previously unnoticed signalling mechanism in SST-INs (Bossi*, Dhanasobhon* et al., 2022). However, how these unusual receptors control neuronal function, particularly in cortical microcircuits, in the adult brain remains unclear.
In the neocortex SST-INs are known to be a highly heterogeneous neuronal population. Despite this diversity, we now observed that eGlyRs are consistently found in most SST-INs throughout all cortical layers and cortical regions, including prefrontal (PFC) and primary visual cortices (V1). In all tested cortical regions blocking GluN1/GluN3A receptor desensitization (with CGP

78608) reveals tonic currents. This finding suggests that glycine levels around SST-INs are sufficiently high to activate eGlyRs. Interestingly in V1, this eGlyR-mediated tonic currents are altered by sensory deprivation indicating that neuronal activity controls GluN1/GluN3A receptor function in an experience dependent manner. We are presently using in vivo two-photon calcium imaging to investigate how eGlyRs and extracellular glycine levels control the processing of visual information in V1. Overall, our results reveal unique molecular and functional properties of eGlyRs, opening new avenues on the diversity of NMDAR signaling and glycinergic neurotransmission in the neocortex.

Disclosures: D. Dhanasobhon: None. **M. de Brito Van Velze:** None. **Y. Zerlaut:** None. **N. Rebola:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.11/C3

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

Support: NINDS NS111619
NICHD HD082373
Emory University Pharmacology & Chemical Biology Innovation Catalyst Award 2021

Title: Glun2d-selective n-methyl-d-aspartate receptor modulators regulate excitatory postsynaptic currents in dorsal-lateral striatal cholinergic interneuron

Authors: *H. XING[1], N. S. AKINS[1], D. C. LIOTTA[1], H. YUAN[2], E. J. HESS[2], S. F. TRAYNELIS[3];
[1]Emory Univ., Atlanta, GA; [2]Emory Univ. Sch. Med., Emory Univ. Sch. Med., Atlanta, GA; [3]Emory Univ. Sch. of Med., Sch. of Med., Atlanta, GA

Abstract: Striatal cholinergic interneuron (ChI) has critical functions by releasing acetylcholine to regulate the activity of striatal afferents and efferents. The dysfunction of ChIs contributes to the movement disorder of Parkinson's disease and dystonia. ChIs activity could be modified via regulating certain subunit-containing N-methyl-D-aspartate receptors (NMDARs) because the GluN2D subunit appears to be expressed in ChI but not in other striatal neurons. To achieve a subunit-selective regulation of GluN2D-containing NMDARs on ChIs, we utilized positive and negative allosteric modulators [(+)1180-453 and NAB-14, respectively] that show strong selectivity for GluN2C/GluN2D over GluN2A/GluN2B. Our goal was to use these modulators to verify the functional expression of GluN2D-containing NMDARs on ChIs, as well as assess their contribution to ChI excitability. We sliced ChAT-eGFP mice (JAX007902) brain to locate cells with positive eGFP, that were identified as cholinergic interneuron from the striatum. Whole-cell patch clamp performed on dorsal-lateral area of striatum to assess ChI properties. We first

recorded under voltage clamp using a picospritzer to apply brief pulses of 1 mM NMDA plus 0.5 mM glycine onto the ChI to generate NMDAR-mediated currents. After 5 min of baseline recording, 10 µM (+)1180-453 or 10 µM NAB-14 were applied for 10 min, then the test drug supplemented with 400 µM DL-APV were applied for 5-8 min to confirm the currents arose from NMDARs. Same protocol were used for current clamp recordings. We found that NAB-14 significantly reduced NMDAR-mediated current amplitude and area in response to pressure-applied NMDA by 31 ± 6.5% ($p = 0.0020$) and 31 ± 6.9% ($p = 0.026$), respectively ($n = 10$). Application of (+)1180-453 increased the half-height width and area of responses by 216 ± 48% ($p = 0.047$) and 122 ± 41% ($p = 0.0089$), respectively (mean ± S.E.M., $n = 10$, paired student's t-test). Neither (+)1180-453 ($n = 9$) nor NAB-14 ($n = 11$) affects the intrinsic membrane properties, suggesting GluN2D-containing NMDARs are not tonically activated at rest condition by low levels of ambient glutamate. When we used picospritzer to apply NMDA onto ChIs under current clamp, we found (+)1180-453 potentiated the firing activity of ChI with prolonged firing duration ($p = 0.019$), increased AP spike count ($p = 0.011$), and lowered depolarization-induced block ($p = 0.034$). These changes were all reversed by co-application of NAB-14 ($n = 10$ cells, repeated measures one-way ANOVA). These findings reveal that ChIs expresses functional GluN2D-containing NMDARs and their excitability can be tuned by GluN2D-selective modulators, which may have therapeutic implications.

Disclosures: **H. Xing:** None. **N.S. Akins:** None. **D.C. Liotta:** Other; member of the Board of Directors for NeurOp Inc., coinventors on Emory-owned Intellectual Property that includes positive allosteric modulators of NMDA receptor function. **H. Yuan:** Other; PI on a research grant from Sage Therapeutics to Emory, co-inventors on Emory-owned Intellectual Property that includes positive allosteric modulators of NMDA receptor function. **E.J. Hess:** Other; member of the Medical and Scientific Advisory Board of the Dystonia Medical Research Foundation and reports research funding from the National Institutes of Health, United States Department of Defense. **S.F. Traynelis:** Other; member of the SAB for Eumentis Therapeutics, Sage Therapeutics, and Combined Brain; member of the Medical Advisory Board for the GRIN2B Foundation and the CureGRIN Foundation; advisor to GRIN Therapeu.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.12/C4

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

Support: Czech Science Foundation 20-12420S
project nr. LX22NPO5107 (MEYS): Financed by EU – Next Generation EU
GAUK 306221

Title: The pathogenic variant GluN1-N650K regulates the surface number and pharmacological properties of NMDA receptors

Authors: *M. HORAK, M. KOLCHEVA, M. LADISLAV, J. NETOLICKY, S. KORTUS, P. BARACKOVA, B. HRCKA KRAUSOVA, A. MISIACHNA;
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Abstract: *N*-methyl-*D*-aspartate receptors (NMDARs) are essential in excitatory synaptic transmission and synaptic plasticity. Conventional NMDARs contain two GluN1 subunits and two GluN2 subunits; in contrast, non-conventional NMDARs also contain GluN3 subunit(s). Here, we characterized the properties of various NMDAR subtypes containing GluN1 subunits with the pathogenic N650K variant associated with seizures and developmental delay. Our microscopy showed that the GluN1-N650K subunit increases the surface expression of GluN1/GluN2A and GluN1/GluN2B receptors but not GluN1/GluN3A receptors. Using electrophysiology, we found that the GluN1-N650K variant increases the sensitivity of the GluN1/GluN2A receptor to its (co-)agonists but decreases its conductance and open probability. Furthermore, the GluN1-N650K subunit does not form functional GluN1/GluN2B receptors but forms functional GluN1/GluN3A receptors. In the presence of extracellular Mg^{2+}, GluN1-N650K/GluN2A receptors exhibit an unaltered or increased response to ketamine and memantine, respectively, while both drugs have a slower onset and offset kinetics compared to WT GluN1/GluN2A receptors. Finally, memantine shows promising neuroprotective effects in neurons expressing the GluN1-N650K variant. Our study provides the pharmacological in vitro characterization of the NMDARs containing the GluN1-N650K variant.

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Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.13/C5

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

Support: NIH Grant NS113991
NIH Grant NS128543

Title: Identifying the function of the NMDA receptor splice variant $NR1^{C2}$via its interaction with Magi-2 in inflammatory pain

Authors: *G. SHEEHAN[1], A. ROSZCZYK[2], A. BHATTACHARJEE[3];
[1]SUNY at Buffalo, Buffalo, NY; [2]Natl. Inst. of Diabetes and Digestive and Kidney Dise, Bethesda, MD; [3]Pharmacol. and Toxicology, SUNY-Buffalo, Buffalo, NY

Abstract: The spinal cord dorsal horn (SCDH) represents the first site of central integration of somatosensory information. Primary afferent neurons, whose cell bodies reside in the dorsal root ganglion, transduce diverse sensory inputs which are conveyed to the CNS via synaptic

connections in the SCDH. These primary afferent neurons, which are classified by sensory modality, terminate in an organized laminar manner within the SCDH. In the Complete Freunds' Adjuvant (CFA) model of chronic inflammatory pain, nociceptive C-fibers, which terminate in the most superficial lamina, become sensitized resulting in thermal hyperalgesia. We found the WW-domain containing synaptic scaffolding molecule Magi-2 to be expressed in the most superficial lamina and utilized a Magi-2 deficient mouse line to determine that Magi-2 deficiency was associated with a loss of the obligate NMDA receptor subunit NR1. Viral knockdown of Magi-2 led to an attenuation of the CFA-induced thermal hyperalgesia following intrathecal administration of AAV2/9-m-Magi-2-shRNA into the lumbar SCDH. In turn, we also found that CFA upregulated the levels of NR1 in the SCDH ipsilateral to the injected hindpaw and that this increase was blocked by Magi-2 shRNA. Eight splice variants of NR1 have been identified. Two of these, C2 and C2' differ with respect to their most distal carboxy-terminal sequence. The C2' variant was previously shown to contain a PDZ binding motif that can bind to PSD-95 (PMID: 11163274) and was recently shown to be the more abundantly expressed isoform in the brain and spinal cord (PMID 36913980). The function of the C2 variant, which exhibits activity dependent expression (PMID 11740502), has remained elusive. Sequence analysis of the C2 variant revealed a previously unidentified Group 3 WW-binding domain. WW domains are typically used by ubiquitin ligases to recognize substrates. We found the C2 variant was susceptible to Nedd4-1 dependent protein degradation *in vitro* whereas the C2' variant was not. Mutation of this WW-binding domain blocked the ability of Nedd4-1 to promote the loss of $NR1^{C2}$ protein. Analysis of NR1 levels following CFA administration revealed a C2-specific increase which was abolished by viral knockdown of Magi-2. C2' levels were unaffected by either CFA or Magi-2 knockdown. Based on these results we hypothesize that the $NR1^{C2}$ isoform is a labile form of NMDA receptors. It confers susceptibility to ubiquitin ligases and represents a novel plasticity mechanism whereby NMDA receptor levels can be modulated in response to synaptic activity.

Disclosures: G. Sheehan: None. **A. Roszczyk:** None. **A. Bhattacharjee:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.14/C6

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

Support: Santa Casa da Misericórdia (MB-7-2018
ANR 257341
Fundação para a Ciência e a Tecnologia

Title: Age-dependent NMDA receptor function is regulated by the amyloid precursor protein

Authors: J. RAJÃO-SARAIVA[1], A. RIBERA[2], J. DUNOT[2], J. E. COELHO[3], H. MARIE[2], P. POUSINHA[2], ***L. V. LOPES**[3];

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Abstract: N-methyl-D-aspartate receptors (NMDARs) are critical for the maturation and plasticity of glutamatergic synapses. In the hippocampus, NMDARs mainly contain GluN2A and/or GluN2B regulatory subunits. The amyloid precursor protein (APP) has emerged as a putative regulator of NMDARs, but the impact of this interaction to their function is largely unknown. By combining patch-clamp electrophysiology and molecular approaches, we unravel a dual mechanism by which APP controls GluN2B-NMDARs, depending on the life stage. We show that APP is highly abundant specifically at the postnatal postsynapse. It interacts with GluN2B-NMDARs, controlling its synaptic content and mediated currents, both in infant mice and primary neuronal cultures. Upon aging, the APP amyloidogenic-derived C-terminal fragments, rather than APP full-length, contribute to aberrant GluN2B-NMDAR currents. Accordingly, we found that the APP processing is increased upon aging, both in mice and human brain. Interfering with stability or production of the APP intracellular domain normalized the GluN2B-NMDARs currents. While the first mechanism might be essential for synaptic maturation during development, the latter could contribute to age-related synaptic impairments.

Disclosures: J. Rajão-Saraiva: None. **A. Ribera:** None. **J. Dunot:** None. **J.E. Coelho:** None. **H. Marie:** None. **P. Pousinha:** None. **L.V. Lopes:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.15/C7

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

Support: NIH grant EY031411
NIH grant T32 GM141013

Title: Nmda receptor transmission differentially shapes the light response properties of distinct retinal ganglion cell subtypes in the mammalian retina

Authors: *A. SCHULTZ[1,2], M. HOON[3,1,4], R. SINHA[1,3,4];
[1]Neurosci., [2]Mol. & Cell. Pharmacol. Training Program, [3]Ophthalmology and Visual Sci., [4]McPherson Eye Res. Inst., Univ. of Wisconsin, Madison, Madison, WI

Abstract: Encoding of visual stimuli in the retina is performed by functionally distinct classes of retinal ganglion cells (RGC). RGCs receive glutamatergic input from bipolar cells via AMPA and NMDA receptors, yet little is known about how NMDA receptors contribute to function. In ON RGCs (which depolarize in response to light increments), NMDA receptors are *perisynaptic*—their activation is limited by synaptic mechanisms that curtail glutamate spillover, such as presynaptic inhibition and glutamate reuptake. Conversely, in OFF RGCs (which

hyperpolarize with light increments), NMDA receptors intermingle with AMPA receptors at synaptic sites, suggesting their contribution to synaptic transmission is broader. However, to what extent NMDA receptors differentially shape the light response properties of ON and OFF RGC subtypes has not been extensively investigated. To evaluate how NMDA receptor transmission shapes the light response properties of functionally distinct RGCs, we leveraged extracellular and whole-cell patch clamp electrophysiology in current clamp configuration to measure spikes in mouse alpha RGCs subjected to contrast-varying light stimulation. Alpha RGCs are classified based on their light-evoked response profiles: ON versus OFF and transient versus sustained (i.e., short-lived spike bursts at stimulus onset or prolonged spike trains that last the duration of a stimulus). To assess contrast-evoked NMDA receptor transmission, we applied either a selective NMDA receptor antagonist - AP5 - in bath solution or a use-dependent NMDA receptor pore blocker, MK-801, delivered intracellularly via the patch pipette. In ON-sustained RGCs, both AP5 and MK-801 diminish spike responses to low contrasts. Surprisingly, both drugs also enhance firing at high contrasts, suggesting that NMDA receptors may constitute an important adaptation mechanism in this cell type. In OFF-transient RGCs, spike responses to the entire range of negative contrasts are impaired by intracellular MK-801 and AP5, substantiating the presence of postsynaptic NMDA receptors that are broadly recruited for contrast encoding. Finally, in OFF-sustained RGCs, while AP5 attenuates spike output in response to light decrements, MK-801 does not, suggesting that only presynaptic NMDA receptors are important for contrast tuning. Together, our studies reveal that, amongst different functional classes of RGCs, pre- and postsynaptic NMDA receptors shape different aspects of visual processing in the mammalian retina.

Disclosures: **A. Schultz:** None. **M. Hoon:** None. **R. Sinha:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.16/C8

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

Support: NIH Grant 2R01NS089435 (MRN and SK)

Title: Alterations in glutamatergic signaling in rat prefrontal cortex underlie spatial learning and memory impairment following infusion of HIV-1 Tat protein

Authors: *B. C. DUFFY[1,3], K. M. KING[2], M. R. NONNEMACHER[1,3,4], S. KORTAGERE[1,3]; [1]Microbiology & Immunol., [2]Drexel Univ. Col. of Med., Philadelphia, PA; [3]Ctr. for Mol. Virology and Translational Neuroscience, Inst. for Mol. Med. and Infectious Disease, Drexel Univ. Col. of Med., Philadelphia, PA; [4]Sidney Kimmel Cancer Center, Thomas Jefferson Univ., Philadelphia, PA

Abstract: HIV-1-associated neurocognitive disorders (HAND) affect up to fifty percent of people living with HIV (PLWH). HAND is marked by impairment in at least two domains of executive function and varies in severity - from asymptomatic, to mild impairment and dementia. Mild forms of HAND are predominant due to the consistent use of combination anti-retroviral therapies. It is critical to identify clinically relevant mechanisms of HIV-1 toxicity that drive this mild neurocognitive disorder. The HIV-1 transactivator of transcription (Tat) protein is found in the cerebrospinal fluid of patients adherent to anti-retroviral therapy and Tat-based models recapitulate the cognitive symptoms seen in PLWH experiencing HAND. Prior studies have demonstrated that Tat interacts directly with the N-methyl-D-aspartate receptor (NMDAR) to potentiate receptor signaling, thus enhancing glutamatergic transmission. To identify changes in regional glutamatergic circuitry underlying defects in cognitive function, we infused recombinant Tat or saline to the medial prefrontal cortex (mPFC) of male Sprague-Dawley rats. Rats were then assessed in the novel object recognition, spatial object recognition and temporal order tasks at 1 and 2 post-operative weeks. Following completion of behavioral testing, mPFC tissue was collected and analyzed by RT-PCR. Results from the study showed Tat (40ng) infused in mPFC induced impairment in the spatial object recognition task while sparing performance in novel object recognition and temporal order tasks. These behavioral alterations coincide with upregulation of Grin1 and Grin2a transcripts in mPFC of rats infused with recombinant Tat (n=14, 86-amino acid isoform), when compared to saline-infused rats (n=12). The detected impairment of spatial learning and memory, combined with upregulation of Grin1 and Grin2a, suggest that exposure to Tat protein drives local adaptation in cells of the mPFC, potentially altering the function of mPFC-hippocampus circuitry. To further understand the mechanism of Tat mediated toxicity in mPFC, RNA-seq will be pursued using the clinically relevant isoform of Tat (101aa, 40ng).

Disclosures: B.C. Duffy: None. **K.M. King:** None. **M.R. Nonnemacher:** None. **S. Kortagere:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.17/C9

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

Title: Mechanism and target engagement of SAGE-718, an NMDA receptor positive allosteric modulator

Authors: *T. K. AMAN, J. T. BECKLEY, M. C. LEWIS, A. C. SMITH, B. J. FARLEY, C. M. JOHNSON, T. M. KAZDOBA, M. A. ACKLEY, A. J. ROBICHAUD, J. J. DOHERTY, M. C. QUIRK;
Sage Therapeut., Cambridge, MA

Abstract: SAGE-718 and SGE-550 are positive allosteric modulators (PAMs) of N-methyl-D-aspartate receptors (NMDAR) with similar pharmacology as 24(S)-hydroxycholesterol (24(S)-HC). SAGE-718 is in development for the treatment of cognitive impairment in neurodegenerative diseases. Here we describe NMDAR modulation by SAGE-718 and SGE-550 and demonstrate target engagement of SAGE-718 using a variety of in vitro and in vivo electrophysiology platforms. SAGE-718 and SGE-550 potentiated all GluN2 diheteromeric receptors (20-500 nM potency; 86-370% efficacy across 2A-2D). Both compounds also potentiated NMDAR responses in medium spiny neurons in rat brain slices (>300% potentiation with either SAGE-718 [n=9] or SGE-550 [n=9]). SGE-550 increased neuronal output (n=6), as measured by increased spike rate, burst frequency, and burst duration in multielectrode array (MEA) recordings from cultured iPSC-derived neurons. Similarly, SAGE-718 (n=9) dose-dependently increased spike rate and burst duration compared to vehicle (n=8) in an MEA study with cultured rat cortical neurons. Next, to demonstrate target engagement, we investigated the in vitro and in vivo interaction with the NMDAR open channel blocker, ketamine. Because other NMDAR PAMs, including 24(S)-HC, have been shown to increase NMDAR open probability, we hypothesized that SGE-550 and SAGE-718 also increase open probability and accelerate the rate of ketamine block and unblock, since ketamine binding requires open channels. We confirmed that SGE-550 increases the open probability of GluN2B-containing receptors (10 µM, 20% increase over baseline, N=10). Furthermore, 10 µM SAGE-718 and SGE-550 significantly accelerated the time constant of unblock by 0.6 µM ketamine on GluN2A NMDAR currents (6.0±1.4 ms vehicle; 2.1±0.6 ms SGE-550; 1.6±0.4 ms SAGE-718). We next evaluated whether SAGE-718 (1-10 mg/kg) alters the effects of ketamine (15 mg/kg) on gamma frequency activity in awake behaving rats using electroencephalography (EEG). As expected, ketamine increased gamma power, and importantly, SAGE-718 significantly decreased the time to return to baseline levels (n=12-13 per group), providing evidence that SAGE-718 modulates NMDAR activity in vivo. Together, these data indicate SAGE-718 can potentiate both in vitro and in vivo NMDAR-mediated currents and has potential to treat indications with NMDAR hypofunction. Further, the interaction of SAGE-718 with ketamine not only provides mechanistic insight into how SAGE-718 can potentiate NMDAR but also functions as a clinical translational biomarker.

Disclosures: **T.K. Aman:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **J.T. Beckley:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **M.C. Lewis:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **A.C. Smith:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **B.J. Farley:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **C.M. Johnson:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **T.M. Kazdoba:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **M.A. Ackley:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **A.J. Robichaud:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **M.C. Quirk:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.18/C10

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

Title: Novel, brain penetrant, GluN2A selective antagonists reveal a structural basis for negative allosteric modulation of GluN2ARs

Authors: D. DUDA[1], S. SIMAVORIAN[2], B. LORD[2], N. KARPOWICH[3], R. NARLAWAR[4], P. BONAVENTURE[2], F. BISCHOFF[4], ***R. NEFF**[2];
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Abstract: NMDARs are a fundamental mediator of excitatory neurotransmission in the CNS, and NMDAR dysfunction is well known to underlie the pathology of a broad array of CNS diseases. Selective modulators of NMDAR subtypes are powerful tools that facilitate understanding of NMDAR subtype biology. Here, we present the discovery of novel, modulators of the GluN2A subtype. Like previously identified GluN2A selective antagonists, these molecules bind to the GluN1/2A LBD dimer interface through common interactions and function as negative allosteric modulators (NAMs) of glycine binding. These molecules are distinct from existing GluN2A selective compounds in that they bind to the GluN1/2A interface in a novel manner. In addition, these molecules are brain penetrant, and achieve GluN2A engagement in the CNS after subcutaneous dosing. Lastly, we show that these molecules reveal a structural basis for GluN2A NAM activity, where binding of these NAMs stabilized the open conformation of the GluN1 LBD domain and prevented glycine binding**.**

Disclosures: **D. Duda:** A. Employment/Salary (full or part-time):; Seed Therapeutics. **S. Simavorian:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **B. Lord:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **N. Karpowich:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **R. Narlawar:** A.

Employment/Salary (full or part-time):; Janssen R&D, LLC. **P. Bonaventure:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **F. Bischoff:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **R. Neff:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.19/C11

Topic: B.04. Synaptic Transmission

Support: NIH Grant F31MH133285
NIH Grant 5T32MH115886
NIH Grant 5P50MH119569
University of Minnesota Undergraduate Career Opportunities in Neuroscience Grant

Title: Functional, structural, and computational consequences of NMDA receptor ablation at medial prefrontal cortex synapses

Authors: *R. M. DICK[1], H. AHMED[2], A. J. SEDERBERG[3], P. E. ROTHWELL[2];
[1]Grad. Program In Neurosci., [2]Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN; [3]Dept. of Psychology, Georgia Inst. of Technol., Atlanta, GA

Abstract: Decreased expression and function of N-methyl-D-aspartate (NMDA) receptors in the prefrontal cortex (PFC) is a notable biomarker of schizophrenia that may contribute to cognitive symptoms in patients. NMDA receptors play an important role in working memory function by mediating slow excitation in neurons, thus enabling the reverberant neural activity that allows information to be retained in working memory. Previous studies in humans and nonhuman primates have demonstrated reduced neural synchrony and increased working memory errors following acute and systemic NMDA receptor blockade. Over time, NMDA receptor hypofunction is thought to cause activity-dependent disconnection, a process by which synapses become progressively weakened due to loss of coordinated input timing. However, it is unknown how synaptic function and architecture change following chronic and cell type-specific NMDA receptor deletion from excitatory neurons in the PFC, which may be particularly vulnerable to disconnection. My preliminary computational model has demonstrated that decreasing the strength versus density of synapses produces different effects upon working memory. In this firing rate network, synaptic weakening reduces memory capacity, while synaptic loss decreases memory stability. It is therefore important to understand whether one or both cellular processes occur following NMDA receptor ablation in order to better understand disease pathophysiology and subsequently develop improved treatments. These two phenomena can be parsed using ex vivo slice electrophysiology and confocal imaging of dendritic spines, which are the primary sites of excitatory synaptic contact. I have successfully ablated NMDA receptors at excitatory

PFC synapses using clustered regularly interspaced short palindromic repeat (CRISPR) genome editing technology in transgenic mice. Infected pyramidal cells have a significant reduction in NMDA receptor-mediated current (two-tailed t test, p=0.022) in mice 6 weeks after surgery. We are currently measuring the amplitude and frequency of miniature excitatory postsynaptic currents, which reflect spontaneous neurotransmission and provide information about synapse strength and density respectively. These functional measurements are being integrated with morphological analysis of dendritic spines in the same neurons, and both datasets will be incorporated into a spiking network model that creates and tests predictions regarding brain activity stability and synchrony.

Disclosures: R.M. Dick: None. **H. Ahmed:** None. **A.J. Sederberg:** None. **P.E. Rothwell:** None.

Poster

PSTR186. NMDA Receptors: Pharmacology, Circuit Specificity, and Disease Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.20/C12

Topic: B.04. Synaptic Transmission

Support: 1ZIANS002994-21

Title: Rare Variants Implicate NMDA Receptor Signaling and Trafficking

Authors: *H. RYU;
NIH, Rockville, MD

Abstract: Rare Variants Implicate NMDA Receptor Signaling And Trafficking

Hye Young Ryu[1 *], Saerom Lee[1], Marta Mota Vieira[1], Shuxi Liu[2] Katherine W Roche[3]
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Rare variants in synaptic proteins are implicated in a range of neurological disorders,including autism spectrum disorders (ASDs), schizophrenia (SCZ) and epilepsy. One of the recurrently affected gene, which encodes for GluN2B, a subunit of N-methyl-D-aspartate receptors (NMDARs). Functional studies of rare variants associated with neurodevelopmental disorders have found that both hyper- and hypofunction of NMDARs have damaging effects that potentially contribute to disease etiology, and that both *GRIN2A* and *GRIN2B* are highly intolerant to variation. We have focused on variants residing in the C-terminal domain (CTD) of these subunits, which is a finely modulated region of NMDARs important for mechanisms of trafficking of NMDARs, anchoring at synaptic sites, and establishment of protein-protein interactions. We investigated the effect of an extended variants found in the CTD of GluN2B

identified, which were identified in a patient with ID and epilepsy. We determined that GluN2B-1485CextX1 and 1485CextX7 have decreased surface expression in hippocampal neurons. We also observed that the, GluN2B-1485CextX1 and 1485extX7 resulted in trafficking defects and receptor loss-of-function. Both variants led to impair their binding to synaptic partners such as SAP102 and PSD-95. Overall, our study underscores the significance of understanding the functional implications of NMDAR rare variants identified in neurological disorder patients to better understand disease etiology.

Disclosures: H. Ryu: None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.01

Topic: B.05. Synaptic Plasticity

Support: The General Insurance Association of Japan Medical Research Grants

Title: Ex vivo gene therapy using human-induced pluripotent stem cell-derived neural stem/progenitor cells to deliver synaptic organizer CPTX for spinal cord injury

Authors: *Y. SAIJO[1], *Y. SAIJO[1], N. NAGOSHI[1], Y. SUEMATSU[1], M. NAKAMURA[1], H. OKANO[2];
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Abstract: *Introduction*Although our laboratory has reported efficacy of human iPS-NS/PCs transplantation for spinal cord injury (SCI), the functional recovery is not fully achieved yet. Recent studies using chemogenetic stimulation on graft cells have shown that synaptic connections between the graft and host cells significantly influence lower limb movements. To enhance the result, we planned ex vivo gene therapy by inducing synapse organizer protein CPTX, which is an artificial synaptic organizer protein containing the domains of Cbln1 and NP-1. The aim of this study is to examine whether ex vivo gene therapy using CPTX contributes to further improvement of lower limb function compared to conventional transplantation therapy. *Method* We designed the Lenti viral vector to express the CPTX protein under the CAG promoter. A contusive SCI was induced at the thoracic vertebral level of the immunodeficient rat. Cell transplantation was performed in subacute phase. The lentivirus was transfected in hiPS-NS/PCs 5 days before transplantation. The follow-up period was set for 13 weeks after transplantation, during which we evaluated the motor function by the Basso, Beattie and Bresnahan (BBB) score and Treadmill gait analysis (Digigait system). Motor evoked potentials and Allodynia assessment were performed at the endpoint. *Results* CPTX-expressing cells were identified in various types of graft neural cells. CPTX protein was localized around the epicenter, with no observed migration to the brain or serum. There was no increase in immature cells for the observed period. The histological analysis presented an increase in synaptic-related proteins

in the CPTX group. Furthermore, synaptogenesis was found to be increased around the transplant site. Tracing experiments using Glycoprotein gene-deficient rabies virus were performed to confirm the connection with higher-level neurons from the transplant site. The CPTX group also showed increased synaptic connections to the superior descending neuronal tract. The CPTX group achieved higher BBB scores compared to the control group and significant improvements in their paw angle. Electrophysiological assessment using motor-evoked potential showed a significant increase in amplitude. They did not show significant differences compared to the control group in the evaluation of allodynia. *Conclusions* Expression of CPTX protein from hiPS-NS/PCs promoted synapse formation and maturation, which reached the recovery of motor function and neuronal conduction. No adverse effects such as tumorigenesis or allodynia were observed. Thus, this gene therapy is expected to be a useful therapeutic strategy for clinical applications

Disclosures: **Y. Saijo:** None. **Y. Saijo:** None. **N. Nagoshi:** None. **Y. Suematsu:** None. **M. Nakamura:** None. **H. Okano:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.02/C13

Topic: B.05. Synaptic Plasticity

Support: ZIAMH002881

Title: Disrupting caspase-3 mediated LTD and autophagy alters CA1 dendritic spine properties

Authors: *K. M. KEARY, III[1,2], E. SOJKA[3], M. GONZALEZ[3], Z. LI[2];
[1]Brown Univ. NIH Grad. Partnership Program, Bethesda, MD; [2]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Colgate Univ., Hamilton, NY

Abstract: Synaptic plasticity is a cellular substrate for learning and memory in the brain, a key component of which involves alterations to dendritic spines, structures serving as the post-synaptic component of the synapse. Changes to dendritic spines can be classified as structural plasticity. This form of plasticity is considerably linked to functional plasticity of synapses, processes through which the functional output of a neuron changes such as long-term potentiation, long-term depression (LTD), and homeostatic scaling. New spine formation and elimination, as well as the enlargement and shrinking of spines, is often observed following functional synaptic plasticity induction. While previous studies have identified some important mechanisms of structural plasticity such as autophagy, a cellular protein degradation mechanism, the complement cascade, and microglia pruning, it is still unclear how structural plasticity is controlled at different segments of the same dendrite which often receive inputs from diverse neural circuits. Previous work from our lab has shown clear differences in both autophagosome localization and LTD inducibility at the distinct circuits of the CA1 apical dendrite. As these

processes are key components of structural plasticity, it raises the intriguing question of if structural plasticity could be differentially regulated at the proximal and distal dendrites in CA1. To address this question, we analyzed the volume, density, and subtype proportions of spines at the CA1 proximal and distal apical dendrites in THY1-eYFP mice. In order to probe the role of LTD and autophagy in structural plasticity, we crossed the THY1-eYFP line with caspase-3 and ATG5 knockouts previously shown to have impaired LTD and autophagy respectively. While we do not observe any major differences between proximal and distal dendrites, our study shows that both caspase-3 and ATG5 knockout mice have larger spines than wild-type mice, with ATG5 knockout mice having a reduced spine density. The larger spine volume was present in each subtype, with significant alterations to the proportion of the subtypes themselves as well. Both caspase-3 and ATG5 knockout mice have a significantly smaller proportion of thin, and significantly higher proportions of stubby and mushroom spines than wild-type mice. These dendritic spine phenotypes support the interplay between long-term depression, autophagy, and structural plasticity. Future works will revolve around elucidating mechanisms mediating these changes, as well as investigating dynamic synaptic turnover within the two dendritic compartments.

Disclosures: **K.M. Keary:** None. **E. Sojka:** None. **M. Gonzalez:** None. **Z. Li:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.03/Web Only

Topic: B.05. Synaptic Plasticity

Support: KAKENHI Grant Number JP22K06428

Title: Interaction of postsynaptic density (PSD)/PSD lattice with microtubules observed by electron microscopy.

Authors: *T. SUZUKI[1], T. FUJII[2], K. KAMETANI[3], K. TABUCHI[4];
[1]Mol. Cell Physiol, Shinshu Univ., Matsumoto, Japan; [2]Fac. Textile Sci. & Tech., Shinshu Univ., Ueda, Japan; [3]Vet. Anat., Vet. Medicine, Rakuno Gakuen Univ., Ebetsu, Japan; [4]Biol Sci. for Intractable Neurolog. Dis., Inst. Biomed Sci, Interdisciplinary Cluster for Cutting Edge Res. Shinshu Univ., Matsumoto, Japan

Abstract: [Background] In a previous paper, we purified and examined a PSD lattice (PSDL) structure, which is an essential skeletal structure for PSD, and found that non-microtubule tubulin was a major component of purified PSDL (Suzuki et al. 2018, 2021). We also reported the distribution of tubulin in and around the synaptic regions in mouse brain by immunoelectron microscopy (Suzuki et al., 2021). Our previous studies suggest a fundamental role for tubulin in the structure of the PSD at excitatory synapses. On the other hands, little is known about the roles for tubulin and microtubules in the postsynaptic region, while periodic and transient

invasion of microtubules from dendrites to spines during expression of synaptic plasticity (Gu et al., 2008; Hu et al., 2008; Mitsuyama et al., 2008) suggest a role for microtubules in in the regulation of spine morphology and growth and development of synaptic plasticity. In this paper we investigated interaction between PSD and microtubule using in vitro system to make clear the linkage between tubulin/microtubule and PSD. **[Method]** We investigated the interaction of PSD and PSDL with polymerizing tubulin at the electron microscopic level. Microtubule formation was induced either in the presence or absence (control) of PSD or PSDL in vitro. Changes in the specimen were investigated by negative staining EM. **[Results]** Association between PSD/PSDL and polymerizing microtubules was observed. The PSDLs were in contact with both ends of microtubules. **[Conclusion & Discussion]** This study demonstrated interaction of PSD/PSDL with polymerizing tubulin in vitro and suggests their interaction in vivo when microtubule transiently enter into dendritic spines during expression of synaptic plasticity. This study suggests that the PSD/PSDL may trap transiently invading microtubules.

Disclosures: **T. Suzuki:** None. **T. Fujii:** None. **K. Kametani:** None. **K. Tabuchi:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.04/C14

Topic: B.05. Synaptic Plasticity

Support: R37DA015014
R01DA032444
R21DA056309

Title: The chemokine CXCL12 tunes cortical dendritic spine dynamics

Authors: *C. HO[1], E. IROLLO[1], A. SACAN[4], O. MEUCCI[1,2,3];
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Abstract: Dendritic spines are structural substrates for learning and memory and their pruning is associated with cognitive decline in conditions like HIV-associated neurocognitive disorders (HAND). Our past studies in a rodent model of HAND (HIV-transgenic rat) revealed that dendritic spine loss and the related cognitive deficits can be rescued by treatment with the chemokine CXCL12. This requires activation of the chemokine receptor CXCR4 and its downstream Rac1/PAK signaling, which regulates actin polymerization in spines. Hence, we hypothesized that CXCL12 may alter dendritic spines turnover, maturation, and clustering in ways that ultimately supports cognitive function. Here, we used a combination of live-cell imaging and computational studies on fixed brain tissue to evaluate the effect of CXCL12 on spine dynamics. First, we investigated CXCL12 effects on spine turnover in cultures of rat

primary cortical neurons. A single CXCL12 treatment (20nM) boosted spine density with a peak effect after 2.5 hours (N=4, $p<0.0001$), which was attenuated by the CXCR4 antagonist AMD3100 (100ng/mL) (N=5, $p=0.7$). CXCL12 enhanced spine formation and decreased elimination, with a net effect of increased spinogenesis. Continuous exposure to CXCL12 maintained a higher spine density (N=3, $p<0.05$). Next, we examined expression and distribution of markers of mature spines in these cultures. CXCL12 consistently increased the density and percentage of postsynaptic density protein 95 (PSD-95) within thin spines and the overall spine population. We saw similar trends for spines containing phospho-PSD-95^{Ser295} and GluA1. Concurrently, we are exploring spine stabilization in cultures infected with AAV-hsyn-PSD95.FinGR-eGFP by tracking endogenous PSD-95 puncta. Finally, we evaluated CXCL12's effect on spine clustering both in neuronal cultures and in medial prefrontal cortex pyramidal neurons of wild-type and HIV-Tg male rats. Clustering was assessed using interspine distance and the nearest neighbor index, which quantifies the deviation of observed interspine distances from a random distribution. CXCL12-treated HIV-Tg rats had a significantly shorter interspine distance and decreased index. We observed similar results in thin spines of CXCL12-treated cultures. These results suggest that CXCL12 induces spine clustering and functional maturation. Overall, this study shows that CXCL12 regulates multiple components of spine dynamics that favor a more efficient and robust network. Ongoing work in live brain tissue aims to validate these conclusions and further define the structural and functional basis of CXCL12-induced cognitive performance in the context of HAND.

Disclosures: **C. Ho:** None. **E. Irollo:** None. **A. Sacan:** None. **O. Meucci:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

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Program #/Poster #: PSTR187.05/Web Only

Topic: B.05. Synaptic Plasticity

Support: Canadian Institutes of Health Research (CIHR) Canada Graduate Scholarships Doctoral Award (CGS D)
Canadian Institutes of Health Research (CIHR) Foundation Grant #154276
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Title: Clustered activity enables de novo spinogenesis in the adult hippocampus

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Abstract: Learning requires rewiring of synaptic circuits in the brain. Formation of new dendritic spines, or spinogenesis, is thought to be critical for this process. New spines have been shown to arise in response to learning-related activity and contribute to the storage of encoded information. However, mechanistic understanding of how spines are formed at the single-spine level in the adult brain has remained incomplete. We used 2-photon (2P) imaging and glutamate uncaging to interrogate the activity signals required to evoke the formation of individual spines in acute hippocampal slices from adult mice. Activation of a single point on the dendritic shaft, which has been shown to elicit spine formation in the developing brain, failed to induce spinogenesis in area CA1 of adult mice (0/20 trials). The lack of spinogenesis persisted with increased stimulation intensity, increased stimulation frequency, and repetitive stimulation, as well as in different CA1 subregions and the dentate gyrus. This was not attributed to a general lack of structural plasticity as structural long-term potentiation occurred readily in response to the same stimulation. Additionally, dendritic stimulation reliably elicited spinogenesis in early development (P7-14), in line with previous reports. We next asked whether stronger postsynaptic activation via quasi-synchronous stimulation of a cluster of spines along with the dendrite could enable spinogenesis in the adult hippocampus. In contrast to dendritic activation alone, clustered stimulation led to successful spine formation in 36% of trials. New spines arose between 20-60 minutes after stimulation and varied with respect to morphological features and persistence. Increasing the number of clustered stimulation episodes did not significantly affect the spinogenesis success rate (40%). Stimulation of a cluster of spines alone without dendritic stimulation did not evoke spinogenesis (0/15 trials), suggesting the importance of coincident activity between the dendrite and neighbouring spines. Our findings provide new mechanistic insights regarding the synaptic activity signals required to trigger spinogenesis in the adult brain and highlight the importance of clustered activity in this process. Ongoing work is aimed at elucidating the molecular mechanisms at play and how different patterns of clustered activity influence propensity for spinogenesis.

Disclosures: **A. Abbasian:** None. **J. Georgiou:** None. **G.L. Collingridge:** None.

Poster

PSTR187. Structural Plasticity: Synapses

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.06/C15

Topic: B.05. Synaptic Plasticity

Support: T32 Institutional Training Grant
Whitehall Foundation
NIH, Common Fund Program, R03DC019290

Title: Trans-synaptic Adhesion Mediated by C1QL3

Authors: *K. CARO[1], T. RELIGA[1], M. STICCO[1], S. ALAM[1], S. RESSL[2], D. MARTINELLI[1]; [1]Neurosci., Univ. of Connecticut Hlth. Ctr., Farmington, CT; [2]Neurosci., The Univ. of Texas at Austin, Austin, TX

Abstract: Trans-synaptic adhesion mediated by C1QL3Keaven Caro (caro@uchc.edu)[1], Matthew Sticco[1] (sticco@uchc.edu), Trevor Religa[1] (trevor.religa@uconn.edu), Shanawaz Alam[1] (shalam@uchc.edu) Susanne Ressl[2] (suressl@utexas.edu), David Martinelli[1] (davidmartinelli@uchc.edu) [1]Department of Neuroscience, University of Connecticut Health, Farmington, CT, USA[2]Department of Neuroscience, The University of Texas at Austin, Austin, TX, USA

Chemical synapses allow for information transfer between neurons of the brain and their dynamic properties are crucial for proper brain function. Synaptic plasticity and homeostasis (formation and pruning) are implicated in learning and post-natal brain development. Synaptic adhesion molecules (SAMs) make a specialized cell-cell junction across the synaptic cleft, and various complexes have been shown to control synapse formation, dendritic spine morphology, and synaptic plasticity. Dysfunction of SAMs have been implicated in neuropsychiatric disorders such as autism and schizophrenia. Complement component 1, Q subcomponent-like 3 (C1QL3) is a novel potential SAM that has been shown to bind to a post-synaptic receptor adhesion G protein-coupled receptor B3 (ADGRB3). *C1ql3* is expressed in many regions of the brain including the suprachiasmatic nucleus, cerebral cortex, and limbic system. *C1ql3* knock out (KO) in the basolateral amygdala leads to loss of excitatory synapses projecting to the PFC and a subsequent deficit in fear conditioning consistent with a role of C1QL3 in promoting synapse maintenance. We previously demonstrated that C1QL3 promotes cell-cell adhesion in an ADGRB3 and neuronal pentraxin dependent manner in heterologous cells, consistent with the role of a SAM. We show with confocal microscopy and stimulated emission depletion (STED) super-resolution microscopy in mouse primary neuron cultures that C1QL3, NPTX1, and ADGRB3 co-localize at synapses, suggesting the presence of this trans-synaptic adhesion complex. We also show that colocalization of the pre- and post-synaptic binding partners is lost after *C1ql3* KO, suggesting that C1QL3 is required to complete the transsynaptic adhesion complex. This research may elucidate a molecular mechanism underlying C1QL3's role in synaptic maintenance.

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Disclosures: K. Caro: None. **T. Religa:** None. **M. Sticco:** None. **S. Alam:** None. **S. Ressl:** None. **D. Martinelli:** None.

Poster

PSTR187. Structural Plasticity: Synapses

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Topic: B.05. Synaptic Plasticity

Support: CIHR, MOP111220
CIHR, PJT156103
NSERC (2017-06444)

Title: The role of CaMKIIβ in fear memory

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Abstract: Reactivation of the neural ensemble activity is critical for memory recall. However, the molecular underpinnings of this process in the hippocampal CA1 remain an active area of study. This project explores the impact of a central synaptic enzyme for synaptic plasticity, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), in the hippocampal CA1 pyramidal neurons for memory retrieval.
CaMKII is a serine/threonine protein kinase critically involved in synaptic plasticity underlying learning and memory. The alpha & beta subunits form hetero-oligomer and are highly concentrated in the postsynaptic density fraction. We have showed that activation of beta subunit of CaMKII (CaMKIIbeta) leads to its dissociation from filamentous actin (F-actin) to trigger reorganize synaptic component and that this process was required for structural and functional synaptic potentiation in the hippocampal CA1 pyramidal neurons. However, how its synaptic roles serve in the memory process are elusive.
To study the CaMKIIbeta function *in vivo,* we virally suppressed endogenous CaMKIIbeta by shRNA and replaced by CaMKIIbeta mutants with a silent mutation against the shRNA target. After validation of the viral molecular replacement approach in the murine hippocampal CA1 pyramidal neurons, we further utilized an activity-dependent (c-fos/tTA/TRE) expression system to specifically coexpress them in the subpopulation of neurons activated during the learning when mice were doxycycline (Dox) OFF. We observed the number of positive neurons were dramatically increased by fear memory induction in Dox OFF, but not in Dox ON, indicating the specific expression in the neurons activated during the memory induction.
Using this viral expression system, we examined the effect of a CaMKIIbeta mutant which prevents its activity-dependent F-actin dissociation while retaining its kinase function by mutating all the serine and threonine residues in the F-actin binding domain (all A). After viral injection of the CaMKIIbeta mutant with beta specific shRNA (Dox ON), we induced fear memory under 2 days Dox OFF (activity-dependent expression) and then measured the freezing responses by returning the arena without foot shock as a contextual fear memory recall after 24 and 48 hours.
We found that there was a significant reduction of the freezing by CaMKIIbeta all A mutant than control during both the first and second retrieval of fear memory, indicating the involvement of a CaMKIIbeta function for its activity-dependent synaptic reorganization.
We will further discuss the results from another series of experiments testing the role of CaMKIIbeta mutants to explore how it affects the memory recall.

Disclosures: J. Iglar: None. **J. Rai:** None. **H. Li:** None. **K. Okamoto:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

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Program #/Poster #: PSTR187.08/C17

Topic: B.05. Synaptic Plasticity

Support: RX002999-01 Center for Restoration of Nervous System Function
RX002969-01A1 Deconstructing Spasticity after Spinal Cord Injury

Title: Pak1 inhibition with Romidepsin attenuates H-reflex excitability after spinal cord injury

Authors: *S. KAUER;
Yale Univ., New Haven, CT

Abstract: In individuals living with spinal cord injury (SCI), spasticity is prevalent with up to 70% of these individuals experiencing clinically significant complications that negatively affect daily activities and quality of life. Our research has shown a contribution of abnormal dendritic spine profiles on motoneurons and spasticity. Rac1-PAK1 mediated dendritic spine remodeling can lead to nociceptive and spinal motor neuron hyperexcitability underlying neuropathic pain and spasticity, as we've published previously. In these studies, disrupting Rac1 and its downstream activity normalizes dendritic spine dysgenesis and reduces spinal cord hyperexcitability disorders after SCI. PAK1 is a promising druggable target for neurological disease. Importantly, we identified romidepsin, a clinically available drug and potent histone deacetylase (HDAC) inhibitor that reduces PAK1 activity without affecting the molecule's protein expression. To assess the utility of targeting PAK1 to attenuate H-reflex hyperexcitability, we administered romidepsin, in Thy1-YFP reporter mice. We performed longitudinal electromyogram (EMG) studies in a study design that allowed us to assess pathological H-reflex changes and drug intervention effects over time, before and after contusive SCI. As expected, our results show a significant loss of rate-dependent depression (RDD)—a indication of hyperreflexia and spasticity--one month following SCI as compared with baseline, uninjured controls (or before injury). Romidepsin treatment reduced signs of hyperreflexia in comparison with control cohorts, and in pre- and post-drug intervention in SCI animals. Neuroanatomical study further confirmed drug response, as romidepsin treatment also reduced the presence of SCI-induced dendritic spine dysgenesis on alpha-motor neurons. The bioavailability of romidepsin within the spinal cord ventral horn also produced significant tissue response in motor neurons, including elevated drug-response biomarker expression of H3 histone deacetylate, and reduced p-Raf1 expression. Importantly, animals appeared to tolerate romidepsin without exhibiting adverse effects in sensory-motor testing with BMS or CatWalk assays. While contusive SCI produced significant inflammation within and around the injury site, we did not observe changes in the degree astrogliosis or microgliosis with acute romidepsin treatment. Taken together, our present study provides compelling evidence that inhibition of PAK1 using romidepsin, a clinically available inhibitor, may provide a promising therapeutic approach for attenuating spasticity induced by spinal cord injury (SCI).

Disclosures: S. Kauer: None.

Poster

PSTR187. Structural Plasticity: Synapses

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Program #/Poster #: PSTR187.09/C18

Topic: B.05. Synaptic Plasticity

Support: NIH/NIA 1R01AG069433 to GT
NIH/NIA R21AG082230 to AF
AARF AARF-22-973974 to AF

Title: Cognitive integrity in Non Demented individuals with Alzheimer's Disease Neuropathology is associated with preservation and remodeling of dendritic spines.

Authors: J. GUPTARAK[1], P. SCADUTO[1], D. JUPITER[2], G. TAGLIALATELA[1], ***A. FRACASSI**[1];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia. The correlation between the accumulation of Aβ plaques and neurofibrillary tangles with the progression of dementia has been questioned in the past decade by the emergence of a particular cohort of individuals recently classified as A+T+N- that present with full AD signature pathology but remain cognitively intact (here referred to as "Non-Demented with AD Neuropathology" - NDAN). We previously described that NDAN are characterized by phagocytic microglia able to remove damaged synapses around plaques and most likely providing protection from greater damage along the axons and dendrites. To test the efficiency of microglia to preserve dendrites and dendritic spines in NDAN, we characterized dendrites and dendritic spines morphology in frontal cortex of NDAN vs. AD, and aged-matched healthy subjctes (controls). We performed an accurate study of the synaptic structure in the plaque area using Thioflavin S to detect Aβ plaques, and DiI dye staining, a fluorescent lipophilic cationic indocarbocyanine dye, to detect axons and dendrites. We identified one region of interest (ROI) composed of a proximal region (around plaques) and a distal region (far from plaques). Using Imaris software, specifically the Filament tracer and Classify Spines XTension, we quantified dendrite length, dendrite diameter, and spine density. We did not find any significant differences in the proximal ROI in all the studied parameters among controls, AD, and NDAN suggesting that Aβ plaques have an overall damaging effect on dendrites and spines regardless the pathological condition. Conversely, when we analyzed the distal ROI, we found AD dendrites diameter significantly bigger than NDAN dendrites, possibly suggesting an enlargement due to an abnormal accumulation of vesicles. We also found significantly higher spine density in NDAN as compared to AD in the distal ROI possibly suggesting that the damage did not spread through the axons thanks to the phagocytic activity of microglia. Interestingly, we measured the relative abundance of four different types of spines (mushroom, stubby, filopodia and long thin), with the stubby being the most common type within all the groups. Mushroom spines, the least dynamic, were significantly increased in AD vs. NDAN and control subjects. Conversely, NDAN individuals showed higher density of stubby, filopodia, and long thin spines

than AD. These results suggest that the rearrangement of dynamic dendritic spines we observed in NDAN might underlie the ability of these individuals to replace damaged synapses and preserve cognitive integrity.

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Poster

PSTR187. Structural Plasticity: Synapses

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Topic: B.05. Synaptic Plasticity

Support: NIMH Award R01MH124997 to S.F

Title: The effect of mitochondrial calcium uniporter deletion in CA2 neurons on dendritic spines and mitochondria ultrastructure

Authors: K. PANNONI[1], R. TARANNUM[1], M. CAWLEY[1], Q. S. FISCHER[2], M. M. ALSALMAN[1], *S. FARRIS[1];
[1]Virginia Tech., Roanoke, VA; [2]Fralin Biomed. Res. Inst. At VTC, Roanoke, VA

Abstract: Hippocampal area CA2 is critical for forming social memories and exhibits a distinct plasticity profile compared to neighboring hippocampal subregions. Specifically, synapses in CA2 distal dendrites are prone to long-term potentiation (LTP), whereas synapses in proximal dendrites are resistant to LTP. We previously showed that the mitochondrial calcium uniporter (MCU) is selectively enriched in CA2 distal dendrites and the mitochondria there are larger and more tubular compared to proximal dendrites. However, the functional significance of this is unknown. MCU is a channel in the inner mitochondrial membrane that regulates calcium influx into the mitochondria, which in turn regulates mitochondrial morphology and bioenergetics. We hypothesize that the asymmetric localization of MCU-enriched mitochondria in CA2 distal dendrites supports LTP by tuning local ATP production to power changes in spine synapses. Consistent with this, we found that MCU is required for LTP at CA2 distal dendrite synapses using a CA2-specific conditional MCU knockout (cKO) mouse. In the current study, we analyzed Golgi-impregnated CA2 neurons from cKO and control (CTL) mice to test whether MCU prevents LTP by altering spines. Preliminary data from 4 mice per genotype suggest a potential decrease in spine density in the cKO compared to CTL; however, more data are needed to substantiate this finding (mean CTL: 10.1 (± 0.02) vs. cKO: 8.5 (± 0.11) spines / 10 microns of dendrite; unpaired one-tailed t-test, p=0.114). We next assessed the impact of MCU loss on mitochondria ultrastructure with scanning electron microscopy. We used the AI platform Biodock to develop an unbiased analysis of mitochondrial morphology across CA2 dendritic layers. Our AI analysis confirmed larger and longer mitochondria in CA2 distal dendrites compared to proximal dendrites. In contrast to the manual analysis, we trained the AI to

selectively segment mitochondria in dendrites, resulting in a more precise quantification of dendritic mitochondrial morphology. Using AI, we analyzed an area of 123,600 µm2 over 2 days, with an error rate of 4 errors / 100 µm2 area, a 77-fold increase in the area analyzed compared to the manual analysis. Comparing mitochondria in CA2 SLM from three mice per genotype, we preliminarily found a small but significant decrease in mitochondria area (Mann-Whitney test, p = 0.03; N = ~5000 mito / group) after MCU KO, with no significant change in the number of mitochondria per 100 µm2 area (CTL: 23 vs. cKO: 24; Mann Whitney test, p = 0.11). Collectively, our preliminary data suggest that MCU regulates layer-specific forms of plasticity in CA2 via an alteration in dendritic spines and mitochondrial area.

Disclosures: K. Pannoni: None. **R. Tarannum:** None. **M. Cawley:** None. **Q.S. Fischer:** None. **M.M. Alsalman:** None. **S. Farris:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.11/C20

Topic: B.05. Synaptic Plasticity

Support: NIH/NINDS F31 Diversity Grant 1F31NS127511-01
NIH R01 NS120746

Title: Bdnf to astrocyte trkb.t1 signaling on the development and plasticity of perisynaptic astrocyte processes at glutamatergic synapses.

Authors: *B. T. C. PINKSTON, J. L. BROWNING, S. BASAK, K. R. REUWER, M. L. OLSEN;
Virginia Polytechnic Inst. and State Univ., Blacksburg, VA

Abstract: Perisynaptic astrocyte processes (PAPs) are specialized structures located on the distal processes of astrocytes that are intimately associated with synapses. PAPs contain numerous neurotransmitter receptors, ion channels, cell-adhesion molecules and the ability to release synaptogeneic molecules, allowing them to contribute to synaptic function and plasticity. However, despite decades of research demonstrating their importance, the mechanisms that attract PAPs to a synapse or induce their structural plasticity in response to neuronal activity remain poorly understood. Our recent published work demonstrated that astrocytes predominately express TrkB.T1 relative to other CNS cell populations. Both global and conditional deletion of TrkB.T1 in astrocytes is sufficient to reduce astrocyte morphological maturation *in vitro* and *in vivo*, alters mature astrocyte gene expression *in vivo*, and reduces excitatory synapse formation *in vitro.* Using neuron-astrocyte co-cultures we demonstrate that TrkB.T1 knockout (KO) astrocytes show a 60% reduction in PAP formation at ‘active’ synapses - defined as co-localization of presynaptic VGluT and postsynaptic Homer. Further, in response to 10 mM KCl exposure, WT astrocytes increase PAP coverage at glutamatergic synapses, a

result not observed in KO astrocytes. These data suggest TrkB.T1 activation in astrocytes may serve to regulate astrocyte structural plasticity at the PAP in development and in the context of experience dependent plasticity. *In vivo*, we demonstrate TrkB.T1 is enriched in the PAP, which may enable astrocytes to respond to synaptically released BDNF. Our preliminary data utilizing 3-D reconstructions of pAAV.GfaABC1D.Lck-GFP expressing astrocytes in layer IV somatosensory cortex show increased association of astrocytes with synaptic elements following 48 hours of environmental enrichment, a paradigm of experience dependent plasticity that involves upregulated BDNF and synapse formation. Ongoing work is aimed at reassessing these findings in conditional Aldh1l1-TrkB.T1 KO mice. This work may provide insights into the mechanisms underlying astrocyte-synapse interactions.

Disclosures: B.T.C. Pinkston: None. **J.L. Browning:** None. **S. Basak:** None. **K.R. Reuwer:** None. **M.L. Olsen:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.12/C21

Topic: B.05. Synaptic Plasticity

Support: G34655

Title: Single-synapse resolution molecular mapping reveals both widespread and highly targeted experience-dependent synaptic plasticity in the mammalian brain

Authors: *H. WOODS[1], Z. QIU[1], B. NOTMAN[1], N. H. KOMIYAMA[1], F. SENGPIEL[2], S. G. GRANT[3];
[1]The Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Cardiff Univ., Cardiff, United Kingdom; [3]Edinburgh Univ., EDINBURGH, United Kingdom

Abstract: Systematic mapping of the molecular, morphological and protein half-life properties of individual excitatory synapses on a brain-wide scale in the mouse has revealed high synapse diversity. Excitatory synapse diversity expands during postnatal development and each brain region develops a unique signature of synapse composition (Science 369, 270-2752020). These findings have given rise to the terms ‘synaptome’, which describes the full diversity of brain synapses, and ‘the synaptome architecture’, which describes the spatial distribution of these synapses in neurons, brain regions and the whole brain. Genetic mutations cause widespread changes in synaptome architecture (Nat. Commun. 13, 6836) indicating that it is genetically programmed. The extent to which daily activity and experience shape the synaptome architecture is poorly understood, with large parts of the brain remaining unexplored. We asked whether the development of synaptome architecture was modified in two well-established, complementary paradigms used to study how experience modifies synaptic properties: environmental enrichment (EE), which stimulates many sensory inputs; and monocular deprivation (MD), which

specifically alters visual inputs. Psd95$^{eGFP/eGFP}$;Sap102$^{MKO2/MKO2}$ mice expressing fluorescently labelled post-synaptic proteins underwent MD from P25-31 ([MD n = 13 (6F); control n = 14 (7F)]) or EE rearing until P90 ([EE n = 23 (12F); control (standard-caged) n = 29 (15F)]) and synaptome mapping was performed at single-synapse resolution on a brain-wide scale. Both MD and EE modified the synaptome architecture in many brain regions, and beyond the areas typically explored in the literature. Specific excitatory synapse types and subtypes, as dictated by their protein composition and protein lifetime were targeted among the experience-dependent changes. For example, whereas 3-month EE rearing reduced the density of long-lifetime PSD95+ synapses across the sensory cortices and hippocampus, 7-day juvenile MD increased the density of shorter-lifetime PSD95+/SAP102+ synapses in these same brain regions in both hemispheres. That experience plays a role in modulating the extensive synapse diversity of the developing mammalian brain has key implications for existing models of learning during development and adulthood.

Disclosures: H. Woods: None. **Z. Qiu:** None. **B. Notman:** None. **N.H. Komiyama:** None. **F. Sengpiel:** None. **S.G. Grant:** None.

Poster

PSTR187. Structural Plasticity: Synapses

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.13/C22

Topic: B.05. Synaptic Plasticity

Support: LF experiment

Title: Reverse engineering of synaptic tagging and capture

Authors: *M. GOMEZ DE SALAZAR, M. KJAERGAARD;
Univ. of Aarhus, AARHUS C, Denmark

Abstract: Memory and learning require fine-tuning of the connections between neurons. To strengthen a connection, a neuron needs to deliver hundreds of proteins from a range of different signaling pathways, but only to synapses that have been specifically stimulated. These proteins, known as plasticity-related proteins (PRP) can be locally recruited and produced in response to synaptic stimulation. Despite decades of research, the molecules and signaling pathways that participate in this mechanism, known as "synaptic tagging" have not been categorically identified, possibly because there are many different tags. Instead of asking “What is the synaptic tag?”, we have focused on “What does it take to be a synaptic tag?” aiming to identify the biophysical characteristics that PRPs should have. To address these questions, we engineered artificial proteins, Synthetic PRPs (SynPRPs), to test mechanisms of activity-dependent targeting. *In vitro*, we showed that SynPRPs are phosphorylated by CaMKII and consequently bind postsynaptic density protein 95 (PSD95) in a phospho-dependent manner *in vitro*. In mouse hippocampal cultured neurons, we observed by ExM that SynPRPs are located in dendritic

spines. Synaptic clusters of SynPRPs + PSD95 increased about 2-fold their synaptic localization after chemical long term potentiation (cLTP), demonstrating that SynPRPs can be captured in activated synapses. Moreover, SynPRPs changed synaptic activity measured by MEA and affected synaptic protein levels. Overall, we demonstrated how synthetic PRPs are captured by a synaptic tag in activated synapses and affect endogenous synaptic plasticity mechanisms. Our results could help to further understand the synaptic tag and capture mechanisms and could lead to prevent alterations on synaptic tagging in cognitive dysfunctions.

Disclosures: **M. Gomez de Salazar:** None. **M. Kjaergaard:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.14/C23

Topic: B.05. Synaptic Plasticity

Title: The Role of Genioglossus Muscle in the Pathogenesis of Obstructive Sleep Apnea

Authors: G. BORGER, S. MEDHARAMETLA, M. VAID, K. LINK, K. DINOVO, ***I. MARTINEZ-PENA Y VALENZUELA**;
Physiol., Midwestern Univ., Downers Grove, IL

Abstract: Obstructive sleep apnea (OSA) is a frequent breathing disorder characterized by the recurrent relaxation of the tongue and soft palate during sleep. OSA results in impaired sleep and has major consequences on the endocrine, cardiovascular, and nervous systems. Obesity can restrict the airway and increases the chance of developing OSA. The genioglossus (GG) muscle is the largest extrinsic tongue muscle, crucial for maintaining the patency of the upper airway while sleeping, and responsible for tongue depression and protrusion. OSA patients have decreased muscular tone in the GG muscle. Due to this, the tongue pulls back into the throat while the patient sleeps, obstructing the airway, preventing airflow, and lowering the oxygen levels of the body. In obesity-related hypoventilation and OSA pathogenesis, the leptin hormone (the hormone that suppresses hunger) plays a fundamental role in the central regulation of upper airway patency and diaphragmatic control. Leptin-deficient obese (ob/ob) mice exhibit pharyngeal collapsibility, hypoventilation, and hypercapnia which can be alleviated by leptin replacement treatment. Ob/ob mouse is a great model to study the effects of OSA in different tissues as previous research suggested. Furthermore, studies show that leptin receptor ObRb is expressed in skeletal muscle, exhibits sexual dimorphism, and protects the muscle against excessive fat accumulation, which can cause lipotoxicity. The goal of this study is to explore the impact of OSA combined with obesity on the extrinsic tongue muscle genioglossus (GG). By using leptin-deficient obese (ob/ob) male and female mice, we study GG muscle structure and function in the OSA environment, with a specific focus on the neuromuscular junction (NMJ), the synapse responsible for breathing and voluntary movements and critical for survival. Our preliminary results show that GG muscles from male ob/ob mice exhibit central nuclei as an

indicator of myofiber regeneration, the number of nicotinic acetylcholine receptors at their NMJs is decreased, and their lipid rafts density is lower than their age-matched controls. Furthermore, both Schwann cell and axon terminal morphologies are altered in ob/ob males. In contrast, other skeletal muscles from ob/ob males were not affected. Additionally, ob/ob females did not show any alterations in their GG muscles. These results suggest that GG muscle and the presynaptic and postsynaptic components of NMJs are affected in leptin-deficient male mice.

Disclosures: **G. Borger:** None. **S. Medharametla:** None. **M. Vaid:** None. **K. Link:** None. **K. DiNovo:** None. **I. Martinez-Pena y Valenzuela:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.15/C24

Topic: B.05. Synaptic Plasticity

Support: PAPIIT IN206521

Title: Changes in synaptophysin expression induced by partner preference and sexual motivation in male rats in brain regions involved in the control of sexual behavior.

Authors: *Z. MIER QUESADA[1], **L. GAYTAN**[2], R. G. PAREDES[3];
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Abstract: Synaptophysin is a protein localized in presynaptic vesicles involved and used as a marker of synaptic plasticity. Sexual behavior and sexual experience induce brain plastic changes but there are few studies that have evaluated possible changes in synaptic plasticity associated with sexual behavior. We evaluated two aspects of sexual behavior, motivation, and execution. We used the Partner Preference Test (PPT) and the Sexual Incentive Motivation (SIM) test to determine by immunofluorescence techniques, plastic changes induced by these behaviors in brain structures related with sexual behavior. 15 male Wistar rats (300-350 gr) were divided into 3 groups: PPT, SIM, and CTRL group. Ovariectomized female rats and sexually experienced males were used as stimulus animals for the tests. Subjects were tested once a week for 10 weeks in either the PPT or the SIM test. After that, subjects were sacrificed and the brains extracted. Brains were cut in 30 µm slices and different regions of interest (ROI) analyzed. Images were obtained with confocal microscopy from the amygdala, hippocampus, ventromedial hypothalamus and olfactory bulb. To avoid bias, the measuring parameters were established in the Control Group and applied to the experimental groups. The ROIs in each image were delimited and the volumetric intensity of each structure was obtained by averaging the intensity values of 4 coordinates per ROI. As expected, the results revealed that sexually experienced

males spent more time with the receptive female in both, the PPT and SIM tests. We found a significant increase in synaptophysin expression in the dentate gyrus in the PPT group vs the control group. We also found an increase in synaptophysin expression in the amygdala and the main olfactory bulb in the SIM group in comparison with the control group. These results suggest that sexual behavior as motivated behavior induces plastic changes in brain structures controlling sexual behavior.

Disclosures: Z. Mier Quesada: None. **L. Gaytan:** None. **R.G. Paredes:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.16/C25

Topic: B.05. Synaptic Plasticity

Support: NIH R21NS116316
Baruchowitz Family Fellowship for Dysautonomia Research
W.M. Keck Foundation

Title: Satellite glial cells regulate cultured sympathetic neuronal activity states through influencing cholinergic transmission

Authors: *J. HARRISON, M. HABURCAK, J. WANG, N. VIEUX-GRESHAM, S. BIRREN; Brandeis Univ., Waltham, MA

Abstract: Sympathetic neurons directly influence the normal physiological function of the organs they innervate. Chronic increases to sympathetic neuronal activity (SNA) precede and drive the development of diseases such as diabetes and hypertension. We have shown that isolated sympathetic neurons from neonatal spontaneously hypertensive rats (SHR), a model used to study human neurogenic hypertension, have increased synaptic charge and synapse formation compared to Wistar Kyoto (WKY) rats; suggesting that intrinsic activity-dependent potentiation may be enhanced in hypertension-prone rats. Further, we have also demonstrated a role for satellite glial cells (SGCs) in the regulation of neuronal function. We therefore investigated the role of SGCs in homeostatic regulation of SNA by coculturing WKY and SHR sympathetic neurons with SGCs and quantifying the number of cholinergic synapses using immunocytochemistry. Surprisingly, the SHR neurons formed fewer synapses than WKY when cultured with SGCs; however, this trend was reversed in the absence of SGCs, with SHR neurons forming more synapses than WKY, indicating a role for SGCs in homeostatic regulation. Since SHR neurons are in an intrinsically high activity state, we asked if chemo-genetically manipulating WKY SNA in the presence of SGCs would induce a similar decrease in cholinergic synapses. Thus, we chronically increased SNA by expressing an excitatory Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) in sympathetic neurons cultured with or without SGCs. Chronic activation of neurons cultured with SGCs decreased the

number of cholinergic synapses. Interestingly, there was an increase in the number of synapses formed in the absence of SGCs, suggesting SGCs are also able to sense neuronal activity states and act to lower high SNA. We therefore asked if manipulating the activity of SGCs is sufficient to induce homeostatic regulation of SNA. We chronically activated SGCs with an excitatory glial DREADD and observed a decrease in the number of cholinergic synapses in response to glial activation. Together, these data demonstrate an activity-dependent role of SGCs in detecting and regulating neuronal activity states. An important outstanding question is whether the homeostatic role of the SGC is seen in prehypertensive rats and lost as the animal develops high blood pressure. Future work will focus on understanding how SGC regulation influences the physiological output of the sympathetic system *in vivo*. This work suggests that selectively targeting regulators of SNA can be a potential therapeutic tool to treat forms of drug-resistant hypertension as well as other autonomic diseases.

Disclosures: **J. Harrison:** None. **M. Haburcak:** None. **J. Wang:** None. **N. Vieux-Gresham:** None. **S. Birren:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.17

Topic: B.05. Synaptic Plasticity

Support: JSPS 20H05685
JST JPMJCR1652
JSPS 21K15203
JSPS 21K20682

Title: Quick short-term potentiation (qSTP): A newly identified mode of associative presynaptic plasticity routed in mechanical synaptic transmission

Authors: H. OKAZAKI[1], F. ETO[2], H. UCAR[2], Y. MORIMOTO[2], ***H. KASAI**[2];
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Abstract: The classical view of dendritic spine synapses as strictly chemical has recently been challenged by findings demonstrating that they can generate a force similar to smooth muscle contractions through actin polymerization during spine enlargement (Ucar et al., Nature, 2021). This mechanical force is sensed by presynaptic terminals, initiating a rapid and sustained surge in glutamate release lasting approximately 20 minutes, thus unveiling an unexplored mode of synaptic transmission: mechanical transmission. The discovery of mechanical transmission brings new insights into the mechanisms propelling short-term potentiation (STP) in spine synapses, as it provides a logical account for the postsynaptic induction and presynaptic expression of STP, removing the need for elusive retrograde chemical messengers. Despite this

breakthrough, STP presents additional conundrums, including its resistance to CaMKII inhibitors and the requirement for high-frequency stimulation, which are problematic considering STP's potential significant role in working memory processes (Kasai et al., Curr Opin Neurobiol, 2023). This study introduces the concept of quick short-term spine enlargement (qSTE), a phenomenon provoked by minimal stimulation (20Hz, 20 times or less) using spike-timing-dependent protocols (STDP) or 0 Mg glutamate uncaging. Crucially, qSTE showed sensitivity to CaMKII inhibitors, setting it apart from conventional STP/STE. Rapid spine enlargement occurred in hippocampal CA1 pyramidal neurons within mere seconds of qSTE induction. In addition, applying optogenetic STDP to presynaptic terminals and postsynaptic cells led to spine enlargement, causing the pushing of the presynaptic terminal and boosting presynaptic function for up to 20 minutes. Therefore, our research uncovers a new form of associative plasticity coined as quick short-term potentiation (qSTP), which involves alterations in presynaptic function. Due to its inducibility with minimal stimulation, we propose that qSTP is likely the most common form of associative plasticity in the brain, aligning with its anticipated role in working memory processes.

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Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.18/C26

Topic: B.05. Synaptic Plasticity

Support: NIA Grant 1R56AG072676
NIA Grant 5R01AG047296

Title: Volumetric volatility of apical dendritic spines is decreased in layer 5 pyramidal neurons of the aging somatosensory cortex

Authors: *A. DUCOTE[1,2], R. VOGLEWEDE[3], R. MOSTANY[1,4];
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Abstract: Cognitive performance progressively declines with age even in the absence of neuropathological conditions, impacting diverse aspects of normal life and reducing quality-of-life in the elderly, but the mechanisms mediating this decay in brain performance remain poorly defined. We have shown that apical dendritic spine dynamics of layer 5 pyramidal neurons (L5 PNs) in mouse somatosensory barrel cortex are altered with age, exhibiting higher density and turnover, decreased long-term survival at baseline, and a reduction in volatility in response to

plasticity induction compared with young mice. However, while gain or loss of spines affects network connectivity, neurons are also constantly fine-tuning existing connections via modulation of synaptic strength. The present study extends previous findings by examining differences in longitudinal volumetric dynamics of spines at baseline and in response to plasticity induction. We tracked spine volumes over 24-hour intervals in apical dendrites of L5 PNs in vivo at baseline and in response to whisker stimulation in young (2-6 months, n=6) and aged (18-24 months, n=5) Thy1-GFP line M mice using two-photon imaging. As female mice have shown changes in spine dynamics depending on estrous cycle stage, our study was restricted to males. We found that the distributions of both persistent spine volumes and volume differences between time points were more stable in aged mice. Changes in ranking by volume over time of individual spines was also decreased in aged mice as measured by rank-biased overlap, and this effect appeared to be due to decreased ranking volatility of the largest spines. These results suggest that, while aged mice exhibit higher turnover in terms of the gain and loss of spines, the system may attempt to compensate for this increased volatility through stabilization of a subset of existing synaptic weights within the network. Our findings demonstrate opposing effects of aging on discrete and volumetric dynamics of a specific subset of dendritic spines and suggest mechanisms by which the aging brain may attempt to balance an ongoing need for learning with maintenance of existing connectivity, while also providing a baseline against which to compare spine volume dynamics in age-related neurodegenerative disorders.

Disclosures: **A. Ducote:** None. **R. Voglewede:** None. **R. Mostany:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

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Program #/Poster #: PSTR187.19/C27

Topic: B.05. Synaptic Plasticity

Support: NSF 1707356
NSF 2014862
R01 5R01MH095980

Title: The distribution and structural plasticity of excitatory synapses along interneuron dendrites in adult hippocampal area CA1

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Abstract: Hippocampal interneurons form a heterogeneous group with differing structural, physiological, and chemical phenotypes. Most CA1 interneuron dendrites lack dendritic spines.

Nonetheless, interneuron dendrites demonstrate spatial restriction of calcium transients and NMDA-receptor-dependent LTP, suggesting plasticity of synapses might be influenced by differences in underlying ultrastructure. We used three-dimensional (3D) reconstructions from serial section electron microscopy (EM) to analyze synaptic structural plasticity along aspiny dendrites in CA1 stratum radiatum at 2h during LTP induced with theta-burst stimulation (TBS-LTP) in adult (P60-61) Long-Evans rats. Given the relative sparsity of interneurons in CA1 stratum radiatum, we re-imaged EM series that had previously been analyzed for synapse structure along spiny dendrites (3) in a scanning electron microscope in transmission mode (4). In total, 43 aspiny dendritic segments (23 LTP, 20 control, ranging 1.90-18.57 µm in length) and their synapses were identified and reconstructed in 3D. Aspiny dendrites appear smooth or varicose ("beaded") in light microscopy. We categorized reconstructed aspiny dendrites as smooth or varicose based on dendritic volume contours along each segment. We further sub-segmented varicose dendrites into varicosities and inter-varicose regions. We found =synapses and total synaptic input per length were distributed uniformly along smooth dendrites. Synapses along varicose dendrites, however, occurred preferentially at varicosities. While synaptic input per length along varicosities was similar to that along smooth dendrites, both synapse number and total synaptic input per length along inter-varicose regions were lower. Dendritic segments were also analyzed for mitochondria and glycogen content. We found that mitochondria and glycogen were also distributed uniformly along smooth dendrites. In varicose dendrites, however, mitochondrial volume and glycogen granule numbers were highest in varicosities, while inter-varicose regions were comparatively devoid of these resources. The spatial distribution of synapses and dendritic resources in both smooth and varicose dendrites was preserved at 2 h during TBS-LTP when compared to control conditions. These findings suggest that synapses occur along interneuron dendrites where dendritic resources are available. When dendritic resources are distributed uniformly throughout the dendrite, as occurs in smooth dendrites, synapse distribution is also uniform. Varicosities, on the other hand, represent resource-rich sites along varicose dendrites where synaptic clustering occurs.

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Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.20/C28

Topic: B.05. Synaptic Plasticity

Support: PRIN 2017HPTFFC

Title: A cartography of learning- and memory- related translation-dependent synaptic potentiation

Authors: *F. LATINI[1], A. JACOB[1], M. DI CAPRIO[1], M. MAINARDI[1,2], A. CATTANEO[1]; [1]Scuola Normale Superiore, Pisa, Italy; [2]Neurosci. Institute, Natl. Res. Council (IN-CNR), Pisa, Italy

Abstract: Investigating the role of synaptic plasticity has been a core component of neuroscientific research. However, currently available methods are dependent on either somatic activation, identifying all synapses of a neuron regardless of potentiation status, or require a simulated activation of distinct synapses. Our aim is to provide a cartography of physiologically induced synaptic potentiation following a learning-related paradigm. This paradigm shift from a cell-centered to a spine-centered perspective can be achieved using SynActive (SA), a genetic tool which allows the expression of a protein of interest specifically at synapses subjected to activity- or learning -dependent potentiation by exploiting regulatory sequences from the Arc mRNA. Here, we combined GFP Reconstitution Across Synaptic Partners (GRASP; Choi et al., 2018) with SA. The post synaptic moiety of split GFP was placed under the control of SA regulatory elements, conferring GFP reconstitution only at potentiated synapses to obtain SA-GRASP. After extensive validation in cultured neurons, we used SA-GRASP to map synaptic potentiation at specific monosynaptic circuits in vivo. We employed two pairs of AAVs, encoding: (a1) tetracyclin-responsive element (TRE3g)-controlled presynaptic half of GRASP; (a2) presynaptic label (mTurquoise-2 blue fluorescent protein) and the reverse tetracyclin-responsive transactivator (rtTA); (b1) TRE3g- and SA-controlled postsynaptic half of GRASP; (b2) postsynaptic label (tdTomato) and rtTA. The “TetON” system allowed control over the temporal window for SA-GRASP transcription. We performed stereotaxic injections in mice hippocampi, delivering AAVs a1-a2 to the CA3, b1-b2 to the CA1. To handle the data generated, we have designed a semi-automated pipeline for image analysis of CA1 apical dendrites. Using this technique, we have been able to determine the spatial distribution of learning-associated CA3-CA1 potentiated synapses following a contextual fear conditioning associative learning protocol. Our analysis indicates a spatially nonuniform and clustered pattern, lending support to the notion that specific memories are encoded in neurons through the recruitment of specific subsets of dendritic spines. Moreover, our approach has allowed us to compare the distribution of CA3-CA1 potentiated spines after the encoding and recall phases of contextual fear conditioning.

Disclosures: F. Latini: None. **A. Jacob:** None. **M. di Caprio:** None. **M. Mainardi:** None. **A. Cattaneo:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

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Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01 NS062736
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ARCS Foundation Fellowships

Title: Prior potentiation initiates a synapse-specific refractory period for plasticity at individual dendritic spines

Authors: *J. FLORES[1], K. M. ZITO[2];
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Abstract: Learning is crucial for survival. One intriguing aspect of learning in humans is that the efficiency of learning can be improved when there are breaks between episodes of learning. One area of the brain important for formation of long-term memories is the hippocampus. In hippocampal circuits, it has been shown that long-term potentiation (LTP) of synaptic strength, a cellular mechanism proposed to underlie learning, is more effective when repeated stimuli are temporally spaced. Excitatory synaptic connections in the hippocampus occur at small protrusions on dendrites called dendritic spines, which increase in size as synaptic strengths are increased during learning. We propose that the spaced timing requirement for learning is the result of a synapse-specific temporary refractory period induced by prior LTP at individual dendritic spines. Here, we use 2-photon glutamate uncaging and time-lapse imaging to show that individual dendritic spines, which exhibit long-term growth and AMPAR insertion in response to LTP-inducing glutamatergic stimuli, are unable to exhibit further plasticity in response to the same stimulus 30 minutes later. Importantly, we observed that size-matched spines on the same cell undergo normal plasticity, supporting that the refractory period is restricted to stimulated spines. We also observe that when the interval between stimuli is increased to 60 min, plasticity is recovered, indicating that the required molecular signaling pathways have recovered within this time frame. Notably, several labs have shown that a subset of postsynaptic molecules regain their normal expression levels at newly potentiated synapses only after a delay of around 60 min. We thus hypothesized that the refractory period for further spine growth and synaptic strengthening may be due to a delay in the arrival of critical postsynaptic scaffolding molecules in newly potentiated spines. Indeed, we show that increasing the expression level of the synaptic scaffolding molecule PSD95, but not PSD93, allows spines to grow in response to a second glutamatergic stimulus. Importantly, enhanced PSD95 is able to overcome the refractory period for plasticity independent of AMPAR function. Our results at single spine synapses are consistent with recent work on saturation of plasticity at the circuit level from the lab of Kristen Harris. This work furthers our understanding of how the relationship between timing and synaptic composition influence plasticity at individual synapses.

Disclosures: J. Flores: None. **K.M. Zito:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.22/C30

Topic: B.05. Synaptic Plasticity

Support: DFG EXC 2049: NeuroCure
DFG OR 547/2-1

Title: Role of Synapsin in hippocampal mossy fiber plasticity

Authors: F. BRUENTGENS[1], L. MORENO-VELASQUEZ[1], A. STUMPF[1], F. BENFENATI[2], D. MILOVANOVIC[3], D. SCHMITZ[1,3], ***M. ORLANDO**[1];
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Abstract: Hippocampal mossy fibers form giant, very plastic synapses that provide a strong and sparse input onto CA3 that is thought to be important for learning and memory (Rolls, 2018). These synapses have a low basal release probability and display frequency facilitation, post-tetanic potentiation (PTP), and a cAMP-dependent presynaptic form of long-term potentiation (LTP) (Nicoll and Schmitz, 2005; Shahoha et al., 2022; Weisskopf and Nicoll, 1995).
Synapsins are the most abundant neuronal phosphoproteins. Due to their phosphorylation-dependent association with synaptic vesicles (Chi et al., 2003; Sansevrino et al., 2023) they play an important role in synaptic transmission and plasticity (Cesca et al., 2010).
Mossy fiber physiology and ultrastructure have been investigated in Synapsin I, II double knock-out mice (Owe et al., 2009); but not in mice lacking all Synapsins: Synapsin triple-knock-out (SynTKO).
Since mossy fiber boutons, differently from most synapses, retain Synapsin III expression in the mature brain (Pieribone et al., 2002), we sought to determine if the additional knockout of Synapsin III would influence mossy fiber boutons structure and function. To this aim we combined transmission electron microscopy and local field potential recordings of acute hippocampal slices from SynTKO and wild type (WT) mice.
In line with previous experiments from Synapsin double knock-out animals (Owe et al., 2009), we observed that the response to 1 Hz frequency stimulation and the PTP after high-frequency stimulation were lower in SynTKO animals. This phenomenon is likely a consequence of the smaller reserve pool of vesicles observed at the electron microscope.
We also measured LTP and saw that, 30 minutes after induction, SynTKO mossy fibers displayed an increased potentiation when compared to WT. We had previously found an increase in the active zone density in WT animals after chemical induction of presynaptic LTP with Forskolin (Orlando et al., 2021). Here, we performed a similar ultrastructural analysis and found an even greater structural remodeling of chemically-potentiated SynTKO mossy fiber boutons. This finding could - at least partially - explain the increased potentiation seen in electrophysiological recordings. Such changes in long-term potentiation have not been described before in any other Synapsin knock-out model, suggesting a possible Synapsin III specific role as a “brake” for mossy fiber potentiation.

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Poster

PSTR187. Structural Plasticity: Synapses

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.23/C31

Topic: B.07. Network Interactions

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JST-CREST JPMJCR21E2
JSPS KAKENHI Grant Grant 21H00217
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The Naito Foundation

Title: Thalamo-cortical synaptic network in motor cortex analyzed with ATUM-SEM

Authors: *Y. KUBOTA[1,2,3], E. KURAMOTO[4], N. EAGAWA[1], T. ETO[1], Y. KAWAGUCHI[1,5]; [1]Natl. Inst. Physiol Sci. (NIPS), Okazaki, Japan; [2]Dept Physiological Sci., The Grad. Univ. for Advanced Studies (SOKENDAI), Okazaki, Japan; [3]Ctr. for Brain Sci., RIKEN, Wako, Japan; [4]Kagoshima Univ., Kagoshima Univ., Kagoshima, Japan; [5]Brain Sci. Inst., Tamagawa Univ., Machida, Japan

Abstract: It is well known that the cortex receives signals from the basal ganglia and cerebellum via thalamo-cortical afferents. Our study using in vivo imaging of primary motor cortex showing spine dynamics during motor leaning demonstrated that the thalamic inputs on tuft dendrites of layer 5 pyramidal tract neuron play an important role in execution of automated hand movement (Sohn *et al*., *Science Advances,* 2022, 8(30): eabm0531). To understand this intriguing cortical microcircuit architecture further, we investigated how the thalamo-cortical axon terminals participate in the cortical microcircuit in rat motor cortex. The axon arborization pattern across cortical layers varies significantly among thalamocortical afferents from three motor-related thalamic nuclei: the ventral medial nucleus (VM), the ventral anterior (VA) and the ventral lateral (VL) thalamic complex, which relay motor information from the basal ganglia (VM/VA) and the cerebellum (VL), respectively (Kuramoto *et al.*, *Cereb Cortex* 2015, 25: 221-235). We hypothesized that the synaptic connections of VM/VA and VL afferents in the cortical microcircuits are different. A viral vector (pal-GFP AAV) was injected into each of three motor-related thalamic nuclei. Their target structures in the motor cortex was investigated using a correlated light and electron microscopy (CLEM) with a laser confocal microscopy and automated tape-collecting ultramicrotomy (ATUM) with scanning electron microscopy (SEM). To identify the GFP labeled axonal fibers subsequently at the electron microscopy, we stained blood vessels with lectin, and cellular nuclei with DAPI, and used them as landmarks in cortical tissue sections. Firstly, images were taken with a laser confocal microscopy. Then the tissue sections were embedded in plastic and sectioned with ATUM for SEM observation. GFP-labeled thalamo-cortical fibers and their target structures were identified in serial electron micrographs, and reconstructed three-dimensionally. Our preliminary results indicated that the VA fibers mainly targeted dendritic spines of the layer 5 pyramidal cell.

Disclosures: **Y. Kubota:** None. **E. Kuramoto:** None. **N. Eagawa:** None. **T. Eto:** None. **Y. Kawaguchi:** None.

Poster

PSTR187. Structural Plasticity: Synapses

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Program #/Poster #: PSTR187.24/C32

Topic: B.05. Synaptic Plasticity

Support: NSF GRFP
NSF NeuroNex

Title: Dendritic circuit reorganization in the adult dentate gyrus

Authors: *A. SOROKINA[1,3], V. SAMPATHKUMAR[1,3], N. MEDINA[1], N. B. KASTHURI[2,3]; [2]Neurobio., [1]Univ. of Chicago, Chicago, IL; [3]Argonne Natl. Lab., Lemont, IL

Abstract: While the majority of synaptic reorganization is thought to occur in the first few weeks of life, regions like the dentate gyrus (DG) of the hippocampus have been shown to be particularly plastic and structurally dynamic further into adulthood. A better understanding of how these synaptic rearrangements occur could lead to a better appreciation of how the DG processes info for learning and memory. Here we address that gap, using large volume serial-section electron microscopy, ‘connectomics’, to investigate how dendritic circuitry undergoes age-dependent changes in the DG of adult mice. Specifically, we focused on changes in connectivity between dendrites through multi-synaptic boutons (MSBs) - axonal boutons that form synapses with more than one synaptic partner. MSBs are more enriched in DG circuits than in other brain regions, so we hypothesized that changes in their connectivity patterns would be important for DG. We reconstructed dendritic fragments, their associated spines and synaptically connected boutons, and the other postsynaptic partners of those boutons at P56 and P115 in the DG. We found distinct patterns of connectivity between neighboring dendrites mediated by MSBs in different age groups. Analysis of a younger adult mouse (P56) showed a greater degree of shared input among neighboring dendrites through MSBs compared to an older adult (P115). These results indicate a remodeling of dendritic circuitry during adulthood, suggesting a potential age-related refinement of neuronal connectivity within the dentate gyrus. For example, at P56, axons are more likely to innervate the same "cohort” of dendrites compared to P115. We found these changes cannot be explained by simple geometry, suggesting a specificity to these rearrangements. Future work will include an additional time point at P30 to provide a trajectory of dendritic connectivity within the dentate gyrus as the adult brain matures and ages. Overall, our study highlights age-dependent alterations in dendritic circuitry, and the dynamic nature of adult neural connectivity. These findings shed light on processes involved in the formation and refinement of adult neuronal networks in the dentate gyrus, which may have significant implications for our understanding of learning, memory, and age-related cognitive decline.

Disclosures: **A. Sorokina:** None. **V. Sampathkumar:** None. **N. Medina:** None. **N.B. Kasthuri:** None.

Poster

PSTR187. Structural Plasticity: Synapses

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.25/C33

Topic: B.05. Synaptic Plasticity

Support: Brain Initiative NIH

Title: Exploring Somatic Inhibition in the Mouse Cortex: A Connectomics Approach

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Abstract: Cortical interneurons play a critical role in information processing in neural circuits. However, despite their importance, the connectivity rules of inhibitory neurons are relatively understudied compared to their excitatory counterparts, despite having greater neuronal and synapse diversity. In this study, we use large-volume serial section scanning electron microscopy (EM) “connectomics” to address that gap. Specifically, we explore differences in the connectivity patterns of a single interneuronal type, the parvalbumin expressing interneuron (PV), which innervates the soma of excitatory neurons. We focus our initial work on how PV innervation is similar and different across two sensory areas, primary visual (V1) and somatosensory (S1). First, we find the excitatory neurons in layer 2/3 of V1 receive 2 fold more synapses per soma than putatively similar neurons in layer 4 and by larger boutons. In S1 we find the inverse relationship, with S1 L4 excitatory neurons receiving more synapses per soma than S1 L2/3 neurons. Surprisingly, this difference does not correlate with differences in the density of PV soma in those cortical areas. Thus, we hypothesized that differences in PV axonal arborizations could explain differences in somatic innervation, e.g. perhaps PV neurons in V1 L2/3 branch and synapse more than L4 PV neurons (and vice versa in S1). To further dissect PV arborization patterns, we employed APEX, a genetically encoded EM marker, to label and provide detailed reconstructions of PV axons in multiple cortical areas. We conclude that interneuron innervation patterns are complicated and not easily predicted from the density of interneurons in a cortical area.

Disclosures: **N. Medina:** None. **G. Wildenberg:** None. **K. Koster:** None. **N. Kasthuri:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.26/C34

Topic: B.05. Synaptic Plasticity

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Title: Developing multiplex imaging for deciphering dysfunctional Ca^{2+}-dependent biochemical signaling under neuropathological conditions

Authors: *H. FUJII[1], H. KIDOKORO[2], Y. KONDO[1], M. KAWAGUCHI[2], S.-I. HORIGANE[4], J. NATSUME[2,3], S. TAKEMOTO-KIMURA[4,5], H. BITO[1];
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Abstract: Ca^{2+} transients are triggered by various neuronal events and their precise measurements are essential to investigate synaptic transmission, local dendritic spikes and action potential firing. Among downstream Ca^{2+}-dependent effectors, CaMKIIalpha and calcineurin stand out as they are critical for regulating neuronal plasticity, learning and memory. Thus, better understanding of how Ca^{2+} and the downstream kinase and phosphatase signals are activated during cognitive processes, and deciphering the dynamics of their spatial and temporal codes, are fundamental, yet unanswered, questions in neuroscience. To begin to address this issue, we previously developed a dFOMA (dual FRET imaging with Optical Manipulation) method for simultaneous measurements of two distinct biochemical signals, and demonstrated that CaMKIIalpha and calcineurin activations operated as distinct chemical decoding readouts of different parameters contained in the patterned neuronal input. To further explore this, an updated dFOMA2.0 method was generated by integrating brighter and more selective donor/acceptor FRET pairs, while also developing new/improved fluorescent probes for Ca^{2+}, CaMKIIalpha and calcineurin signaling. By combining a CaMKIIalpha FRET probe and a linearly performing red-color Ca^{2+} indicator, we developed a FRET-based kinase phenotyping system that is selective, sensitive, quantitative, and scalable platform for gaining functional insights into disease-causing rare gene mutations found in causative kinase coding regions. We applied this system to an intellectual disability (ID)-causing mutation in CAMK2A gene and

discovered that in many rare variants, Ca^{2+}/CaM-dependent activation in neurons was aberrantly facilitated and showed faster activation and more delayed inactivation. The altered kinetics was accompanied by a leftward shift in the CaMKIIalpha input frequency tuning curve. This is compelling evidence suggesting that abnormalities in biochemical decoding indeed underline the pathogenesis of CAMK2A-related ID, and illustrates the power of advancing multiplex imaging of biochemical signaling to deciphering the etiology of the neuropathological diseases.

Disclosures: H. Fujii: None. **H. Kidokoro:** None. **Y. Kondo:** None. **M. Kawaguchi:** None. **S. Horigane:** None. **J. Natsume:** None. **S. Takemoto-Kimura:** None. **H. Bito:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.27/C35

Topic: B.05. Synaptic Plasticity

Support: CIHR PJT-168958
CRCP 950-232476

Title: Intersecting vertebrate-specific ubiquitination and splicing programs tune aggression

Authors: A.-A. MULLIN[1,2], R. NIIBORI[2], B. J. BLENCOWE[3], ***S. P. CORDES**[4,5];
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Abstract: Hyper-aggressiveness can be problematic for patients with neurodevelopmental disorders, such as Cri du Chat Syndrome (CdCS) and autism spectrum disorder (ASD), or ones suffering from neurodegenerative conditions, such as Alzheimer's disease. Protein modification by attachment of linear ubiquitin (Met1Ub) chains, in which one ubiquitin is fused to the N-terminal tail of another, is unique to animals. Met1Ub chains are generated by the linear ubiquitin assembly complex (LUBAC), comprised of its catalytic component HOIP along with HOIL and SHARPIN [4-7], and can be removed by the Met1Ub deubiquitinase OTULIN. While OTULIN is often reduced in CdCS patients and genome-wide association studies implicate disruption of Met1Ub homeostasis in Alzheimer's disease, the neurobiological roles of Met1Ub remain to be elucidated.Here we report that Met1Ub modification of select excitatory neurotransmitter receptors is linked to increased reactive aggression. We found that reducing *Otulin* in male mice causes increased aggression as assessed by the resident-intruder test (n>15/genotype). Increased levels of glutamate receptors have been shown to mediate reactive aggression in human and mice. Accordingly, immunofluorescent analyses of primary cortical neuronal cultures and immunoblot analyses of cortical synaptosomes revealed increased excitatory glutamate receptors, cincluding glutamate ionotropic receptor NMDA type subunit 1 (Grin1), at synapses in $Otulin^{+/-}$ mice and ones lacking *Otulin* in neurons ($Otulin^{Neuro\text{-}K0}$) (n= 3

animals/genotype/experiment). Consistent with a role for dynamic Met1Ub modification of synaptic proteins, both HOIP and OTULIN are enriched in synaptosomes. Supporting the possible attachment of Met1-Ub to excitatory receptors, a Met1-Ub-specific pulldown assay (UBAN Pulldown) captured Grin1. Finally, we discovered an intersection between the Met1Ub pathway and a vertebrate- and neuron-specific microexon splicing pathway, which is down-regulated in a substantial fraction of ASD cases. We identified microexons that specifically regulate Met1Ub modified synaptic protein levels and thereby excitatory-inhibitory neurotransmission balance, which is often disrupted in ASD. These intersecting programs allow 'fine-tuning' of excitatory neurotransmission and provide insight into how nuanced behaviors like reactive aggression can be regulated in vertebrates.

Disclosures: **A. Mullin:** None. **R. Niibori:** None. **B.J. Blencowe:** None. **S.P. Cordes:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.28/C36

Topic: B.05. Synaptic Plasticity

Support: NIH Grant MH095980

Title: Ultrastructure evidence for hyperexcitability in the hippocampus of cognitively impaired aged rats

Authors: *L. M. KIRK, J. FALCO, D. HANKA, K. HARRIS;
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Abstract: The hippocampus, a brain structure important for learning and memory, is vulnerable with aging. Previous studies have shown that CA3 pyramidal neurons in the aged hippocampus have increased firing rates, while their primary target cells (CA1 pyramidal neurons) fire less frequently. These differences result in dysfunctional hippocampal circuitry that leads to impaired learning in aged animals. Functional changes during synaptic plasticity, learning, and memory are reflected through changes in spine and synapse structure. Although individual synapses are dynamic, the balance of excitatory and inhibitory (E/I) synaptic input onto dendrites is tightly regulated. Coordination of E/I synaptic function prevents runaway excitation and preserves associative learning in young adults. Here, we used a spatial navigation task to characterize hippocampal learning capacity in aged and young adult rats. 3D reconstruction (3DEM) of excitatory and inhibitory synapses along hippocampal dendrites from these animals revealed important effects of cognitive decline during aging on E/I balance. Our data show excitatory synapses from hippocampal CA1 stratum radiatum dendrites were larger in aged learning impaired rats than in young adult controls, while inhibitory synapse size did not change. Conversely, excitatory synapse density was unaltered while inhibitory synapse density decreased. Together, these changes led to an overall increase in the E/I ratio in CA1 dendrites

from learning impaired aged rats compared to young adults. These structural findings provide new understanding about how hyperexcitability could diminish cognitive capacity in aged animals.

Disclosures: **L.M. Kirk:** None. **J. Falco:** None. **D. Hanka:** None. **K. Harris:** None.

Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.29/C37

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01EY025613

Title: Prey capture induces structural remodeling of the mouse binocular visual cortex through higher spine dynamics and increased spine density

Authors: *D. BISSEN, B. A. CARY, S. D. VAN HOOSER, G. G. TURRIGIANO; Brandeis Univ., Waltham, MA

Abstract: The ability to learn and consolidate new knowledge and skills is crucial for survival and is mediated by the ability of neuronal connectivity to change in response to external stimuli. Such adaptations are proposed to be mediated through Hebbian changes in synapse number and/or strength, morphologically implemented by changes in spine density and size, thus leading to novel microcircuits embodying the new memory. Learning-induced structural plasticity has been primarily studied in stereotypical motor tasks or auditory fear conditioning; however, it remains virtually unexplored in visually-driven behaviors and more naturalistic settings. An ideal paradigm combining both aspects is prey capture in mice. This naturalistic, intrinsically rewarding and ethologically relevant learned behavior is heavily reliant on binocular vision. We have found in rats during the visual system critical period, when binocular vision is refined, that learning to hunt requires the primary visual cortex (V1) and induces dramatic changes in neuronal activity within binocular V1 (V1b). To assess the contribution of structural plasticity to these changes, here we follow spine density and dynamics onto layer 5 pyramidal neuron apical dendrites in V1b, using chronic *in vivo* imaging in awake critical-period mice as these animals learn to hunt.

We found that spine density starts increasing immediately after the first hunting session and reaches a plateau as the mice become experts at hunting. This increase is not observed in control mice, which undergo the same paradigm but receive immobilized prey during their “hunting” sessions. Careful examination of behavioral videos revealed that hunting and control mice show similar behavioral traits during prey approach, capture and consumption, thus strongly suggesting that the spine density increase is induced by the acquisition of new visually driven skills. This increase is mediated by an immediate and dramatic augmentation of spine formation, followed by a slower and more gradual increase in spine loss. Both spine formation and loss

remain elevated even when spine density reaches a plateau, suggesting that the network remains in a high dynamic state, even as spine density stabilizes at a higher value. Importantly, neurons in extrastriate visual areas remain unaffected, indicating that V1b plays a distinct role in prey capture learning. Taken together, our data show that prey capture learning induces structural remodeling within L5 of the mouse binocular visual cortex and shifts the local excitatory network into a more dynamic state. These changes within V1b are likely to contribute to behavioral improvement during this V1-dependent task.

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Poster

PSTR187. Structural Plasticity: Synapses

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: B.05. Synaptic Plasticity

Support: NIH Grant MH104536
NIH Grant NS117588

Title: Characterization of Synaptic Properties of Enteric Neurons Innervating the Drosophila Hindgut

Authors: *S. JETTI[1,2], J. T. LITTLETON[3];
[2]The Picower Inst. for Learning and Memory, [3]MIT, [1]MIT, Cambridge, MA

Abstract: The enteric nervous system is formed by a network of neurons that regulate multiple aspects of gut function, including digestion, motility, absorption, secretion, and excretion. Despite the functional significance, how enteric neurons regulate these properties is poorly understood. To further examine the *Drosophila* larval enteric nervous system, we characterized structural and functional synaptic properties of hindgut neurons by combining genetics, Ca^{2+} imaging, *in vivo* whole-cell patch-clamp physiology, and STED nanoscopy. To genetically label hindgut neurons, a Gal4 driver screen was performed that identified several driver lines that label distinct sub-populations of hindgut neurons. Unlike motoneurons, the enteric neuronal soma are located exclusively in the terminal A9 segment in the ventral nerve cord and innervate the hindgut to form bilateral enteric neuromuscular junctions (eNMJs). Unlike typical 3rd instar NMJs, eNMJs exhibit distinct structural properties. Immunohistochemical analysis suggests that subpopulations of enteric neurons use distinct excitatory neurotransmitters (glutamate & acetylcholine) and neuromodulators, suggesting hindgut synapses might employ multiple co-transmitters to communicate with the hindgut. eNMJs show two distinct innervation patterns that include elongated longitudinal eNMJs that innervate the hindgut and parallel eNMJs that branch out and innervate the rectum. Both eNMJs types form smaller boutons (~2µm) than typical larval NMJs and contain 2-4 active zones (AZs) per bouton. In addition, the density of AZs in the

hindgut is significantly lower and AZs are more spread apart. STED imaging revealed hindgut AZs show both similar and distinct characteristics compared to tonic and phasic abdominal NMJs. While AZs of longitudinal eNMJs have a variety of donut, triangular and square geometries, most parallel eNMJ AZs have triangular geometry. As such, hindgut synapses exhibit significant diversity in nanoscopic organization that might be important in diversifying their synaptic properties. We are examining the nanoscopic organization of key synaptic proteins and voltage-gated calcium channels to compare the molecular architecture of hindgut AZs with tonic (Ib) and phasic (Is) AZs. Somatic Ca^{2+} imaging revealed that pairs of hindgut neurons show correlated activity that might be relevant for driving hindgut motility. Current efforts are underway to characterize intrinsic and synaptic properties of these neurons using *in vivo* electrophysiology. Together, these findings suggest hindgut neurons have some unique properties that may facilitate their function within the enteric nervous system.

Disclosures: S. Jetti: None. **J.T. Littleton:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.01/C39

Topic: B.08. Epilepsy

Support: Dravet Syndrome Foundation Grant
NIH Grant 5R01NS094186
NIH Grant 5R35NS111573

Title: Adult Scn1a recovery improves and adult disruption impairs behavioral comorbidities inmouse models of Dravet Syndrome

Authors: *C. S. CHEAH[1], M. A. BECKMAN[2], W. A. CATTERALL[2], J. C. OAKLEY[1]; [1]Neurol., [2]Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Dravet Syndrome (DS) is caused by loss-of-function mutations in *SCN1A* encoding Type-1 brain sodium channel Nav1.1. DS causes febrile seizures in infancy and persistent cognitive and behavioral comorbidities. Genetic therapies have been most successful when initiated prior to seizure onset; however, perinatal treatment may not be possible in most cases. Here we leverage two parallel DS mouse models to determine the therapeutic window for initiating treatment and assess the impact of withdrawal in adulthood.
We have developed a novel mouse model utilizing Cre-Lox mouse genetics to correct *Scn1a* expression at any age ($Scn1a^{GTS/+}$) by excising a stop cassette inserted before exon 25, which fully restores expression upon Cre excision. Mice with globally expressed, tamoxifen-inducible Cre-recombinase (ERT2) were crossed with our $Scn1a^{GTS/+}$ and $Scn1a^{FLX/+}$ mice. Tamoxifen (TAM) was administered at $\geq$P90 and two weeks later, behavioral testing for hyperactivity in the open field, spatial memory assessed via contextual fear conditioning (CFC), and thermal

sensitivity to seizures were assayed. Comparisons were made between ERT2 positive and Cre-negative (WT) animals within each genotype.Following TAM treatment, *Scn1a* recovery animals (N=9) showed a trend to decreased hyperactivity in the open field compared to functional DS mice (N=9; p=0.27) and conversely, *Scn1a* disruption mice (N=12) showed a trend toward increased hyperactivity compared to functional WT (N=9; p = 0.28). In CFC, *Scn1a* recovery animals showed a significant improvement in long-term memory at one week (p = 0.003) compared to functionally DS, unrecovered controls. Conversely, *Scn1a* adult disruption animals showed a significant impairment at 24 hours (p = 0.02) and 1 week (p = 0.006) when compared to functionally WT animals. Following TAM treatment 4/16 ERT2:*Scn1a*$^{GTS/+}$animals had seizures prior to 41°C (40.4 +/- 0.3°C), compared to 12/12 WT:*Scn1a*$^{GTS/+}$ (39.3 +/- 0.2°C; p= 0.01, t-test). Conversely, 0/25 WT:*Scn1a*$^{FLX/+}$ animals had seizures compared to 28/34 ERT2:*Scn1a*$^{FLX/+}$ (39.9 +/- 0.8°C; p=4.3e-48, one-sided t-test).
Overall, adult recovery of *Scn1a* in previously DS animals improves, but does not fully recover, seizure susceptibility and behavioral comorbidities, whereas adult disruption of *Scn1a* in previously unaffected animals induces seizures and impairs cognition. These findings suggest that, even in adulthood, a significant portion of the seizures and behavioral co-morbidities of DS correlate with function of *Scn1a* rather than seizure history and indicate that the therapeutic window for treatment extends beyond the immediate postnatal period into young adulthood.

Disclosures: C.S. Cheah: None. **M.A. Beckman:** None. **W.A. Catterall:** None. **J.C. Oakley:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.02/C40

Topic: B.08. Epilepsy

Title: Activation of Hippocampal CA1 Parvalbumin-Positive Interneurons is Gradual in an Optogenetic Kindling Model in Mice

Authors: *F. C. TESCAROLLO[1], S. CHEN[2], H. SUN[2];
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Abstract: Impairments in the γ-aminobutyric acid (GABA) inhibitory signaling performed by Parvalbumin-positive interneurons (PV-INs) are described to occur during epileptogenesis, and are a critical factor for the increased seizure susceptibility observed in temporal lobe epilepsy (TLE). A dysfunctional GABAergic signaling as result of increased neuronal intracellular chloride concentration ([Cl^-]i) may generate abnormal synchronization of excitatory neurons, leading to overactivation. To investigate the activation pattern of PV-INs during epileptogenesis, we assessed the Ca^{2+} fiber photometry (FPh) of PV-INs in the hippocampus of freely moving mice across the progression of a focal optogenetic kindling protocol (OpK). Homozygous PV-cre

mice (n=4) expressing hChR2 in putative glutamatergic neurons in the left ventral hippocampal CA1 (for optogenetics), and CaMKIIα-RCaMP and hsyn-GCaMP in the bilateral dorsal CA1 (for simultaneous pyramidal and PV-positive neurons Ca^{2+} FPh, respectively), were submitted to an OpK model consisting of light-stimulation epochs at 50 Hz delivered every 30 minutes, 6x/day until each animal became fully kindled. ΔF/F was recorded from each epoch and the values were analyzed in 3s blocks ranging from -30s prior to the seizures onset (baseline) to 90s after the onset. The analysis of Ca^{2+} FPh revealed that PV-INs activate gradually across the progression of OpK, while excitatory neurons show constant levels of activation since the first optical stimulation in comparison to baseline ΔF/F ($P<0.0001$, 2-Way ANOVA). Significant ΔF/F deviation from baseline in PV-INs Ca^{2+} FPh first occurred in the stimulation epoch 10 (Total of 26 ± 2 epochs; $P<0.01$, 2-Way ANOVA) reaching maximum ΔF/F values in RS7 seizures. We also observed a significantly increased ΔF/F of PV-INs in relation pyramidal neurons 6-3s before the onset of RS7 seizures ($P<0.05$, Unpaired t-test), despite the selective activation of CaMKIIα-positive pyramidal neurons. The gradual PV-INs activation throughout kindling observed here is likely a response to a progressive chloride accumulation in neurons that occurs in kindling (Wang et al., 2018 - Brain Research) and epileptogenesis (Weiss, 2023 - Neurobiology of Disease), which might be contributing to the seizure aggravation observed in our experiment. This mechanism might also explain our observations about PV-INs activation prior to pyramidal neurons in the initiation of RS7 seizures, reinforcing the idea that excessive $[Cl^-]i$ promotes pro-epileptic roles of PV-INs in TLE (Magloire et al., 2019 - Nature Communications).

Disclosures: F.C. Tescarollo: None. **S. Chen:** None. **H. Sun:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.03/C41

Topic: B.08. Epilepsy

Title: Cell autonomous Purkinje cell hypoexcitability after cell-specific deletion of Scn1b in a mouse model of Dravet syndrome

Authors: *F. I. GUILLEN, M. A. HOWARD;
The Univ. of Texas at Austin, Austin, TX

Abstract: β1, encoded by *SCN1B*, is an auxiliary subunit that modulates aspects of voltage-gated ion channel behavior and plays critical roles in controlling neuronal excitability and brain development. While most known for interacting with $Na_v1.1$, β1 interacts with other voltage-gated Na^+, K^+ and Ca^{2+} channels, acts as cell adhesion molecule, and can be cleaved to enter the nucleus or be released extracellularly. Mutations in *SCN1B* have been reported in patients with a range of epileptic encephalopathies including Dravet syndrome (DS), a catastrophic infantile epileptic syndrome associated with life-threatening consequences (“comorbidities”) such lack of motor coordination (ataxia), autism-associated behaviors, and memory deficits that continue to

even when their seizures are medically controlled. The cerebellum is known to be involved in movement, cognition/memory, and social behavior, and shows high expression of *SCN1B* in both cerebellar Purkinje and granule cells, but its role in DS and epilepsy is understudied. Previous electrophysiological data from our lab showed that global loss of *Scn1b* causes hypoactivity in the cerebellar Purkinje cells in mice (P15-19). Similar hypoactivity of this cell type can cause movement disorders and autism in other neurological diseases. The relation between Purkinje cell dysfunction and neurological deficits of DS has been difficult to study due to the early age at which *Scn1b* knockout mice die (~P22). Our aim was to determine the role of β1 in cerebellar Purkinje cells (PCs) in the adult mouse brain. We crossed conditional *Scn1b* knockouts with a Purkinje cell-specific Cre line. These mice survived into adulthood, but at ~10 weeks began showing lack of motor coordination (ataxia) and other odd behaviors. We used whole-cell current clamp recordings of PCs in acute cerebellar slices from male and female Purkinje cell-specific *Scn1b* knockout (KO) and wild-type (WT) littermate mice, aged P70-98. We found that *Scn1b* KO PCs have decreased excitability compared to WT. KO PCs showed a reduction on the spontaneous and evoke firing. We also recorded voltage sag during injection of hyperpolarizing current. Compared to WT, KO PC voltage sag increased and input resistance decreased. Our findings of PC hypoexcitability in adult PCs are similar to our previous findings of Purkinje cell firing deficits in the *Scn1b* knockout mouse model. This indicates that this change is cell autonomous and due to loss of β1 rather than seizure driven changes. Thus, changes in cerebellar physiology may in part contribute to the motor, social, and cognitive deficits of DS even when seizures are controlled.

Disclosures: F.I. Guillen: None. **M.A. Howard:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR188.04/C42

Topic: B.08. Epilepsy

Support: R01NS036692
R01AG065836

Title: Pilocarpine induced status epilepticus causes de novo expression of perineuronal nets and altered excitability of CA1 neurons

Authors: *A. M. WOO[1], D. C. PATEL[2], B. P. TEWARI[2], H. SONTHEIMER[1];
[1]Neurosci. Dept., [2]Univ. of Virginia, Charlottesville, VA

Abstract: Epilepsy is a chronic disorder defined by spontaneous recurrent seizures, believed to be due to excitation-inhibition imbalances. Irregular neuronal activity is often associated with other cellular and molecular aberrations including neurodegeneration, astrogliosis, and malfunction of inhibitory interneurons. Recently, alterations in the extracellular matrix (ECM)

have been associated with acquired epilepsy in both human patients and experimental models. Tightly condensed structures of the ECM called perineuronal nets (PNNs) are of particular interest, as they mainly develop around inhibitory fast-spiking interneurons and are likely to contribute to the normal functioning of these cells. In this study, we examined PNNs in a pilocarpine mouse model of temporal lobe epilepsy (TLE), looking both at changes in the overall brain and hippocampal structure and at the individual cellular level. We observed a population of cells distributed through the *stratum radiatum* and *stratum lacunosum moleculare* (SLM) layers of the CA1 region of the hippocampus that appear to acquire PNNs *de novo* following pilocarpine-induced *status epilepticus* (PISE). Notably, mice that receive pilocarpine but fail to undergo SE and thus do not acquire epilepsy fail to express PNNs in this region, suggesting that the induction of PNNs is due to seizure activity. Patch-clamp recordings from these neurons in PISE epileptic mice show increased excitability compared to sham mice and pilocarpine-treated mice that did not develop *status epilepticus*. We propose that the formation of PNNs around these cells is associated with a compensatory or causative mechanism in the wake of PISE, with implications for other acquired forms of epilepsy.

Disclosures: A.M. Woo: None. **D.C. Patel:** None. **B.P. Tewari:** None. **H. Sontheimer:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR188.05/C43

Topic: B.08. Epilepsy

Support: T32 DA039080
F31 DA053796

Title: Interneuron FGF13 Regulation of Seizure Susceptibility

Authors: *S. LIN, A. GADE, H. WANG, A. GALANTE, P. MITRANO-TOWERS, A. M. RAJADHYAKSHA, G. S. PITT;
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Abstract: Fibroblast growth factor homologous factor 13 (FGF13) is a non-canonical member of the fibroblast growth factor (FGF) superfamily that is not a secreted growth factor but instead functions intracellularly as a voltage-gated sodium channel auxiliary protein. P**atients with disruptions or mutations in the X-linked *FGF13* have early onset cognitive impairment and febrile seizures. In mice, FGF13 has two major alternatively-spliced isoforms in the cerebral cortex, one (*Fgf13-S*) which is predominantly expressed in excitatory and another (*Fgf13-VY*) in inhibitory cells.** To study cell-type specific *Fgf13* expression, we generated mice lacking FGF13 throughout *Emx1*-expressing glutamatergic excitatory neurons or *Gad2*-expressing GABAergic interneurons. Female mice heterozygous for *Fgf13* in interneurons suffer spontaneous seizures, consistent with the human phenotype, and hemizygous male knockout

mice die perinatally. Mice with *Fgf13* deficiency in *Emx1*-targeted glutamatergic cells survive through adulthood without evidence of seizures. *Gad2*^{Fgf13} KO mice are susceptible to hyperthermia-induced seizures, while *Emx*^{Fgf13} KO mice are not. To further define the functional role of interneuron FGF13, we recorded action potentials in *Gad2*^{Fgf13} KO interneurons alongside wildtype littermates. Interneurons in *Gad2*^{Fgf13} KO mice showed decreased excitability, likely driving the seizure phenotype. Only *Fgf13-S* rescued excitability. Our findings reveal cell type-specific roles of *Fgf13* in cortical development, likely driven by distinct splice variants and suggest that loss of *FGF13-S* in inhibitory neurons may underly the seizure phenotypes.

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Poster

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Topic: B.08. Epilepsy

Support: NIH Grant R01NS065020

Title: Modeling two-hit epileptogenesis: combining mTOR hyperactive excitatory neurons with interneuron loss

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Abstract: With consistent rates of medically refractory epilepsy and no preventative therapies, there is a pressing need to better elucidate the mechanisms underlying epileptogenesis. Hippocampal inhibitory interneurons have garnered significant interest for their potential role in epileptogenesis. Interneuron loss has long been identified as a hallmark pathology of epilepsy in both patients and animal models. Hippocampal somatostatin (SST) interneurons, specifically, have been shown to be reduced in temporal lobe epilepsy. Our lab previously developed a mouse model of epilepsy in which deletion of phosphatase and tensin homologue (Pten) from a subset of dentate granule cells (DGCs) constitutes the epileptogenic insult. In this model, the percentage of Pten KO granule cells can be experimentally manipulated, yielding low KOs (≈5% of DGC) with focal seizures, to high KOs (>10%) with convulsive seizures and interneuron loss. These findings led us to postulate that interneuron loss - evident in high KOs - is a critical second hit that leads to the development of severe epilepsy. To test this hypothesis, we have piloted a strategy to ablate SST interneurons from Pten mice with low KO rates, predicting that this combination will lead to convulsive seizures. To determine whether a “two-hit” model would be effective and not result in excess mortality, triple transgenic Gli1-CreER^{T2}, Pten$^{fl/fl}$, SST-FlpO

mice underwent stereotaxic injection of a FlpO dependent AAV9-diphtheria toxin receptor (DTr) expression vector. Selective expression of DTr in SST interneurons enabled their subsequent ablation with diphtheria toxin (DT). Key groups include low Pten knockout alone, SST interneuron ablation alone, and two-hit mice. Following baseline EEG recording, animals received treatments of either DT or saline. Monitoring continued for a minimum of two weeks post-treatment. All mice in the SST ablation and two-hit groups (n=6 per group) exhibited seizures in the two weeks following DT treatment. Additionally, 6/6 two-hit and 5/6 SST ablation-only mice had periods of protracted epileptiform activity. The two-hit and SST ablation only-groups each had one instance of seizure-related mortality. Findings demonstrate that this approach can yield a consistent epilepsy phenotype with limited mortality, providing a viable two-hit model for investigating how epilepsy phenotype is impacted by combinatorial epileptogenic insults. Ongoing work is being conducted to further analyze EEG data and evaluate epilepsy phenotypes longitudinally.

Disclosures: A.W. Drake: None. **M. Dusing:** None. **C.L. LaSarge:** None. **J.V. Ruksenas:** None. **M. Wesley:** None. **S.C. Danzer:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.07/C45

Topic: B.08. Epilepsy

Support: MOST 109-2314-B-182A-086-
MOST 109-2314-B-182-079-

Title: Optimization and Characterization of a Rat Model of Status Epilepticus and Chronic Epilepsy mimicking Human Mesial Temporal Lobe Epilepsy

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Abstract: Chronic epilepsies are more complicated than acute seizures that may involve different pathophysiological mechanisms. There is a need to develop reproducible animal models of chronic epilepsies relevant to human epilepsy syndromes for the comprehensive studies of epilepsy, anti-epileptic drugs, and discovery of novel therapeutic strategies. Mesial temporal lobe epilepsy (mTLE) is the most common type of focal epilepsy in adult humans and is refractory to medical treatment. Kainic acid (K.A.) rodent model of epilepsy is the most frequently used animal model for exploring the pathophysiology and mechanisms of human status epilepticus (SE) and mTLE. To develop a limbic epilepsy model that better reflects the characteristics of human mTLE and avoid wide-spread brain damage comparing to systemic administration of K.A., stereotactic surgery was performed on adult male Sprague-Dawley rats (250-300g). A

depth recording electrode was placed in the right CA1 region with a guide cannula positioned ipsilaterally, and a reference electrode in the contralateral frontal cortex. Wireless electrocorticography (ECoG) with local field potential transmitters were implanted for continuous long-term telemetry recordings. One week after surgery, we tested different strategies of K.A. application into right basolateral amygdala nucleus to optimize this animal model. Seizure activity, electrographic features, the epilepsy phenotypes and natural history of epilepsy were analyzed. SE and chronic limbic epilepsy was induced by a moderate dose K.A. injection. Complex partial SE with intermittent GTCs started 5-10 minutes after K.A. injection and persisted for 10-12 hours followed by intermittent cluster seizures for 12-24 hours, and gradually subsided then entered a latent period. Chronic spontaneous recurrent seizures emerged 1-3 weeks following K.A. administration, predominately presented as complex partial seizure-like behaviors and might accompanied with GTCs. The histology showed classical pathology features of mTLE with apparent unilateral hippocampal atrophy and obvious mossy fiber sprouting on ipsilateral side of epileptogenic zone. Take together the epilepsy phenotype and the pathohistological features, suggest our rat model more closely resembles human mTLE comparing with the mouse model or the systemic K.A. model. This optimized limbic epilepsy model exhibits a more consistent latent period, sustained and reproducible seizure frequency and low mortality rate that is more suitable for the application in epilepsy research and the development of novel therapies.

Disclosures: **P. Kao:** None. **W. Chang:** None. **B. Chang:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.08/C46

Topic: B.08. Epilepsy

Support: NIH Grant R01 NS092552

Title: Impact of tau expression on the development of acquired temporal lobe epilepsy

Authors: ***M. C. MOSELEY**[1], R. A. CLOYD[3], Y.-J. KANG[2], S.-H. LEE[2], B. N. SMITH[2]; [2]Biomed. Sci., [1]Colorado State Univ., Fort Collins, CO; [3]Physiol., Univ. of Kentucky, Lexington, KY

Abstract: Temporal lobe epilepsy (TLE) is the most common focal epilepsy in adults and is often resistant to anti-epileptic drugs. Understanding the mechanisms that drive the development of TLE is crucial to developing new specific therapies. A potential target for treatment and/or prevention of epilepsy is microtubule-associated tau protein. For example, genetic deletion or suppression of tau expression improves seizure outcomes in channelopathy models of genetic epilepsies. Tau expression may alter synaptic function and neuronal excitability, but the mechanism(s) by which lack of tau expression influences seizure susceptibility or TLE

development is unclear. To determine the impact of tau expression on epileptogenesis of acquired TLE, we treated tau$^{-/-}$ and C57BL/6 mice with an intrahippocampal injection of kainate (IHK). The IHK model induces status epilepticus (i.e., SE) in rodents and, after a delay, the development of TLE with spontaneous recurrent seizures (SRS; i.e., epileptogenesis). In this study we used mice that lack native murine tau (i.e., tau$^{-/-}$) and C57BL/6J mice. We assessed the severity of SE, epileptiform activity, and the development of SRS using video-electroencephalography (v-EEG). We further measured the impact of SRS on cellular excitability of dentate granule cells (DGCs) using in vitro whole-cell patch-clamp electrophysiology. We found that lack of tau expression is associated with resistance - but not elimination -- of SE induction and epileptogenesis. Strikingly, development of SRS in mice that lack tau expression resulted in greater synaptic dysfunction and cellular excitability compared to IHK-treated C57BL/6 mice. Results from this study highlight the role of tau in the development of acquired TLE. Subsequent studies will further investigate how tau alters DGC neurotransmission.

Disclosures: **M.C. Moseley:** None. **R.A. Cloyd:** None. **Y. Kang:** None. **S. Lee:** None. **B.N. Smith:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.09/C47

Topic: B.08. Epilepsy

Support: BT/PR20392/MED/122/26/2016 DBT ,Govt. India
BMI/11(58)/2022, ICMR, Govt. of India

Title: Altered semaphorin3F (SEMA3F) levels contributes to enhanced glutamatergic synaptic transmission in temporal lobe epilepsy (TLE)

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Abstract: Temporal lobe epilepsy (TLE), the most common form of drug-resistant epilepsy, is associated with enhanced glutamate receptor-mediated hyperexcitability in the temporal lobe structures. The mechanism of regulation of aberrant glutamatergic activity in TLE is still elusive. In this study, we investigated the contribution of semaphorin3F (SEMA 3F) to glutamatergic synaptic activity in the hippocampus and anterior temporal lobe (ATL) of TLE rats. We used Sprague-Dawley rats for developing a pilocarpine model of TLE and collected samples from the hippocampus and ATL. Seizure frequency in TLE rats was recorded using EEG device. TLE rats and control rats were injected with scrambled siRNA and with siRNA for Semaphorin 3F.

mRNA levels of SEMA 3F and its downstream effectors were evaluated using quantitative real-time PCR and immunohistochemistry was performed to assess protein expression. Whole-cell patch clamp technique was used to measure spontaneous glutamatergic synaptic transmission in the hippocampus and ATL. Golgi-Cox staining was conducted to visualize alterations in neuronal morphology, including their soma, axons, dendrites, and spines. Sholl analysis was performed to measure the length of apical and basal dendrites and spine density using Neurolucida software. Increased glutamatergic activity was observed in the hippocampus and ATL samples from TLE rats, with a more pronounced increase in the hippocampus. At mRNA level, significant upregulation of SEMA3F and AMPA receptor subunit GLUR4 was observed in the hippocampus and ATL of the TLE rats. We observed a significant reduction in the seizure frequency in TLE rats injected with siRNA for SEMA 3F. Although, no changes in SEMA3F mRNA levels or AMPA receptor GLUR4 expression were observed in the hippocampus and ATL in TLE rats injected with siRNA for SEMA 3F. But protein expression of SEMA3F and GLUR4 was decreased in these siRNA-injected rats. Furthermore, in the hippocampal pyramidal neurons of TLE rats, we observed altered lengths of apical and basal dendrites, changes in spine density, and modifications in soma architecture. This study provides the first direct evidence that altered levels of SEMA3F could be contributing to enhanced AMPA receptor-mediated glutamatergic synaptic transmission in TLE rats. This explorative study suggests a tight association between the inhibition of SEMA 3F and a reduction in seizure frequency in TLE rats.

Disclosures: V. Dubey: None. **A. Srivastava:** None. **A. Aryan:** None. **A. Dixit:** None. **M. Tripathi:** None. **P. Chandra:** None. **J. Banerjee:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.10/C48

Topic: B.08. Epilepsy

Title: A region-specific alteration in the expression and activity of Protein Tyrosine Kinase 2 in temporal lobe epilepsy

Authors: *O. SHANKER[1], S. KUMAR[2], J. BANERJEE[3], M. TRIPATHI[4], P. CHANDRA[5], F. SIRAJ[6], A. DIXIT[2];
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Abstract: Epilepsy is among the most prevalent neurological diseases affecting more than 65 million people worldwide. Temporal lobe epilepsy (TLE) is one of the most common forms of drug-resistant epilepsy and its precise etiology is still unknown making it imperative to

understand the molecular pathways contributing to the disease. Previous studies have shown Pyk2 activation in stress-mediated pathways in the brain and an alteration in tyrosine-phosphorylated Pyk2 in activated microglia in response to seizures but the region-specific changes in the expression and activation of Pyk2 are unknown which is crucial to be investigated as TLE is a distributed network disorder. We thus aimed to assess the region-specific alteration and activation of Pyk2 using the lithium pilocarpine model of epilepsy and TLE patient samples. For this purpose, lithium pilocarpine was used to induce status epilepticus in adult Male Sprague Dawley rats (n=15) and the results were compared to control rats that received saline (n=15). The hippocampus, Anterior temporal lobe (ATL) and neocortex were resected to assess region-specific variations. qRT-PCR analysis revealed a significant upregulation (*$p<0.005$) in Pyk2 mRNA only in the epileptic hippocampus as compared to the control and western blot analysis revealed a significant upregulation (*$p<0.005$) in the levels of Phospho(Tyr402)Pyk2 in the hippocampus and ATL region of TLE rat as compared to control. No significant change was observed in the neocortex. These results were concordant with the cell-specific expression of Phospho-Pyk2 analysed using immunofluorescence. There was no significant change in the levels of the unphosphorylated form of Pyk2 in TLE suggesting an epilepsy-induced activation of Pyk2 which was further evaluated using kinase assay. which revealed a significant increase (*$p<0.005$) in Pyk2 activity in the hippocampus of the TLE model as compared to the control. The findings from patient samples (n=10) as compared to autopsy controls (n=8) also showed a significant upregulation (*$p<0.005$) in Phospho-Pyk2 in epileptic hippocampal and ATL samples. Since Pyk2 can be activated in response to calcium, flow cytometry analysis was done to observe the levels of calcium in all three regions and a positive correlation was obtained between activated Pyk2 and increased calcium levels in the hippocampus and ATL of TLE rat as compared to control. Overall, the results for the first time showed a region-specific alteration and activation of Pyk2 possibly in a calcium-dependent manner emphasizing its role in the generation of independent epileptogenic networks and implying a possible contribution of Pyk2 in the pathogenesis of epilepsy.

Disclosures: **O. Shanker:** None. **S. Kumar:** None. **J. Banerjee:** None. **M. Tripathi:** None. **P. Chandra:** None. **F. Siraj:** None. **A. Dixit:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.11/C49

Topic: B.08. Epilepsy

Support: Council of scientific and industrial research (CSIR), India

Title: Dysregulation of Histone deacetylase 4/Serum response factor axis in a region specific manner in temporal lobe epilepsy

Authors: *S. KUMAR[1], O. SHANKER[2], J. BANERJEE[3], M. TRIPATHI[4], P. CHANDRA[5], F. SIRAJ[6], A. DIXIT[7];
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Abstract: Temporal lobe epilepsy (TLE) is a neurological condition characterized by spontaneous and recurrent seizures and is often associated with pharmaco-resistance necessitating neurosurgical interventions in about one-third of patients. The molecular mechanisms involved in its pathogenesis are poorly understood, demanding further investigations. Previous reports from our lab have shown an altered expression of histone deacetylases (HDACs) in MTLE-HS patients however the precise mechanism by which they contribute to TLE remains elusive. Among class II HDACs, HDAC4 dysregulation is reported in several neurological disorders but its specific molecular substrates and precise mechanism by which it contributes to TLE is unknown. Also, its role in the establishment of distinct epileptogenic networks is not determined. Thus, our study aimed to examine region-specific differences in HDAC4 expression and characterise its interaction with a non-histone substrate. For this purpose, we performed a string network analysis and identified serum response factor (SRF) as a non-histone substrate of HDAC4. Next, a lithium pilocarpine model of TLE was developed using adult male Sprague Dawley rats (n=15) and controls were injected with saline (n=15). The hippocampus, anterior temporal lobe (ATL) and cortex were resected for investigating region-specific variations. qRT-PCR, western blotting and immunofluorescence assay were performed to reveal a significant up-regulation (*$p<0.005$) in the levels of HDAC4 in the hippocampus region of the TLE model as compared to the control. No significant changes were observed in the ATL and cortex. Co-immunoprecipitation performed to characterize HDAC4-SRF interaction revealed a decreased HDAC4-SRF interaction only in the epileptic hippocampus indicating a dysregulated HDAC4/SRF axis caused by TLE. Since HDAC4 phosphorylation is involved in its nuclear export and its dissociation from SRF, the levels of Phospho(Ser-632)HDAC4 were investigated using western blotting and a significant increase (*$p<0.005$) was observed only in the epileptic hippocampus as compared to control. These findings were consistent with the results obtained using surgically resected patient samples (n=10) as compared to autopsy controls (n=6). The results for the first time reveal a region-specific alteration in the levels of HDAC4 and suggest a possible dysregulation of the HDAC4/SRF axis in TLE which might be contributing to its pathogenesis. The results further implicate that the mechanisms underlying the formation of epileptogenic networks vary in the three regions and HDAC4 might be playing a crucial role in this process.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.12/C50

Topic: B.08. Epilepsy

Support: 1R21NS125552-01

Title: Senescence-specific killing compound 1 alleviates seizure burden in a model of temporal lobe epilepsy

Authors: *D. J. MCFALL[1], A. HUSSAIN[2], T. KHAN[3], P. A. FORCELLI[4];
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Abstract: While there are dozens of anti-seizure medications available for patients with epilepsy, up to one third of patients experience seizures refractory to treatment. Identifying treatments that prevent epileptogenesis is an unmet need. Epileptogenesis involves DNA damage, apoptosis, and inflammation, features also relevant to the cellular senescence program. Senescent cells (SCs) arise in response to extreme stress or injury, leading to a decline in normal functioning and increased inflammation. Critically, the role of SCs in epileptogenesis is understudied. Ongoing studies from our group show that both genetic and pharmacologic ablation of SCs normalizes spatial memory and reduces seizure burden in the status epilepticus (SE) mouse model. However, no studies have looked at newer senolytic therapies, such as senescence-specific killing compound 1 (SSK1), a prodrug which requires β-galactosidase action to activate its gemcitibine core (Cai et al., 2020). Here, we investigate the effects of SSK1 on seizure burden and behavioral comorbidities in the SE mouse model.
4 month old wild type, C57Bl/6 mice were injected with pilocarpine to induce SE and were monitored for 2 hours. Seizures were terminated with diazepam. Mice were then randomly assigned to receive either SSK1 (0.5mg/kg, IP) or a vehicle injection, twice a week, for the duration of the study. 2 months after SE, mice were tested in a battery of behavioral tests in order to assay object and spatial memory and anxiety. After behavioral testing, telemeters were implanted in the mice, and video EEG was recorded continuously for 2 weeks. Mice were then euthanized, and their brains were fixed for histology to confirm SC ablation. A similar number of animals were used from each sex. Analysis of behavior, EEGs, and histology were all performed blinded.
Treatment with SSK1 following SE eliminates ~25% of SCs in the hippocampus. SSK1 also appears to reduce a subset of spatial memory deficits seen following SE (object-location memory, but not navigational memory). Finally, SSK1 treatment also leads to a reduction of frequency, average duration, and total seizure burden.
These results demonstrate that SSK1 replicates a majority of the findings of earlier SC ablation methods. Interestingly, SSK1 does not rescue deficits in navigational memory, which may suggest that a higher threshold of SC ablation is necessary to rescue this phenotype. Further work may explore different dosing regimens, the incidence of off-target effects compared to other senolytics, and mechanisms affected by SSK1 (inflammation, plasticity). Overall, this study furthers our understanding of how SCs may be targeted to prevent or slow epileptogenesis.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.13/C51

Topic: B.08. Epilepsy

Title: Differential profile of anti-seizure medications in the rat amygdala-kindling model

Authors: *E. GRONLIER, J. VOLLE, C. HABERMACHER, B. CARABALLO, C. DUMONT, Y. ROCHE, C. ROUCARD;
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Abstract: Several unmet clinical needs remain, including resistance to anti-seizure medications (ASM) in 30% of epileptic patients. Preclinical models play an important role in the early discovery of new therapies for the symptomatic treatment of epilepsy. The rat amygdala-kindling model remains one of the few chronic models available to study epilepsy development. It consists in repetitive subconvulsive electrical stimulations that elicit gradual and progressive enhancement of neuronal activity and behavioral responses, culminating in generalized seizures. This model is highly predictive for detecting effective drugs acting on seizure propagation, from the initial focal seizure to the secondarily generalized convulsive seizure. This study was designed to assess the pharmacological profile of different types of ASM in this model. Sprague Dawley rats were chronically implanted with electrodes in the basolateral amygdala (BLA), parietal and prefrontal cortex. The progression of epileptic activity was evaluated in freely moving rats through electroencephalogram (EEG) recordings, accompanied with synchronized video recordings. Afterdischarges (AD) were defined as spikes and spike-waves appearing in the EEG signal in response to the stimulation. First, we applied a stimulation to determine the AD threshold (ADT) for each rat which is the minimum current intensity needed to evoke an AD. Once ADT was established, the kindling protocol was delivered once a day until the animal reach a stage 5 on Racine's scale for 3 consecutive days. Kindled rats were alternately treated with ASM (or respective vehicles): diazepam, ethosuximide, carbamazepine, retigabine and levetiracetam. Differential pharmacological responses were observed, for instance, diazepam at 3 mg/kg produced seizure control as measured by a reduction in motor components of the seizure and a reduction in afterdischarge duration (ADD). Conversely, ethosuximide at 100 mg/kg did not show any effect on motor components of the seizure nor on ADD. We reported distinct effects between focal and cortical EEG responses for the remaining three ASMs, which will be described in further detail in the results section. This study was intended to highlight translational aspects of the rat amygdala-kindling model to identify new entities with improved tolerability and efficacy on focal and generalized seizures. The combination of this model and a cross-over design will provide a decision-enabling screening platform for the identification of novel compounds for the prevention, treatment, and modification of epilepsy, wherein pharmacoresistant focal seizures constitute the greatest challenge for treatment.

Disclosures: **E. Gronlier:** A. Employment/Salary (full or part-time):; SynapCell Company. **J. Volle:** A. Employment/Salary (full or part-time):; SynapCell Company. **C. Habermacher:** A. Employment/Salary (full or part-time):; SynapCell Company. **B. Caraballo:** A. Employment/Salary (full or part-time):; SynapCell Company. **C. Dumont:** A. Employment/Salary (full or part-time):; SynapCell Company. **Y. Roche:** A. Employment/Salary (full or part-time):; SynapCell Company. **C. Roucard:** A. Employment/Salary (full or part-time):; SynapCell Company.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.14/Web Only

Topic: B.08. Epilepsy

Title: Transcranial Magnetic Stimulation: Antiapoptotic effects in rats subjected to amygdaloid kindling model.

Authors: *D. VÁZQUEZ HUERTA[1], W. MORENO[2], E. URIBE[2], A. PORTILLA[2], C. RUBIO[2], M. RUBIO OSORNIO[2];
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Abstract: Background: Epilepsy is the second most prevalent neurological disease in the world. It affects approximately 70 million people worldwide. One third of patients have poor response to conventional treatment, so the search for new non-invasive and easy-to-adhere therapeutic strategies, such as transcranial magnetic stimulation (TMS) is important to reduce refractoriness to targeted treatment **Objective:** This study aims to elucidate the neuroprotective mechanisms of transcranial magnetic stimulation in apoptosis secondary to epilepsy **Methods:** Five groups of 25 male Wistar Rats were available: control, Sham/Kindling, Kindling, Kindling/TMS, and Sham/TMS. After performing the kindling amygdaloid model, TMS was administered daily at 1 Hz and 50 gauss. Rats were sacrificed by intracardiac perfusion and cerebral cortex, hippocampus and cerebellum were obtained for protein quantification of Bcl-2 and Caspase-3 by immunofluorescence and Western blotting. Protein quantification was analyzed in ImageJ software. SPSS was used for statistical analysis. **Results:** After-discharge time decreased 70% in the group of rats subjected to transcranial magnetic stimulation compared to Kindling rats. In the statistical analysis, a considerable increase of Bcl-2 ($p<0.0003$) was observed, favoring the groups submitted to transcranial magnetic stimulation, in addition to a decrease of caspase-3 ($p<0.05$) but only in cerebellum and cortex in the groups submitted to transcranial magnetic stimulation compared to Kindling groups **Conclusions:** The theory of neuroprotection could be fulfilled under the parameter of increased Bcl-2 in epileptogenic areas, post TMS, these findings may be related to the decreased post-discharge time.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.15/C52

Topic: B.08. Epilepsy

Title: A BEHAVIOURAL AND BIOMARKER ASSESSMENT OF MOUSE CORNEAL KINDLING AS A MODEL FOR EPILEPSY, CNS NEUROINFLAMMATION AND GLIOSIS

Authors: ***G. A. HIGGINS**[1], L. B. SILENIEKS[1], A. BERNARDO[1], D. FENG[1], L. STAM[1], J. VINTUKS[1], J. PRENDERVILLE[2], R. WINTERS[2], M. MITSOGIANNIS[2];
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Abstract: Corneal kindling represents a robust model for testing pharmacological approaches to treat epilepsy and its associated comorbidities. This study aimed to characterize the corneal kindling process in two mouse strains, C57BL/6J (C57) and CD-1 and to evaluate the comparative efficacy of three reference antiepileptic drugs (Carbamazepine (CBZ), Sodium Valproate (VPA), and Levetiracetam (LEV)). In addition, we have examined the mice for concomitant behavioural changes associated with epilepsy-related comorbidities such as anxiety, depression using tests such as light/dark box, canopy test, forced swim, and fear conditioning. Also, we have conducted biomarker and histological assessments of sham vs. kindled brain tissue to examine indices of neuroinflammation and gliosis. Initial strain studies compared C57 and CD1 mice on rate to develop kindled seizures. Both groups (n=30 and 20 respectively) were treated with twice daily kindling sessions each consisting of a 3s 2mA stimulus delivered by corneal electrodes. The number of kindling sessions to Stage 5 seizures (Racine scale) was 15.4±0.4 (C-57) vs. 28.9±2.5 (CD-1). C57 mice also had a lower attrition rate, with 95% of C57 mice reaching and maintaining a kindled state over repeated testing. Drug studies were conducted in fully kindled mice, using a repeated measures design. Calculated ED50's for LEV, CBZ and VPA were found to be similar between each strain (e.g. VPA ED_{50} 305 mg/kg C57; 278 mg/kg CD1). Behaviourally, fully kindled mice were found to be hyperactive in an open field (e.g. C57: Sham 5966±433cm, kindled 10199±364cm; $P<0.05$) with a significant thigmotaxis, indicative of anxiety. Performance in the exploratory based anxiety tests support this interpretation. Based on these findings, our studies have now focussed on the C57 mouse strain. To gain insights into astrocyte and microglial activation, GFAP and Iba1 immunohistochemistry (IHC) was performed. Nissl staining and NeuN IHC was used to investigate neurodegeneration. Fully kindled mice exhibited robust increases in both astroglial and microglial expression - notably in CNS regions such as hippocampus (HC). For example, HC GFAP % area expression: Sham 5±1%; Kindled: 18±3%; $P<0.01$. There was no clear evidence of neurodegeneration in any HC subfield (CA1-4, dentate gyrus) between Sham or fully kindled mice. Cytokine analysis was also performed for biomarkers of inflammation, including

TNFα, IL-1β, IL-6. Taken together, these data provide valuable insights into the corneal kindling process as it relates to behavioural changes and neuroinflammatory biomarkers - and consequently an approach to study novel treatments across these domains.

Disclosures: **G.A. Higgins:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **L.B. Silenieks:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **A. Bernardo:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **D. Feng:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **L. Stam:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **J. Vintuks:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **J. Prenderville:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **R. Winters:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **M. Mitsogiannis:** A. Employment/Salary (full or part-time):; Transpharmation Ltd.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.16/C53

Topic: B.08. Epilepsy

Support: iDream/NARLabs

Title: Utilizing Stereoencephalography Technology to Monitor Epilepsy in Freely Moving Miniature Swine

Authors: *H.-C. PAN[1], C.-M. YEH[1], Y. MA[1], J.-W. YANG[2], Y.-C. WANG[3], H. CHEN[2,4]; [1]Natl. Lab. Animal Ctr. (NLAC), Natl. Applied Res. Labs. (NARLabs),, Taipei City, Taiwan; [2]BioPro Scientific, Taoyuan, Taiwan; [3]Dept. of Neurosurgery, Chang Gung Mem. Hosp., Taoyuan, Taiwan; [4]Inst. of Electronics Engineering, Natl. Tsing Hua Univ., Hsinchu, Taiwan

Abstract: The miniature swine has emerged as a valuable translational research species for investigating various fields such as neuroscience, immunology, and metabolism. With their high genetic, systematic, and structural similarities to humans, coupled with ease of handling, these animals have proven to be effective in verifying biocompatibility and efficacy. In the context of porcine epilepsy models, electrocorticography (ECoG) is commonly employed to measure epileptic spikes, which are indicative of epileptogenic regions. However, the use of ECoG grid electrodes, positioned beneath the dura mater, primarily captures signals from the cortical surface and fails to provide sufficient information from deeper brain areas, including the hippocampus and thalamus. To address this limitation, we introduced stereoelectroencephalography (sEEG) technology, enabling the detection of epileptic spikes originating from deeper brain regions. We developed a refinement procedure for epileptogenic surgery, combining brain imaging, a stereotaxic frame, and 3D-printed customized parts to precisely guide the implantation of sEEG depth electrodes, micro-injection needles into the target brain areas, successfully establish epilepsy swine model by intracerebral chemicals injection and acquire electrical signals during

the operation in Lanyu minipigs. Furthermore, our methodology allowed the minipigs to survive for at least four weeks post-surgery, and we were able to wirelessly measure the local field potential (LFP) from sEEG leads in an open field arena, enabling freely moving recordings. This advancement opens up new possibilities for studying epileptic phenomena in a more comprehensive and naturalistic manner in miniature swine models.

Disclosures: **H. Pan:** None. **C. Yeh:** None. **Y. Ma:** None. **J. Yang:** None. **Y. Wang:** None. **H. Chen:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.17/C54

Topic: B.08. Epilepsy

Support: NIH R01 NS066974

Title: Reduced subcortical cholinergic firing associated with impaired consciousness in an awake behaving mouse model of temporal lobe seizures

Authors: ***S. LIU**[1], P. PASZKOWSKI[2], L.-A. SIEU[1], J. LIU[1], M. VALCARCE-ASPEGREN[1], W. KHAN[1], S. MCGILL[1], D. LEE[2], A. DUQUE[1], H. BLUMENFELD[1];
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Abstract: Temporal lobe epilepsy (TLE) is a debilitating disorder characterized by focal seizures originating from the limbic system and associated deficits in consciousness. While the prevailing consensus suggests that TLE affects consciousness by propagating seizures to bilateral temporal lobes, our alternative “network inhibition hypothesis” proposes that seizure activity infiltrates subcortical regions, leading to reduced arousal and inducing a state of slow-wave activity similar to deep sleep or encephalopathy within the neocortex. To test this hypothesis, we developed a new awake, behaving mouse model for in vivo electrophysiological recordings, which also allowed for the simultaneous assessment of pupillary diameter and behavioral changes associated with loss of consciousness during TLE seizures. In this study, mice were head-fixed but free to run on a wheel. We measured local field potentials using chronically implanted bipolar electrodes in the right lateral orbitofrontal cortex and bilateral hippocampi. Focal limbic seizures were induced by applying current pulse trains (2 s, 60 Hz) into the unilateral hippocampus. Juxtacellular single unit activity (SUA) recordings were performed using glass capillaries filled with 4% Neurobiotin in the nucleus basalis of Meynert (NBM) and brainstem pedunculopontine tegmental nucleus (PPT). Animal running behavior and pupillary changes during seizures were also recorded. Following recordings, a double immunofluorescence procedure was performed to confirm the cholinergic nature of the Neurobiotin-filled neurons (stained with Cy3-conjugated streptavidin) using anti-choline acetyltransferase antibodies. We found that focal limbic seizures suppressed running wheel behavior, while orbitofrontal local field potentials exhibited

synchronized slow-wave activity resembling coma or deep sleep. Pupillometry analysis showed pupil dilation at seizure onset and fluctuations during slow waves. SUA recordings during focal limbic seizures displayed diverse firing pattern changes in NBM (n=37) and PPT (n=77), with some neurons exhibiting reduced firing, others showing increased firing, and some displaying no change. Noteworthily, cholinergic neurons, particularly in the PPT (n=10), exhibited significantly reduced firing during focal limbic seizures. These results show that single-neuron activity recordings from deep brain areas in awake, behaving mice during seizures are feasible and reproducible. Importantly, the inhibition of cholinergic neurons in subcortical arousal nuclei, such as NBM and PPT, may play a crucial role in regulating consciousness during focal TLE seizures.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.18/C55

Topic: B.08. Epilepsy

Support: NIH R01 NS066974

Title: Correlation between Increased GABAergic Neuronal Activity in the Lateral Septum, Cortical Slow Waves, and Impaired Consciousness in an Awake Mouse Model of Temporal Lobe Seizures

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Abstract: Patients with temporal lobe epilepsy (TLE) often experience loss of consciousness during seizures. Studies have shown that cortical slow wave activity is present during temporal lobe seizures, which is associated with impaired consciousness. Previous research with human neuroimaging and rodent models has suggested that decreased subcortical arousal is responsible for the depressed cortical function and impaired consciousness in TLE focal limbic seizures. However, precise mechanisms underlying this decreased subcortical arousal in TLE remain incompletely understood. One potential mechanism involves increased activity of GABAergic neurons in the lateral septum (LS). Previous studies using rat models have demonstrated that focal hippocampal seizures lead to increased neuronal activity and blood flow in the LS. Moreover, LS stimulation without seizures has been shown to induce cortical slow oscillations. Based on these findings, we hypothesize that the hyper-excited state of the hippocampus during seizures spreads to the LS, which increases GABAergic inhibition from the LS to subcortical arousal systems. This cascade of events results in neocortical deactivation through indirect

pathways. We utilized an awake-behaving mouse model in which focal limbic seizures can be induced by stimulating the hippocampus. We recorded local field potential (LFP) from the hippocampus, simultaneously measuring LFP in the orbitofrontal cortex (OFC), and recording multi-unit activity (MUA) in the LS. Additionally, we conducted cell-specific recordings (targeting GABAergic neurons) in the LS using fiber photometry, employing a genetically encoded calcium indicator (GCaMP) in Gad2-IRES-Cre knock-in mice. Mice running wheel behavior was also monitored. Our findings revealed that impaired consciousness, indicated by decreased running speed (n=9), was associated with slow wave activity in the OFC, increased MUA firing in LS neurons (n=9), and increased fluorescent intensity of the GABAergic neuronal-specific calcium indicator during seizures (n=5). These results establish a direct correlation between neocortical deactivation, behavioral arrest, and heightened LS activity in the presence of a hyper-excited hippocampal state within a temporal lobe seizure model. To gain further insights into the network and neurotransmitter mechanisms at play, we have initiated measurements of neurotransmitters in the nucleus basalis. By continuing our investigation, we aim to uncover the mechanisms responsible for impaired consciousness in focal temporal lobe seizures, potentially leading to the development of innovative treatments for this disorder.

Disclosures: **J. Liu:** None. **L. Sieu:** None. **S. Liu:** None. **H. Blumenfeld:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.19/C56

Topic: B.08. Epilepsy

Support: CONAHCYT / 781259

Title: Histopathological analysis of the neuroprotective and cardioprotective effect of dapsone in a model of status epilepticus induced with kainic acid in rats.

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Abstract: Status epilepticus (SE) is a serious condition resulting from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally, prolonged seizures. During the ictal period, the activity of the autonomic nervous system is altered, with an increase in sympathetic nerve activity, increased cardiac stimulation, arrhythmogenesis, and cardiac remodeling increasing the risk of sudden cardiac arrest. Currently, the treatment is the use of benzodiazepines; however, they can contribute to the development of

drug-resistant seizures. Dapsone (DDS) is an antileprosy drug that has been shown to have a neuroprotective action due to its possible antagonistic effect on NMDA receptors, reducing oxidative stress and inflammation. The objective of this project was to evaluate the neuro and cardioprotective effect of DDS in a rat model of SE induced with kainic acid (KA). Methods. Male Wistar rats weighing 250 to 350 g were used and divided into two groups treated with: (1) KA + Vehicle; and (2) KA+DDS 25 mg/kg i.p. every 24 hours for 3 days. KA was administered 5 mg/kg i.p at 1 h intervals until SE was established. After euthanasia, the animals were perfused with 10% formalin and the hearts and brains were removed. Hematoxylin-eosin, cresyl violet, and immunohistochemical staining (4-Hydroxynonenal) were used. Results. In the hippocampus, neurons with pyknotic nuclei and evident edema in the CA1 and CA3 regions were observed in the group treated with KA+Vehicle, while in the group with KA+DDS a greater preservation of pyramidal neurons was observed. In the cardiac tissue of rats treated with KA+Vehicle, an infiltrate of inflammatory cells was produced, while in the KA+DDS group the infiltrate was of a lower proportion. The immunoreactivity of 4-Hydroxynonenal in cardiac tissue was more evident in the KA+Vehicle group, which decreased in the group treated with KA+DDS. In conclusion, the above results, taken together, suggest that DDS: (1) decreases the inflammatory infiltrates and oxidative stress at cardiac level, and (2) produces neuroprotective effects.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.20/C57

Topic: B.08. Epilepsy

Title: Personalized patient models of rare epilepsies: in vivo Prime editing of GRIN2A

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Abstract: A wide spectrum of genetic variants can cause epilepsy. While it is well understood that there is tremendous diversity in genetic targets and mutation types, there is a lack of available tools to investigate these specific variants in vivo. Consequently, most rare mutations are grouped into larger categories, resulting in a loss of mechanistic detail in disease pathogenesis and potential variant-specific treatments. This project aims to enhance the generation of patient-specific models of epilepsy disorders through the design and delivery of

genome editing tools. To achieve this, we employ Prime editing to introduce patient-specific epilepsy variants into the genomes of wild-type mice, enhancing external validity compared to inbred mouse lines. Proof-of-concept experiments encode a patient-derived variant in NMDA receptor subunit *GRIN2A*, which displays alterations in electrical activity and spontaneous seizures, and demonstrate the feasibility of generating an on-demand mouse model of an individual patient. This approach surpasses the efficiency of traditional disease modeling methods, enabling the generation of mice within a significantly reduced timeframe of just 7 weeks. We have further developed novel techniques to enhance sample enrichment using deliverable, in vivo fluorescent reporters and we have established a streamlined pipeline for the rapid production and delivery of Prime editing ribonucleoproteins. These advancements establish a mouse model for the *GRIN2A* variant to be used for screening existing medications for patient-specific, off-label use, and additionally provide a framework for fast and flexible modeling of rare epilepsy variants for efficient compound screens. This project addresses the need for specific tools to investigate epilepsy-associated genetic variants in vivo. By employing Prime editing and developing complementary techniques, we can accelerate the production of patient-specific epilepsy models and gain valuable insights into disease mechanisms and potential treatments.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.21/C58

Topic: B.08. Epilepsy

Title: Zebrafish embryos with reduced deoxyhypusine synthase exhibit dysmorphology, epileptiform activity, and reduced GABAergic neuronal arborization

Authors: *E. SHOJAEINIA[1,2,3], C. T. CONNORS[4], T. L. MASTRACCI[4,5], O. DEVINSKY[6,7], C. V. ESGUERRA[1,2], A. D. CRAWFORD[3];
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Abstract: The >7000 rare diseases affect >300 million patients worldwide, of which neurodevelopmental disorders (NDDs) are a major category. An ultra-rare NDD, with only 5 patients identified to date, is caused by mutations in DHPS, which encodes the highly conserved enzyme deoxyhypusine synthase. DHPS is essential to synthesize hypusine, a rare amino acid that only occurs as a post-translational modification of eukaryotic initiation factor 5A (eIF5A).

DHPS deficiency syndrome causes epileptiform activity and seizures, cognitive impairment, and mild facial dysmorphology. Recently, a mouse model with a brain-specific genetic deletion of Dhps at birth has been shown to have similar clinical characteristics of human DHPS deficiency, and to have impaired eIF5A^{HYP}-dependent mRNA translation, resulting in altered expression of proteins required for proper neuronal development and function. To further elucidate DHPS' role during early brain development, we generated a zebrafish loss-of-function model by partial knockdown of dhps expression using an antisense morpholino oligomer (MO) targeting the exon 2/intron 2 (E2I2) splice site of the dhps pre-mRNA. dhps knockdown embryos exhibited dose-dependent dysmorphology characterized by microcephaly, axis truncation, and body curvature, as well as developmental delay. These results were confirmed with a second MO targeting the start codon of dhps mRNA, and could be partially rescued by co-injection with in vitro transcribed dhps mRNA. Confocal microscopy analysis of dhps antisense MO-microinjected transgenic reporter larvae with GABAergic-specific expression of mCherry revealed a significant reduction in the arborisation complexity of GABAergic neurons, with an average of 47.55 ± 25.12 (n=7) intersections in dhps knockdown larvae at 5 days post-fertilization (dpf), compared to an average of 108.50 ± 9.31 (n=6) in control MO-microinjected larvae. Electrophysiological analysis was carried out using local field potential (LFP) recordings of 3-dpf larvae, revealing an increase in epileptiform brain activity associated with the partial loss of function of dhps, with 60% of dhps knockdown larvae (n=15) exhibiting 2 or more ictal-like events during a 20-minute recording, compared to 20% of wildtype larvae (n=10). Ongoing experiments will evaluate the potential activity of anti-seizure drugs and drug candidates in this model.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

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Topic: B.08. Epilepsy

Support: NIH NINDS R01-NS126247
VA I01-BX004938
Department of Defense W81XWH-18-1-0598

Title: Immediate early gene cFos expression in the hippocampus of seizure-prone CACNA2D2 knockout mice

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Abstract: The voltage-gated calcium channel subunit gene CACNA2D2 controls calcium-dependent signaling in neurons, and loss of this subunit causes epilepsy and ataxia in both mice and humans. Although this gene is primarily associated with cerebellar Purkinje cell function, it is also expressed in the hippocampus, and homozygous CACNA2D2 mutant mice manifest electroencephalographic spike-wave discharges (SWDs) as well as generalized tonic-clonic seizures. SWDs are typically associated with aberrant thalamocortical activation, but due to the existence of generalized seizure events, we sought to determine whether these mice manifested signs of hippocampal involvement in seizure activity. We analyzed various histopathological correlates of epilepsy in the hippocampal dentate gyrus of juvenile (21-28 do) and adult (56+ do) CACNA2D2 wildtype (WT) and knockout (KO) mice, using immunohistochemical staining and confocal microscopy. Despite intermittent behavioral seizures, juvenile CACNA2D2 KO mice demonstrated no difference in expression of the activity-dependent gene cFos within the dentate granule cell layer (GCL) compared to CACNA2D2 WT mice. Interestingly, even KO mice who displayed handling-induced behavioral tonic-clonic seizures in the hour prior to sacrifice did not demonstrate increased activation of granule cells and putative semi-lunar granule cells when compared with WT littermates. Perhaps most surprisingly, adult (> 3mo) CACNA2D2 KO mice had decreased expression of cFos in the GCL, as well as at the GCL-inner molecular layer border compared to WT mice, perhaps indicating adaptation of circuit activity in the hippocampus over time or an effect of survivorship bias. As previously reported, other histopathological markers of epilepsy in these mice, including markers of altered neurogenesis (Ki67 and doublecortin), glial activation (GFAP AND Mac-2), and mossy fiber sprouting (ZnT3), as well as structural changes such as granule cell layer dispersion, were all minimally changed in juvenile mice, and are being examined in adult mice. Overall, it does not appear that generalized seizure activity involves widespread hippocampal activation on an ongoing basis, suggesting that alternative loci might be involved in the initiation or maintenance of behavioral seizures in these mice.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

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Topic: B.08. Epilepsy

Support: Bundesministerium für Bildung und Forschung (BMBF): Treat-ION network(Project P5)

Title: Towards Novel Therapies for KCNT1-Associated Epileptic Encephalopathy

Authors: *T. BAS[1,2], K. ARAKI[3], F. MORELLINI[4], S. HORNIG[4], A. NEU[4], O. PLESS[5], S. TRÖDER[6], H. BECK[3], M. STOCKEBRAND[7,8], D. ISBRANDT[7,8];
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Abstract: Altered brain development through inherited or de-novo ion channelopathies can lead to lifelong cognitive deficits and therapy-refractory epilepsy. The therapeutic options available for early infantile epileptic encephalopathies (EIEE) are limited, and prophylactic therapies for at-risk patients do not yet exist. However, the plasticity of the developing brain suggests great therapeutic potential. To prevent epileptogenesis and associated comorbidities, treatment is likely most effective when targeted to specific vulnerable periods of brain development. Gain-of-function (GoF) mutations in *KCNT1* encoding the sodium-gated potassium channel $K_{Na}1.1$ (Slack), are associated with early-onset and therapy-resistant genetic epilepsies, mental retardation, and pronounced behavioral changes. No specific KCNT1 channel blockers are available for use in affected patients to date. To address this urgent clinical need, we identified candidate blockers in a high throughput screen (HTS) for repurposing FDA-approved. Furthermore, we generated two novel knock-in mouse lines harboring the patient-derived *KCNT1* mutations p.I296N (IN) or p.R950Q (RQ) causing different GoF levels. To characterize the phenotypes of our mouse lines we used acute slice electrophysiology, telemetric electrocorticogram and multichannel-depth recordings, and a battery of behavioral tests. Heterozygous IN and homozygous RQ mice developed spontaneous generalized seizures. The severity of the phenotypes corresponded to the GoF levels observed in vitro. Both models showed epilepsy-typical changes in the hippocampus such as reactive astrogliosis and increased perineuronal nets and neuropeptide Y (NPY) expression in the dentate gyrus. These changes were associated with a behavioral phenotype including increased locomotion, reduced anxiety, impaired memory performance, and altered interictal network activities such as reduced cortical theta power during REM and non-REM sleep and wakefulness. As our HTS identified potent and brain-permeable KCNT1 blockers that were validated on human and mouse KCNT1 channels in vitro, we sought to characterize their effect on ictal and interictal network activities. Preliminary results indicate that chronic treatment with KCNT1 blockers in adult epileptic mice does not significantly modify the seizure phenotypes and, thus, suggest that treatment has to be targeted to the period of epileptogenesis during brain development. In summary, our results confirmed our lines' face and etiological validity as KCNT1 epilepsy models that are now being used to characterize the disease pathophysiology and test novel treatment options.

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Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

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Topic: B.08. Epilepsy

Support: Deutsche Forschungsgemeinschaft (DFG): CRC1451 (B01)

Title: Developmental and epileptic encephalopathy-linked HCN1 mutations cause epilepsy and affect interictal hippocampal and cortical network activities in mice

Authors: *J. KASEMIR[1,2], A. MERSEBURG[1,2], S. MARGUET[1,2], B. SANTORO[3], D. ISBRANDT[1,2];
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Abstract: Inherited and *de novo* mutations in voltage- and ligand-gated ion channels underlie many developmental and epileptic encephalopathies (DEEs). Affected patients' phenotypes are often resistant to antiseizure medications or even show paradoxical responses. The key to developing effective therapies that also prevent behavioral comorbidities is to understand the general or gene-specific disease mechanisms behind these DEEs. Here, we examined two knock-in mouse models that each carry one patient-derived pathogenic *HCN1* (hyperpolarization-activated cyclic nucleotide-gated cation channel) sequence variation. The human mutations p.G391D and p.M153I (*Hcn1*$^{G380D/+}$ and *Hcn1*$^{M142I/+}$ in mice) are associated with severe neonatal- and early infantile-onset epileptic encephalopathies with daily seizures, high mortality, and neurodevelopmental comorbidities like intellectual disabilities. The corresponding mouse models both display spontaneous generalized tonic-clonic epileptic seizures and comorbidities including locomotor hyperactivity, reduced motor coordination, and deficits in spatial working memory, which were overall more severe in *Hcn1*$^{G380D/+}$ animals. Analysis of HCN1 immunoreactivity in the hippocampus and cerebellum revealed pronounced alterations in the distribution and the levels of HCN1 channels, specifically disrupted targeting to the axon terminals of basket cell interneurons. Treatment with the sodium channel blockers lamotrigine and phenytoin induced epileptic seizures, which was also reported for patients carrying pathogenic *HCN1* sequence variants. Analysis of interictal electrocorticogram (ECoG) recordings revealed reduced low and high gamma power during rapid-eye-movement (REM) sleep. Moreover, hippocampal CA1 local field potentials recorded from awake head-fixed mice in the Mobile Homecage showed a reduced frequency of ripple oscillations. Together, these findings are indicative of impairment in inhibitory neuron function.

Disclosures: **J. Kasemir:** None. **A. Merseburg:** None. **S. Marguet:** None. **B. Santoro:** None. **D. Isbrandt:** None.

Poster

PSTR188. Animal Models of Epilepsy: Genetics and Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.25/C62

Topic: B.08. Epilepsy

Support: Deutsche Forschungsgemeinschaft (DFG) FOR2715 (P7)
DFG CRC1451 (B01)

Title: Hippocampal and Cortical Dynamics in a Scn2a Mouse Model of Developmental and Epileptic Encephalopathy

Authors: *Y. REVA[1,2], D. KIRIANOV[1,2], M. BARBONI[3], K. ULRICH[1,2], H. OELßNER[1,2], B. ENGELAND[1,2], S. MARGUET[1,2], H. BECK[3], T. KELLY[3], D. ISBRANDT[1,2];
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Abstract: Developmental insults to the brain can profoundly disrupt the functioning of neuronal circuits, giving rise to disorders such as Developmental and Epileptic Encephalopathies (DEEs), which manifest very early in life and often persist into adulthood causing life-long disability. DEEs exhibit genetic heterogeneity; however, targeted interventions during their early stages could represent a shared strategy for reversing the pathological phenotype or preventing seizures and associated comorbidities. To investigate this possibility, we generated a mouse model of DEE with a patient-derived p.A263V substitution in the voltage-gated sodium channel Nav1.2 alpha subunit, encoded by Scn2a. Initially, we sought to characterize this model both in vitro and in vivo over the course of the lifespan to understand the underlying network changes. Patch-clamp recordings revealed a transient increase of excitability in hippocampal pyramidal neurons in vitro, reflecting the known developmental shift from Nav1.2 to Nav1.6 at nodes of Ranvier and axon initial segments during the second and third postnatal week. By employing hippocampal in vivo local field potential (LFP) recordings in awake, unanesthetized mice on a CD-1 outbred genetic background during the neonatal period, we discovered that both homozygous and heterozygous Scn2a mutants develop seizures, the former showing electrographic seizures as early as postnatal day P3. Additionally, alterations in hippocampal sharp waves were observed during the interictal periods. Notably, the persistence of seizures into adulthood was observed exclusively in homozygous Scn2a mutants, as supported by our electrocorticographic (ECoG) recordings. The mice exhibited increased mortality after the weaning and severe tonic-clonic seizures with running and jumping, characterized by a rapid onset following a transition from the REM sleep phase. Nonetheless, they displayed only a mild behavioral phenotype, with a slight increase in hyperactivity observed solely in males. Furthermore, preliminary results did not reveal major alterations in interictal (physiological) activity using hippocampal LFP depth profile recordings in awake head-fixed freely moving animals. With a comprehensive understanding of the model's characteristics, we can now explore potential treatment options. To date, we have experimented with chronic administration of the sodium channel blocker phenytoin during postnatal days P1-P21 as a means to prevent seizures and improve outcomes in adults.

Disclosures: Y. Reva: None. **D. Kirianov:** None. **M. Barboni:** None. **K. Ulrich:** None. **H. Oelßner:** None. **B. Engeland:** None. **S. Marguet:** None. **H. Beck:** None. **T. Kelly:** None. **D. Isbrandt:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.01/C63

Topic: B.08. Epilepsy

Support: CIRM TRAN1-11611
CIRM CLIN2-13355

Title: Behavioral analyses in the immunodeficient NOG mouse after intrahippocampal kainate induction of chronic epileptic seizures

Authors: *F. PORKKA, H. K. KIM, P. HAMPEL, E. T. SEVILLA, A. VOGEL, A. BATES, M. B. PAREKH, C. A. PRIEST, C. R. NICHOLAS;
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Abstract: Behavior testing is a critical platform to characterize animal models of neurological disorders such as epilepsy, as well as to evaluate the efficacy and safety of novel therapeutics. Severely immunodeficient mouse strains such as NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac (NOG) are often selected for preclinical model development in the fields of regenerative medicine and cell therapy, as they can support human cell engraftment at a high rate of success. However, their performance on many behavioral tasks is not well characterized. To facilitate the use of NOG mice in our preclinical studies using the intrahippocampal kainic acid (IHKA) model of epilepsy, behavior assays were optimized for exploratory activity, anxiety, and learning and memory in naïve and epileptic NOG mice. Behavioral paradigms were examined for exploratory activity (open field test, holeboard), anxiety (light-dark emergence, elevated zero maze), and learning and memory (novel object recognition, novel object placement, y-maze, Barnes maze). On each test, the performance of naïve NOG mice was assessed and compared to the performance of epileptic NOG mice, and naïve BL6N or BL6J mice. All mice tested were adult males and individually housed at the time of testing. In multiple testing environments, naïve NOG mice exhibited elevated levels of motor activity when compared to naïve BL6N and BL6J mice. While traveling similar distances, NOG mice in the chronic epileptic phase spent significantly less time in the center of an open field than naïve NOG mice. Naïve NOG mice generally exhibited stronger anxiety-associated behaviors than naïve BL6N mice in multiple assays, which should be considered when developing behavioral protocols for this strain. Increased anxiety-associated behaviors in epileptic NOG mice were indicated by the open field, light-dark emergence, and the elevated zero maze assays. In the y-maze and in object-based learning and memory tasks, naïve NOGs exhibited impaired learning when compared to naïve BL6N mice. However, naïve NOG mice performed similarly to naïve BL6J mice in Barnes maze acquisition learning. NOG mice in the chronic epileptic phase showed significant impairment in acquisition and recall in the Barnes maze task. These results recapitulate select behavioral comorbidities of epilepsy as described in immunocompetent mouse strains by other groups. Learning and memory deficiencies in IHKA NOG epileptic mice can be captured by the Barnes maze. Barnes maze, in combination with open field and light-dark emergence assays, appears most useful to assess NOG mice for potential disease-modifying effects on cognitive impairments accompanying epilepsy.

Disclosures: **F. Porkka:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **H.K. Kim:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **P. Hampel:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **E.T. Sevilla:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **A. Vogel:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **A. Bates:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **M.B. Parekh:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **C.A. Priest:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **C.R. Nicholas:** A. Employment/Salary (full or part-time):; Neurona Therapeutics.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.02/D1

Topic: B.08. Epilepsy

Support: NIH Grant R21NS116546 (MVJ)

Title: Deficits in behavioral pattern separation in mice with varying susceptibility to temporal lobe epilepsy

Authors: S. J. REWEY[1], D. J. LASKY[1], J. ISAACSON[2], H. KOSIARA[1], R. K. MAGANTI[2], ***M. V. JONES**[1];
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Abstract: The dentate gyrus (DG) acts as a "gate" on temporal lobe hyperexcitability and also as a "filter" that performs pattern separation during normal episodic memory (i.e., the ability to store, differentiate and recall highly similar memories). We hypothesize that the gate and filter functions of DG are intimately intertwined and that both may simultaneously fail in temporal lobe epilepsy (TLE). We previously showed that C57BL/6 mice display both circuit-level and behavioral pattern separation deficits several weeks following kainate-induced status epilepticus (SE) (Madar et al., 2021). Here, we compared pattern separation memory between epilepsy-resistant C57BL/6 and epilepsy-susceptible DBA/2J mice, with and without an epileptogenic insult. Mice were implanted for EEG to measure epileptiform activity and then induced with SE (repeated low-dose i.p. kainate, KA) or saline (SA) as control. Several weeks after SE, we performed a pattern separation memory test wherein mice explored two identical objects at varying locations. A mouse with normal memory function should spend significantly more time exploring a moved object than an unmoved object. If a mouse cannot differentiate between moved and unmoved objects, this indicates a deficit in behavioral pattern separation and, thus, probably a DG abnormality. We computed a discrimination ratio to quantify pattern separation capabilities: a higher ratio shows more time spent at the moved object and better pattern separation memory. From videos of mouse exploration, we measured movement path, distance traveled, velocity and time spent exploring each object, as well as time spent near walls (an anxiety measure). A one-way ANOVA test found no significant difference between

discrimination ratio averages for C57SA, C57KA, DBASA, and DBAKA groups. However, we found some interesting trends in both strains. The spread of discrimination scores for KA-injected mice was considerably larger than for SA-injected mice. Moreover, the average pattern separation capabilities of DBAKA mice were lower than C57KA mice. Finally, more DBAKA mice developed epilepsy (50%) than C57KA mice (42%). Further work is necessary but our findings suggest that C57BL/6 epilepsy-resistant mice display more precise pattern separation memory than DBA/2J epilepsy-susceptible mice, whether or not they have epilepsy. We speculate that baseline differences in pattern separation memory may reveal a predisposition for developing TLE after an epileptogenic insult, which might allow noninvasive and inexpensive testing for risk of TLE (c.f., Madar et al., 2021).Madar et al., 2021 DOI: 10.1523/JNEUROSCI.2439-20.2021

Disclosures: S.J. Rewey: None. **D.J. Lasky:** None. **J. Isaacson:** None. **H. Kosiara:** None. **R.K. Maganti:** None. **M.V. Jones:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.03/D2

Topic: B.08. Epilepsy

Support: ERANET ANR-20-NEUR-0005 VELOSO

Title: Epileptic seizures are more frequent at transition from activity to rest in GAERS rats

Authors: R. EL MAHZOUM, H. TRAN, A. BONNOT, ***I. COHEN**;
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Abstract: Predicting the probability of occurrence of absence seizure not only can help protect patients, but also better understand factors contributing to the seizure trigger mechanisms. In this study, we recorded seizures over periods of several days in the GAERS rat model of absence epilepsy, while we monitored behavioral activity level using a head accelerometer (ACCEL), an electromyogram of the neck (EMG), and electro-occulograms (EOG). The three markers allowed to consistently discriminate states of activity and rest, with an overall match of 82-89% between any two markers, and transition times between states matching across markers with a variability shorter than 30s. Both GAERS and control non-epileptic Wistar rats spent more time in rest (55-66%) than in activity (34-45%), yet GAERS showed prolonged less fragmented episodes of activity (23 vs 18 min) and rest (34 vs 30 min). On average seizures lasted 13s and were separated by 3.2min. We found that seizures were associated with a decrease in the power of the activity markers from steep for ACCEL, to moderate for EMG and weak for EOG. We found that overall seizures tend to occur in bursts. We measured that the probability to be in a state of seizure is significantly increased around a seizure in a window of +/- 4 min. Finally, we estimated the seizure rate around the behavioral transition times. Seizure rate strongly increased,

for several minutes, when transiting form activity to rest. These results point to mechanisms that control behavioral state as determining factors of seizure occurrence.

Disclosures: R. El Mahzoum: None. **H. Tran:** None. **A. Bonnot:** None. **I. Cohen:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.04/D3

Topic: B.07. Network Interactions

Support: NIH Grant R01 NS126594
NIH Grant R56 NS099586

Title: Neuronal ensemble synchrony during absence seizures in somatosensory and visual cortex

Authors: *S. KILIANSKI, E. DULKO, A. CARNS, M. P. BEENHAKKER;
Pharmacol., Univ. of Virginia, Charlottesville, VA

Abstract: There is ample evidence from human patients and animal models that the spike-and-wave discharges (SWD) that define absence seizures are present broadly across the cortex. Extreme neuronal synchrony is often cited as the source of these large amplitude events, but there are conflicting reports about whether neuronal synchrony, in the form of highly correlated spiking activity of local groups of neurons, actually occurs during SWD. Some report that the synchrony of cortical neurons actually decreases during SWD, while others present evidence that it increases. These conflicting reports were generated using different animal models (*Cacng2*^{stg} "stargazer" mice vs GAERS rats) and included recordings from different cortical areas (visual vs. somatosensory cortex). Furthermore, to date, only three publications report the activity of neuronal ensembles (10-100s of neurons recorded simultaneously) during absence seizures; two studies used microelectrode arrays, and a third used two-photon calcium imaging. To resolve these outstanding inconsistencies and deepen our understanding of ensemble activity during SWD, we used high-density microelectrode arrays to record from somatosensory and visual cortex in a mouse strain that has spontaneous SWD (C3H/HeJ). This experiment will be repeated in *Cacng2*^{stg} "stargazer" mice so we can more directly compare our results to those of previous reports and identify any distinctions between these two strains. Our experiments represent a significant contribution to the base of knowledge about population activity during SWD.

Disclosures: S. Kilianski: None. **E. Dulko:** None. **A. Carns:** None. **M.P. Beenhakker:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.05/D4

Topic: B.08. Epilepsy

Support: Italian Ministry of Health (RRC)
Progetto 5PerMille Ictomyelin RC18.7

Title: Extracellular potassium increases in unmyelinated and myelinated regions and cortical hyperexcitability

Authors: *L. UVA, G. BRUNO, M. DE CURTIS;
Fondazione IRCCS Inst. Neurologico "C. Besta", Milano, Italy

Abstract: The piriform cortex (PC) is a three-layer cortex consisting of the plexiform layer I and the cellular layers II and III. The olfactory fibers of the lateral olfactory tract (LOT) enter the PC in its superficial layer I and lose their myelin coating. In PC layer I a large and fast increase of extracellular potassium concentration ($[K^+]_o$) at the onset of seizure-like event induced by the K^+ channel blocker 4-aminopyridine (4AP) was recorded in the guinea pig brain in vitro (Uva 2017 doi: 10.1523/JNEUROSCI.2239-16.2016). We hypothesize that in a condition of massive discharge the release of K^+ is larger in a region with unmyelinated axons compared to regions where myelinated axons are present, because of the lack of K^+ buffering by the oligodendrocyte wrap around the axons. In the isolated guinea pig brains maintained *in vitro* by arterial perfusion, $[K^+]_o$ augmentations were assessed in LOT and in the superficial PC layer I (PC_s) with ion-sensitive electrodes after arterial application of the proconvulsant 4AP (50µM; 5 min). The larger and prompter $[K^+]_o$ increase was observed in PC_s. Trains of high frequency electrical stimuli (HFS; 30 Hz for 1.5 s; every 2-5 sec) were delivered to LOT, which projects to PC, to mimic the preictal activity induced by 4AP arterial application and the induced $[K^+]_o$ shifts were recorded simultaneously in PC_s and LOT or in PC_s and in deeper PC layer II-III (PC_d). Series of HFS at increasing intensities produced increasing shifts of $[K^+]_o$ that were larger and faster in the superficial unmyelinated PC layer I than in LOT or the deeper PC layers II/III. HFS trains could induce spike afterdischarges that outlasted HFS; these were associated to a prolongation of $[K^+]_o$ increase in the deeper PC sites compared to layer I. During arterial application of the K_{ir} 4.1 channel blocker $BaCl_2$ (100µM; 30 min) to prevent the K^+ buffering carried out by the glia, $[K^+]_o$ shifts and spike afterdischarges were induced in PC by HFS of lower amplitude compared to control conditions, indicating a decrease of the threshold for the generation of hyperexcitability; larger $[K^+]_o$ rises in presence of $BaCl_2$ were also observed. Our results indicate that in a layer with high density of unmyelinated fibers an excessive $[K^+]_o$ increase is favored during activity, suggesting a role of myelin in $[K^+]_o$ buffering. $[K^+]_o$ rise represents a proconvulsant condition that is exacerbated by impairments in K^+ buffering. When myelin loss and gliosis occur, as reported in models of epilepsy and in epileptic patients (de Curtis 2021 doi: 10.111/epi.16824), pathological K^+ changes could support hyperexcitability.

Disclosures: L. Uva: None. **G. Bruno:** None. **M. de Curtis:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.06/D5

Topic: B.08. Epilepsy

Support: NINDS Grant R01NS082046
NINDS Grant R01NS038572

Title: The effects of interictal bursts on spatial coding properties of hippocampal CA1 neurons in epileptic mice during navigation of the VR environment

Authors: *S. A. PARK[1], H. TAKANO[1,2], D. A. COULTER[1,3];
[1]Pediatrics, Div. of Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Neurol., [3]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: In pathological conditions such as temporal lobe epilepsy, a dysregulation of hippocampal activity could affect cognitive abilities. One pathological signature of epilepsy is the presence of interictal burst (IB) events that are observed between seizures and occur more frequently than epileptic seizures. To better understand how epileptic IBs affect spatial processing, we examined neuronal activity in the CA1 hippocampal cells in epileptic mice. For this purpose, we implemented an in vivo approach and performed simultaneous LFP recordings from the dorsal hippocampus and two-photon calcium imaging from the CA1 of awake, behaving mice traversing a virtual reality (VR) environment. Our experimental setup allowed us to monitor the activity of CA1 pyramidal cells to assess their spatial coding with regard to the animal's position within the virtual environment while concurrently detecting IBs. No synchronous network activity was observed in any of the control mice. Compared to controls, the CA1 region in epileptic mice that had experienced pilocarpine-induced status epilepticus (SE), had fewer and less stable place cells coding for place fields, which agrees with previous studies. SE mice (n=3) exhibited multiple synchronous network activities in the CA1 region appearing to be IBs. On examination of ~34 min trials of the SE mice navigating the VR environment, we observed 6-10 IB events of variable intensities. In these trials, on average, 14% of all cells appeared to code a place field. Of these place cells, about a quarter of the cells had altered place fields after the highest intensity IB event compared to before the IB event. For example, post-IB, we observed that pre-IB place fields could shift to a new place field, become unstable, disappear, and in rare cases, a new place field arise when there was no place field pre-IB. We also observed that, in general, CA1 neurons tend to fire less post-IB. Thus, we examined the time it took any cell to fire post-IB and calculated the post-IB silent period to be 1.02 sec. This post-IB silent period suggests that there is a minimum post-IB “refractory period” during which place cells cannot code for space. Taken together, our findings shed light on the contribution of IBs to modify place cell coding in epileptic mice navigating a VR environment.

Disclosures: S.A. Park: None. **H. Takano:** None. **D.A. Coulter:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.07/D6

Topic: B.08. Epilepsy

Support: American Epilepsy Society Seed Grant (12/15/2021-12/14/2022), Kim (PI)
NIH R21NS130250. Kim (PI)

Title: Supt6 deletion in parvalbumin-expressing interneurons induces seizure development and behavioral abnormalities in mice.

Authors: *B. CARABELLI[1], T. LAI[1], M. MURUGAN[1], D. BOISON[1,2], Y. KIM[1,2];
[1]Rutgers Univ., Piscataway, NJ; [2]Brain Hlth. Inst., Piscataway, NJ

Abstract: Rationale: Supt6 is a histone chaperone and a binding partner of RNA polymerase II, the genetic variation of which is implicated in neurodevelopmental disorders with epilepsy. In addition, Supt6 is an interactor of p11, alterations of which are implicated in the etiology of major depressive disorder and antidepressant actions. Because alterations of parvalbumin (PV)-expressing interneurons are implicated in epilepsy and neuropsychiatric disorders, we examined the effects of Supt6 deletion in PV neurons on neuronal function, seizure development and behavioral alterations. **Methods**: We generated PV neuron-specific Supt6 homozygous and heterozygous KO mice. Measurement of behavioral seizures, EEG analyses, immunofluorescence for PV expression, Timm staining for mossy fiber sprouting in the hippocampus, and PV-neuron-specific Translating Ribosomes Affinity Purification (TRAP)/RNA sequencing were performed. Animals were also subjected to the following behavioral tests: Open Field Test, Sucrose Splash Test (SST), Nest Building and Forced Swim Test (FST). **Results**: PV-Supt6 homozygous KO mice displayed a convulsive seizure phenotype and died around 5 weeks of age. These mice also presented mossy fibers sprouting in the hippocampus, one of the classic histopathological marks in experimental models of epilepsy, as well as in humans with epilepsy. PV-Supt6 heterozygous KO mice displayed subclinical electrographic seizures and deficits in grooming behavior in the SST, decreased latency for immobility in the FST, and lower nesting scores, correlates of apathy, passive coping to stress and impaired well-being, respectively. Immunoreactivity for PV was significantly decreased in the hippocampus and cortex of PV-Supt6 heterozygous and homozygous KO mice compared to WT controls. PV neuron-specific TRAP/RNA sequencing followed by gene set enrichment analysis indicates that the Supt6 heterozygous deletion increases a set of genes related to oxidative phosphorylation. **Conclusions**: Supt6 deletion in PV neurons causes seizure development and behavioral deficits that resemble apathy, as well as passive stress coping, and impaired welfare. This model may represent a useful tool for the studies of PV neuronal development and the mechanisms relevant to epilepsy and psychiatric disorders.

Disclosures: B. Carabelli: None. **T. Lai:** None. **M. Murugan:** None. **D. Boison:** None. **Y. Kim:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.08/D7

Topic: B.08. Epilepsy

Support: CURE Epilepsy Award
NIH Grant R21NS122011
NIH Grant K08NS105929

Title: Comprehensive Analysis and Comparison of the Relationship of Seizures to Sleep-Wake and Circadian Rhythm

Authors: *B. J. HARVEY[1,3,4], L. I. ROSENBERG[3], L. M. AIANI[2], N. P. PEDERSEN[3,4]; [1]Grad. Program in Neurosci., [2]Genet., Emory Univ., Atlanta, GA; [3]Neurol., [4]Centers for Neuro-Engineering and Medicine, Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: The relationship between sleep and seizures is complex and bidirectional, including seizure-frequency-associated sleep fragmentation as well as sleep-deprivation-induced increases in seizures. We have developed methods that enable the scoring of large datasets to finally analyze and compare sleep-wake, ultradian, multidien, and circadian effects in epilepsy.
Using the SWISC pipeline (1) to score sleep and seizure, we have completely scored 28 day chronic recordings of electrocorticogram (ECoG), electromyogram (EMG), and bilateral hippocampal depth electrode data from VGAT-ires-Cre mice on a C57BL/6J background (n = 29) implanted using a customized 3D-printed headplate designed in our lab (2). These mice were treated with intra-amygdalar injections of kainic acid (N=23) or saline (N=6) to induce epileptogenesis. Data was scored by the pipeline following AASM and Rechtschaffen and Kales criteria, with an accuracy >93% relative to expert scoring.
This large-scale dataset has allowed us to verify that average total time spent in wakefulness was significantly increased after treatment in IAKA animals when compared to baseline, with parallel decreases in NREM and REM. The same findings were evident in comparing IAKA treated mice to saline controls during the post-treatment period. In addition, sleep fragmentation is increased, as evaluated by number of state transitions from sleep states into wakefulness in both the within-subject and between-subjects comparisons. These results suggest that the IAKA mouse model of TLE is consistent with human TLE with regard to sleep loss and sleep fragmentation.
These results show that our reproducible headplate-based electrophysiology combined with our rapid and accurate classifier for mouse data can improve the accuracy and speed of sleep-wake research in rodent models of epilepsy. The use of these techniques in other rodent models of epilepsy, combined with the use of novel electrophysiological techniques for the acquisition of multiunit data, can vastly improve the throughput of future research into both multi-model comparisons as well as circuit-level mechanisms underlying this sleep and epilepsy relationship.
References
1.Harvey, B. J., Olah, V. J., Aiani, L. M., Rosenberg, L. I., & Pedersen, N. P. (2023). Classifier for the Rapid Simultaneous Determination of Sleep-Wake States and Seizures in Mice. In bioRxiv (p. 2023.04.07.536063). https://doi.org/10.1101/2023.04.07.536063

2.Zhu, K. J., Aiani, L. M., & Pedersen, N. P. (2020). Reconfigurable 3D-Printed headplates for reproducible and rapid implantation of EEG, EMG and depth electrodes in mice. Journal of Neuroscience Methods, 333, 108566-108566.

Disclosures: **B.J. Harvey:** None. **L.I. Rosenberg:** None. **L.M. Aiani:** None. **N.P. Pedersen:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.09/D8

Topic: B.08. Epilepsy

Support: Creighton HSSIF

Title: Effects of pH on autoresuscitation efficacy in a mouse model of Sudden Unexpected Death in Epilepsy

Authors: *J. HINMAN, S. IYER, S. MATTHEWS, K. A. SIMEONE, T. A. SIMEONE; Pharmacol. and Neurosci., Creighton Univ., Omaha, NE

Abstract: During a generalized convulsive seizure (GCS), patients often stop breathing due to tonic muscle paralysis. The autoresuscitation reflex is engaged by the resulting hypoxia and hypercapnia (HH) decreasing pH and triggering a chemoresponse to increase ventilation, expel excess CO_2, and increase pH. If pH is stabilized, eupnea ensues. If too much CO_2 is expelled, respiratory alkalosis-induced hypocapnia triggers apnea to increase CO_2 again (apparent as post-convulsive central apnea). The hypercapnia/hypocapnia oscillation will continue, lessening each cycle until CO_2 stabilizes and eupnea ensues. In sudden unexpected death in epilepsy (SUDEP), individuals fail to autoresuscitate. We hypothesize that the starting pH has a critical role in determining autoresuscitation efficacy. We tested the effects of decreasing or increasing *in vivo* pH on ventilatory chemoresponses and autoresuscitation of wild-type mice and *Kcna1*-null littermates, a well-studied model of SUDEP. For 48 hours, mice were supplemented with ammonium chloride (280 mM) to decrease, or sodium bicarbonate (150 mM) to increase pH. Mice were placed in a whole-body plethysmography and subjected to either a combination of hypoxia (6% O_2) and hypercapnia (3-9% CO_2) to test for ventilatory chemoresponses**,** or an anoxia challenge (97% N_2 and 3% CO_2) to test for autoresuscitation. *Kcna1*-null ventilation response to the HH challenge was variable indicating chemoresponse dysfunction. We found only 25% of *Kcna1*-null mice (3 of 12) mounted a successful autoresuscitation response, whereas 75% of wild-type mice succeeded (9 of 12). In contrast, increasing pH improved autoresuscitation and survival in 72% of *Kcna1*-null (5 of 7) and 66% wild-type mice (6 of 9). In contrast, decreasing pH reduced autoresuscitation success to 0% of *Kcna1*-null (0 of 11) and 62% wild-type mice (5 of 8). Next, we tested whether daily treatment influenced lifespan. Daily treatment with ammonium chloride decreased lifespan, whereas treatment with sodium

bicarbonate increased lifespan. These data suggest that baseline pH may affect the ability to commence a proper chemoresponse and autoresuscitation, which may be particularly detrimental in individuals at high-risk for SUDEP.

Disclosures: J. Hinman: None. **S. Iyer:** None. **S. Matthews:** None. **K.A. Simeone:** None. **T.A. Simeone:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.10/D9

Topic: B.08. Epilepsy

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CURE Epilepsy, The Joanna Sophia Grant
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Title: Time-of-day-dependent mortality in seizure-induced death is coincident with extracellular serotonin and eliminated in serotonin neuron deficient mice.

Authors: *B. L. KREITLOW[1], *B. L. KREITLOW[2], M. J. SUMMERFIELD[2], G. F. BUCHANAN[3];
[1]Neurol., Univ. of Iowa, Iowa City, IA; [3]Neurol., [2]Med. Scientist Training Program, Interdisciplinary Grad. Program in Neuroscience, Iowa Neurosci. Institute, Neurology, Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: *Rationale:* People with epilepsy are at risk of sudden death, or sudden unexpected death in epilepsy (SUDEP). SUDEP is more common during the night and is often attributed to seizures during sleep. Our lab has demonstrated that an independent circadian mechanism may contribute to nighttime risk. Seizures induced during wakefulness in mice housed in constant darkness are more fatal during the subjective night. Mouse models of spontaneous seizure-associated death also tend to die more during the night. These findings suggest an underlying circadian rhythm may influence SUDEP timing. Serotonin (5-HT) is a compelling target of study. Extracellular levels of 5-HT oscillate throughout the twenty-four-hour day, with decreasing levels during the night in humans and rodents. 5-HT neurons have anticonvulsant effects, regulate breathing, and are required to awaken in response to increasing CO_2. *Methods*: Adult male adult (3 - 7 month) *Lmx1b*$^{f/f/p}$ (homozygous floxed *Lmx1b* alleles and hemizygous ePet1 Cre recombinase) and wild-type *Lmx1b*$^{f/f}$ mice were used for this study (N = 12 - 16 per group). Conditional knockout of the *Lmx1b* gene in ePet1 Cre-containing neurons eliminates the majority (>99%) of 5-HT neurons in the brain. Seizure-naïve mice were subject to a single

maximal electroshock seizure (30 or 50 mA, 60 Hz, 200 ms) at six evenly spaced time points (*Zeitgeber Time* (ZT) 2, 6, 10, 14, 18, and 22) during wakefulness. A separate cohort of animals (5 - 8 per group) were instrumented with microdialysis guide canulae to continuously sample extracellular 5-HT levels in the dorsal raphe nucleus (1 µL/min, 60 min/sample, 24 - 54-hour collection). *Results:* Consistent with previous findings from our lab from C57BL6/J mice, wild-type *Lmx1b*$^{f/f}$ mice were more likely to die during the dark phase of the twenty-four-hour day (44% (ZT 18) versus 25% (ZT 10) at 30 mA and 75% (ZT 18) versus 25% (ZT 6) at 50 mA), which is coincident with the nadir of extracellular 5-HT (1.4 pg/µL at ZT 17 by high-performance liquid chromatography). *Lmx1b*$^{f/f/p}$ animals, meanwhile, had high mortality regardless of time of day (75% and 66% mortality at ZT 18 and 6, respectively, at 30 mA, and 75% at both times at 50 mA). *Conclusions:* Nighttime seizures appear to carry increased risk of death. The time-of-day and circadian mechanisms that convey this risk are poorly understood. Preliminary work suggests that a circadian serotonergic mechanism may be related to time-of-day mortality following seizures. Better understanding this serotonergic mechanism may allow researchers to facilitate development of low cost chronotherapeutic strategies to improve serotonergic tone during the nighttime and reduce nighttime risk of SUDEP.

Disclosures: B.L. Kreitlow: None. **B.L. Kreitlow:** None. **M.J. Summerfield:** None. **G.F. Buchanan:** None.

Poster

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.11/D10

Topic: B.08. Epilepsy

Support: San Paolo Foundation

Title: Low glycemic index diet restrains epileptogenesis in a gender specific fashion

Authors: *C. MICHETTI[1,2], D. FERRANTE[1], B. PARISI[1], L. CIANO[2], C. PRESTIGIO[1], S. CASAGRANDE[1], S. MARTINOIA[3], E. MILLO[1], P. VALENTE[1], S. GIOVEDI[1], F. BENFENATI[2], P. BALDELLI[1];
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Abstract: Dietary restriction, such as low glycemic index diet (LGID), have been extensively and successfully used as antiepileptic approach; however, if such diet could also exert a real antiepileptogenic action is still unclear. Here, we investigated in Synapsin (Syn) II KO mice, a mouse model of hereditary temporal lobe epilepsy, whether administration of LGID during the latent period, which precedes the appearance of the first seizure, inhibits epileptogenesis. In these mice, seizures appear around 3 months after birth, offering a window of opportunity in which LGID may affect the epileptogenic process. Pregnant Syn II KO mice were fed either a LGID or

standard diet (StD) during gestation and lactation. Both diets were maintained in weaned mice up to 5 months of age. The latency and duration of the first behavioral seizure, as well as the behavioral analyses of the seizure elements were investigated by video recordings. Notably, LGID delayed the seizure onset and induced a reduction of seizures severity only in Syn II KO female mice. Behavioral seizure analysis was followed by high-density multielectrode array recordings in acute brain slices. Coherently with the behavioral results, a reduction of the frequency, amplitude, duration, covered-area and velocity of propagation of inter-ictal events was observed in the hippocampus of LGID-fed Syn II KO females, but not in males, suggesting a possible involvement of sexual hormones in this gender-specific effect of LGID. ELISA-based analysis of plasmatic and cortical ALLO revealed did not show any gender-influence. Notably, LGID increased cortical but not plasmatic concentrations of ALLO in female, while it remained unchanged in male mice. These results strongly suggest that the *gender-specific* interference with the epileptogenic process induced by LGID finds a fundament in a *gender-specific* increase of cortical ALLO, a neurosteroid known for its capability to strength GABAergic inhibitory inputs, acting as an endogenous benzodiazepine.

Disclosures: C. Michetti: None. **D. Ferrante:** None. **B. Parisi:** None. **L. Ciano:** None. **C. Prestigio:** None. **S. Casagrande:** None. **S. Martinoia:** None. **E. Millo:** None. **P. Valente:** None. **S. Giovedi:** None. **F. Benfenati:** None. **P. Baldelli:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Program #/Poster #: PSTR189.12/D11

Topic: B.08. Epilepsy

Support: FNS grant #31003A_179399

Title: Lactate receptor signaling as a modulator of epileptic seizures

Authors: *M. ALESSANDRI, A. OSORIO-FORERO, A. LUTHI, J.-Y. CHATTON; Lausanne Univ., Lausanne, Switzerland

Abstract: We previously reported, using in situ hybridization and a HCAR1-reporter mouse line, that the lactate receptor HCAR1, a Gi-coupled receptor, is especially enriched in the hippocampal glutamatergic neurons in the CA fields and in the dentate gyrus. Additionally, experiments on primary neuronal cultures and acute brain slices revealed that activation of HCAR1 downmodulates neuronal activity by mobilizing the adenylyl cyclase-cAMP-protein kinase A axis. These data prompted us to investigate the role of lactate in the pathology of epilepsy and its ability to control seizure episodes by acting on the lactate receptor HCAR1.We found that lactate released by astrocytes mediates seizure termination through its action on HCAR1 in a hippocampal slice model of epilepsy. We found that HCAR1-KO mice displayed a lower seizure threshold in the systemic kainate epilepsy model, i.e. a marked increase in their

susceptibility to developing seizures. The power of the frequency bands recorded using LFP electrodes positioned in the CA3 of freely behaving mice was altered in HCAR-KO with increased theta and high-frequency oscillations. Interestingly, a single-dose hippocampal injection of selective non-metabolized HCAR1 agonists at the time of seizure induction failed to reduce seizure severity in the intrahippocampal kainic acid model in WT mice. We hypothesized that the expected increase in tissue lactate concentration during ictal activity would prevent further activation of HCAR1 by exogenous agonists. Overall, we found that HCAR1 is an important player in the modulation and termination of epileptic seizures. Whether HCAR1 pathways can be targeted for seizure control, e.g., using allosteric modulators, still needs to be evaluated.

Disclosures: M. Alessandri: None. **A. Osorio-Forero:** None. **A. Luthi:** None. **J. Chatton:** None.

Poster

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Topic: B.08. Epilepsy

Support: NIH IR15NS124008-01
Regional Alliance of INBRE Network [RAIN]

Title: Novel non-Cannabis derived cannabidiol congeners modulate EEG activity and attenuate seizure severity

Authors: *A. CARRILLO[1], A. CONTRERAS[2], W. MAIO[3], D. J. HINES[4], R. M. HINES[4]; [1]Interdisciplinary Neurosci. PhD Program, Univ. of Nevada Las Vegas, Univ. of Nevada, Las Vegas, Henderson, NV; [2]Interdisciplinary Neurosci. PhD Program, Dept. of Psychological and Brain Sci., Univ. of Nevada, Las Vegas, Las Vegas, NV; [3]Dept. of Chem., New Mexico State Univ., Las Cruces, NM; [4]Psychology, Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Developmental epilepsy syndromes are neurological disorders characterized by recurrent severe seizures and developmental delays. The majority of clinically available antiepileptic drugs target seizures through the regulation of $GABA_A$ receptor signaling, in order to decrease neuronal membrane excitability, however, there are adverse effects for the developing brain, and many patients are refractory. Cannabidiol (CBD), the major non-psychotropic cannabinoid of *Cannabis*, has therapeutic potential as an anticonvulsant. The oral CBD solution Epidiolex is the only FDA-approved CBD-based treatment for severe epilepsy, but its production is costly, and its applicability is limited. In the present study, we use electroencephalography (EEG), behavior, and histology to characterize the effects of non-*Cannabis* derived CBD congeners, with the larger goal of identifying novel therapeutics for childhood epilepsy syndromes with improved production efficiency and therapeutic efficacy,

while causing less harm. We administered the congeners via oral gavage following baseline EEG recording and identified diverse and potent effects on spectral frequency bands. Structure activity relationship assessment suggested that elongated alkyl chains ((+)-*ent*-CBD-8) increased the potency of EEG effects produced by the congeners. Next, we examined the effects of (+)-*ent*-CBD-8 on the effects of the chemoconvulsant kainite in both wildtype mice, and in a mouse model of developmental epilepsy (*Gabra2*-1). We found that pre-treatment with (+)-*ent*-CBD-8 promotes seizure resilience in both wildtype and *Gabra2*-1 mice, by influencing seizure event characteristics and risk of mortality. We further examined the effects of daily oral gavage (5 days) in wildtype and *Gabra2*-1 mice during postnatal development, and found that (+)-*ent*-CBD-8 normalized the aberrant dendritic spine phenotype of *Gabra2*-1 mice. This suggests that variations in the alkyl chain length of synthetic CBD congeners may enhance therapeutic potential for treatment of developmental epilepsy, supporting the development of novel anticonvulsants.

Disclosures: **A. Carrillo:** None. **A. Contreras:** None. **W. Maio:** None. **D.J. Hines:** None. **R.M. Hines:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Title: Network activation and seizure generation reveal the participation of non-hippocampal neuronal networks in temporal lobe epilepsy and the impact of visual and auditory stimulation in seizure induction

Authors: *S. T. BELLO[1,2], S. XU[2], X. LI[2], J. REN[2], P. JENDRICHOVSKY[3], F. JIANG[2], X. CHEN[2], J. HE[2,3];
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Abstract: Epilepsy is a neurological disorder that affects over 45.9 million people globally. Temporal lobe epilepsy (TLE) is the most predominant form of acquired epilepsy which is characterized by hippocampal lesions and is often drug resistant. Although several studies have attributed epileptic activities in TLE to the hippocampus, however, the involvement of non-hippocampal neuronal networks in the pathology of TLE is often neglected. Here, we sought to understand how non-hippocampal neuronal networks participate in the pathology of chronic TLE. In this study, TLE was induced through injection of kainic acid (KA) in the dorsal hippocampus of C57BL/6J mice. Neuronal network activation after spontaneous seizure was assessed using c-Fos expression. Experimental paradigms to induce seizure using light or noise stimulation were developed. Seizure onset zone (SOZ) and frequency of epileptic spikes (ES) were evaluated using multiple-site *in vivo* electrophysiology. To assess seizure recurrence in the absence of hippocampus, unilateral removal of KA-injected hippocampus was conducted via surgical aspiration, and bilateral hippocampi were removed using Ibotenic acid. c-Fos expression after spontaneous seizure in chronic TLE mice showed that both hippocampal and cortical neurons were activated during spontaneous seizures, and the exposure of chronic TLE mice to sensory stimulus in the form of light or noise stimulation resulted in the precipitation of light-induced or noise-induced seizures, respectively. Furthermore, localization of SOZ in light-induced or noise-induced seizures revealed the existence of cortical and hippocampal SOZ in chronic TLE. Additionally, ES were observed in the cortex and hippocampus of mice after intrahippocampal KA injection, and the frequency of ES continued to increase in the cortex and hippocampus from the latent to the chronic phase beyond what was present in baseline physiology showing that TLE pathology goes beyond the hippocampus. Importantly, we found that surgical removal of the KA-injected hippocampus or chemical removal of the bilateral hippocampi of chronic TLE mice does not stop seizure recurrence in chronic TLE mice, revealing that seizures in chronic TLE can occur independently of the hippocampus. This study has shown that network pathologies that evolve in TLE are not localized to the hippocampus alone; instead, remote brain regions are also recruited in the development of TLE. This work, therefore, demonstrates the fundamental role of non-hippocampal neuronal networks in generating epileptic activities in the presence or absence of hippocampus in the chronic stage of TLE.

Disclosures: **S.T. Bello:** None. **S. Xu:** None. **X. Li:** None. **J. Ren:** None. **P. Jendrichovsky:** None. **F. Jiang:** None. **X. Chen:** None. **J. He:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Topic: B.08. Epilepsy

Support: NIH Grant NS111389
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Title: Respiratory chemoresponses in Kv1.1 KO mice, a preclinical model of Sudden Unexpected Death in Epilepsy (SUDEP)

Authors: *S. IYER[1,3], S. DRAVES[2], S. MATTHEWS[3], S. HERR[2], C. BOOTH[2], M. YEH[2], T. A. SIMEONE[3], K. A. SIMEONE[3];
[1]Dept. of Pharmacol. and Neurosci., [2]Sch. of Med., Creighton Univ., Omaha, NE; [3]Dept. of Pharmacol. and Neurosci., Creighton Univ. Sch. of Med., Omaha, NE

Abstract: Sudden unexpected death in epilepsy (SUDEP) is one of the leading causes of death in epilepsy. Retrospective studies indicate that prior to SUDEP, patients experienced a generalized convulsive seizure followed by repeated apnea. Failure to autoresuscitate from apnea-induced hypoxic and hypercapnic (H-H) blood gas fluctuations resulted in death. It is unknown why the autoresuscitation response fails in SUDEP, thus representing a critical knowledge gap. H-H fluctuations activate the hypothalamic chemosensing orexin neurons that project to brainstem respiratory nuclei which then restore normal breathing. **This homeostatic central chemoreception is critical to the autoresuscitation response and maybe impaired in SUDEP.** We have previously reported that the Kv1.1 KO (KO) mice, a model of SUDEP, have increased seizures, apnea, chronic hypoxia and progressively worsening blood gas instability as they approach death. We also found that the KO mice had higher number of orexin neurons. Blocking orexin receptors reduced their apneas and increased longevity. This suggests an augmented orexinergic influence on chemoresponses in these mice. We hypothesize that, a) as KO mice approach SUDEP, they have impaired chemoresponses to hypoxic-hypercapnic challenges and fail to autoresuscitate; b) blocking orexin receptors will improve chemoresponses and autoresuscitation. Using whole-body plethysmography, wildtype (WT) and KO mice were subjected to either an intermittent hypoxia (6% O_2) test, or hypoxia-hypercapnia (9% CO_2, 6% O_2) test, or an anoxia-autoresuscitation test (97% N_2, 3% CO_2). In response to hypoxia/hypoxia-hypercapnia, older high-SUDEP risk KO mice had significantly higher tidal volume, peak expiratory flow and minute ventilation, compared to WT controls ($p<0.01$ each, n=6). These parameters increase in proportion to O_2 debt and are exaggerated in the KO mice. When tested for autoresuscitation, 85% of older WT mice (11 of 13) were able to autoresuscitate and survive. However, 75% of the older high-risk KO mice (9 of 12) failed to autoresuscitate and died. In a younger cohort, both WT and KO were able to autoresuscitate. Blocking orexin receptors with TCS1102 (100mg/kg) improved autoresuscitation and survival in a majority (~85%) of high-SUDEP risk KO mice (12 of 14). These data suggest that as the KO mice approach SUDEP, they have impaired ventilatory chemoresponses and fail to autoresuscitate. Blocking orexin receptors improved autoresuscitation in these mice. Thus, an exaggerated orexin response maybe contributing to central chemoreception dysfunction and autoresuscitation failure in this preclinical SUDEP model.

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Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Program #/Poster #: PSTR189.16/D15

Topic: B.08. Epilepsy

Support: Science Foundation Ireland (SIRG/18/5646)

Title: The RNA modification m^6A contributes to epileptogenesis via dysregulation of synaptic and immune related pathways

Authors: J. MATHOUX[1], M.-M. WILSON[1], J. KESAVAN[1], M. CANAVAN[2], L. VILLALBA-BENITO[3], E. LANGA[1], A. SANZ-RODRIGUEZ[1], M. ALKHAYYAT[3], N. DELANTY[4], D. O'BRIEN[4], T. ENGEL[1], D. C. HENSHALL[1], ***G. P. BRENNAN**[3];
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Abstract: Purpose: The mechanisms governing the generation and maintenance of hyperexcitable networks are poorly understood. Changes in the properties of the neurons which compose these networks constitutes an important aspect of this process and are driven, at least in part, by large scale disruption of gene expression and gene expression regulation. Several layers of gene dysregulation have been identified in epilepsy including at the epigenetic, transcription, and non-coding RNA levels. However it is likely that other gene regulatory mechanisms contribute to the altered gene expression patterns which characterise epilepsy. One mechanism which remains unexplored in epilepsy are RNA modifications. Here we perform the first characterization of N-6methyladenosine (m6A) in epilepsy. m6A is an abundant internal modification of RNA which influences stability, sub-cellular localization and translational efficiency of RNA. We then explore the relationship between m6A and epilepsy-associated pathways and test the potential of targeting m6A to prevent seizures.**Method**: m6A-sequencing, microarrays and mass spec analyses were used to profile m6A transcriptome wide and effects on translation. Human iPSC derived neurons and pre-clinical mouse models of epilepsy were used to explore the functional relevance of m6A in epilepsy using viral overexpression and small molecule inhibition of METTL3 to elevate or deplete m6A. Telemetry EEG recordings and behavioural assays were used to determine the therapeutic potential of targeting this pathway for epilepsy treatment.**Results**: Global m6A hypermethylation was identified in both human and mouse epilepsy and is likely driven by upregulation of the m6A writer enzyme METTL3. Dysregulation of m6A was apparent on mRNA transcripts related to metabolism, glutamatergic signaling and inflammation. Modification of m6A alters these pathways in an in vitro and in vivo setting and contributes to epilepsy development.**Conclusion**: Disrupted m6A tagging of RNA alters hippocampal gene readout and contributes to the pathogenesis of the epilepsy development.

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Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Program #/Poster #: PSTR189.17/D16

Topic: B.08. Epilepsy

Support: CDMRP W81XWH-18-1-0655

Title: Inhibitory Neurons Underly the Excitatory/Inhibitory Imbalance and Seizure Severity in an Optogenetic Mouse Model of Temporal Lobe Epilepsy

Authors: *M. YACUN, S. VADAPALLI, K. ERCAN, F. C. TESCAROLLO, S. C.-Y. CHEN, H. SUN;
Rutgers, The State Univ. of New Jersey, Piscataway, NJ

Abstract: Temporal lobe epilepsy (TLE) is the most common form of seizure disorder and can be a debilitating condition to those affected. The local signaling mechanisms involved in initiating and progressing seizures of TLE are not well understood. One hypothesis is that seizures arise from an excitatory-inhibitory imbalance, which suggests the problem originates when the ratio of excitatory to inhibitory signaling (E/I) in the seizure onset area deviates too far from optimal in either direction.

To test this, we use an optogenetic mouse model of TLE with simultaneous calcium fiber photometry for inhibitory parvalbumin-expressing (PV) neurons and putative excitatory pyramidal neurons expressing CaMKII to evaluate the balance of E/I signaling as seizures initiate and progress. Our method utilizes a stimulating light to initiate seizures in the ipsilateral hippocampus and records both PV and CaMKII Ca2+ activity on the contralateral side. Near the onset of seizures, we observed a dramatic rise in both PV and CaMKII Ca2+ levels. The PV peaking pattern is the most discerning feature between seizures. Specifically, we found an initial PV peak at seizure onset, and a later PV peak after bilateral behavior was observed in the mouse. The initial PV increase peaked before the initial excitatory CaMKII peak and before seizure generalization; whereas the second, post-generalization peak in PV occurred at the same time as the second CaMKII peak. We also showed a gradual decline in the presence of the first peak and an increase in the second with greater seizure severity. This implies that the failure of the inhibitory system to trigger at seizure onset may have led to more runaway excitatory activity before generalization. Then, the seizure would eventually spread and cause simultaneous population discharge of cells regardless of type, including PV and CaMKII cells, creating the second peaks. We also found that the first (naïve) seizure of the recording day had an excitatory-favored E/I signaling ratio prior to generalization than later seizures within the same day while having significantly lower severity. Looking only at non-naïve seizures would create the expectation of a greater severity for these seizures based on their E/I ratio, but the fact that they are less severe shows these seizures may have a disinhibitory role, enabling later seizures to become more severe with a still lower E/I ratio.

Our results show that there is a complex, time-dependent pattern in the E/I signaling ratio as seizures progress. The initial relative inhibitory activity from PV neurons may play a pivotal role in determining the eventual severity of the seizure.

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Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Topic: B.07. Network Interactions

Support: Work supported by (T32 #NS07288 and F32#NS123009) to JMH and (R01 #NS34774) to JRH.

Title: Machine Learning Based System Discovery Identifies Specific Inter-regional Coupling and Synchrony as an Absence Seizure Mechanism

Authors: *J. HULL[1], S. GANGULI[2], J. R. HUGUENARD[3];
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Abstract: Absence seizures (AS) are characterized by synchronized spike wave discharges in multiple cortical areas. Numerous mutations, brain structures, and cell types are identified as generating AS. A focus on single regions or ion channels may however overlook common dynamics which achieve similar effects on inter-regional communication. Due to the brain's complexity, describing these interactions requires a mathematical framework. However, without prior knowledge of the equations this approach is intractable. Recent advancements in machine learning now enable model identification directly from primary data, simultaneously identifying model terms and their combinations which account for many simultaneous observations. We used 16-site electrocorticogram (Ecog) recordings from Scn8a+/- and Hcn2EA/EA mouse models of absence and then used the sparse identification of nonlinear dynamics algorithm (SINDy) to identify a data driven model of AS, SINDy found equations for nonlinear oscillators with phase and amplitude-dependent coupling. When simulated, the model recapitulates 36 measures of oscillation phase, amplitude, and frequency relationships across regions simultaneously. Phase plane analysis identifies that seizure activity largely depends on the coupling strength between somatosensory and frontal/secondary motor regions. Using silicon probes, we find synchronous neuronal firing is highly correlated with the oscillations described by the model in somatosensory and motor integration related brain regions while limbic, prefrontal, visual, and olfactory related regions are less robustly recruited. We detect spike pattern shifts in the posterior thalamic nucleus (PO) 2 seconds before seizure onset, identifying a robust pre-seizure state only apparent when observing numerous neurons simultaneously. Using current source density analysis, we identify strong sink source pairs in layer 1 and layer 5 of cortex during absence seizures corresponding to the projection zones of PO axons. Importantly, PO is known to enhance the strength cortico-cortico communication across the somatosensory/motor axis, providing a physiological basis for the enhanced cortico-cortico

coupling detected by SINDy. Altogether, our approach provides a quantitative mechanism of AS generation, providing multiple therapeutic targets which are not apparent by studying these brain regions in isolation.

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Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.19/D18

Topic: B.08. Epilepsy

Support: NIH D-SPAN 1F99NS125773 (LAB)
NSF GRFP (LAB)
NINDS R37 NS065434
ALSAM Foundation

Title: Reactive NG2glia show lasting alterations in their response to purinergic damage signals and a protective role in survival following viral brain infection and seizures

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Abstract: Viral infection is a major source of epilepsy in humans. As a result of many types of viral infection, the ensuing encephalitis, neuroinflammation, and tissue damage are implicated in development of epilepsy, yet the mechanisms underlying damage and repair responses following infection remain largely unknown. The Theiler's Murine Encephalomyelitis Virus (TMEV) mouse model of infection-induced epilepsy is a preclinical model of infection-induced seizures and acquired Temporal Lobe Epilepsy. Following infection with TMEV, viral tropism for hippocampal pyramidal neurons causes neuronal cell death, triggering the release of damage signals (including purine molecules ATP and ADP), and promoting reactive gliosis and neuroinflammation that contribute to seizure development. Following acute seizures, a seizure-free latent period emerges as the virus is cleared from the brain. However, extensive tissue damage, circuit rewiring, and glial scarring contribute to the development of epilepsy. We recently demonstrated that NG2glia (aka oligodendrocyte precursor cells) become reactive, increase proliferation, and participate in scar formation following TMEV infection. Additionally, NG2glia express the purinergic receptors P2X7 and P2Y1, whose hyperactivation likely contribute to NG2glia reactivity. To investigate functional changes that accompany NG2glia reactivity, we assessed the NG2glia calcium response to focal application of purines (100μM ATP and ADP) in acute brain slices from mice following TMEV infection. Using a triple-transgenic mouse to express a tdTomato reporter and a genetically encoded calcium indicator (GCaMP6f) in NG2glia, we provide evidence of lasting deficits in P2R-mediated intracellular

calcium signaling in reactive hippocampal NG2glia during both acute seizures and the latent period. This deficit was not found in the cortex, indicating that reactive NG2glia specifically have a reduced ability to generate calcium signals upon P2 receptor activation. In addition, using chemogenetics to selectively ablate NG2glia prior to infection, we found that nearly 50% of NG2glia-ablated mice did not survive infection. Additionally, preliminary data indicate that reduced survival observed in NG2glia-ablated mice may be due to reduced infiltration of macrophages and lymphocytes following infection. Collectively, our study provides evidence of a critical role for NG2glia in surviving viral encephalitis and identifies changes in purinergic calcium signaling that may contribute to NG2glia reactivity, proliferation, scar formation, and mouse survival following infection.

Disclosures: L.A. Bell: None. **L. Buxton:** None. **A.B. DePaula-Silva:** None. **J.N. Wagner:** None. **K.S. Wilcox:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

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Topic: B.08. Epilepsy

Support: R01NS103090
R01NS122834

Title: Developmentally-regulated dysfunction of parvalbumin-positive interneurons in a mouse model of SCN8A epileptic encephalopathy

Authors: *R. MIRALLES, A. BOSCIA, S. KITTUR, J. HANFLINK, M. K. PATEL; Univ. of Virginia, Charlottesville, VA

Abstract: *SCN8A* epileptic encephalopathy (EE) is a severe epilepsy syndrome resulting from gain-of-function mutations in the *SCN8A* gene, which encodes the sodium channel $Na_V1.6$. $Na_V1.6$ is expressed in the distal axon initial segment (AIS) of both excitatory and inhibitory neurons in adult mice. However, there is a distinct developmental switch between the second and third postnatal weeks where $Na_V1.6$ replaces $Na_V1.2$ at the distal AIS. Previous studies indicate hyperexcitability in excitatory neurons at early developmental time points. Inhibitory interneurons have been less studied in *SCN8A* EE, specifically parvalbumin-positive (PV) interneurons, which express $Na_V1.6$ and are critical for balancing network excitability. Developmental dysfunction of PV interneurons has been implicated previously in Dravet Syndrome, which results from mutations in $Na_V1.1$, indicating that this characterization is vital to further understanding *SCN8A* EE. To assess the effects of mutant $Na_V1.6$ in PV interneurons, we used a mouse model of *SCN8A* EE where the patient-derived R1872W mutation is expressed in a Cre-dependent manner. We used PV-Cre to generate a mouse that expresses this SCN8A mutation exclusively in PV interneurons, referred to as $Scn8a^{W/+}$-PV mice. We performed whole-

cell patch clamp experiments to assess action potential (AP) properties and excitability of PV interneurons in developing (P18-21) and adult (P42-56) wild-type (WT) and *Scn8a*$^{W/+}$-PV mice. Our results indicate that PV interneurons in developing *Scn8a*$^{W/+}$-PV mice are hypo-excitable compared to WT, and that APs in mutant PV interneurons have significantly decreased upstroke and downstroke velocity, resulting in an increased AP width. In adult mice, mutant PV interneurons were hyperexcitable at lower current injections compared to WT, yet they experience premature depolarization block, a state of action potential failure, at higher current injections, leading to overall hypo-excitability. Interestingly, adult *Scn8a*$^{W/+}$-PV interneurons do not show differences in AP properties. Furthermore, we assessed the seizure phenotype of *Scn8a*$^{W/+}$-PV mice in vivo and show that they have audiogenic seizures at P21. Adult *Scn8a*$^{W/+}$-PV mice exhibit both audiogenic and spontaneous seizures as well as decreased survival. Our data indicate that throughout development, multiple mechanisms of PV interneuron hypo-excitability may elicit a disruption within the inhibitory network in *SCN8A* EE, leading to unchecked cortical excitation. Further recognizing the developmental contributions of PV interneurons is critical in understanding both the initial appearance and chronic stage of epilepsy in *SCN8A* EE.

Disclosures: **R. Miralles:** None. **A. Boscia:** None. **S. Kittur:** None. **J. Hanflink:** None. **M.K. Patel:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.21/D20

Topic: B.08. Epilepsy

Support: NIH U54 NS108874
NIH T32 AG020506

Title: Gene expression pattern contributes to phenotypic diversity in genetic epilepsy

Authors: *W. CHI, E. KISKINIS;
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Abstract: Characterized by recurrent seizures, epilepsy affects approximately 70 million people worldwide. Epilepsy is genetically and phenotypically diverse, with several hundred genes having been identified and a wide range of comorbidities having been reported. It remains unknown whether gene expression patterns contribute to the phenotypic diversity. Here we use publicly available datasets to systematically examine the expression pattern of 247 epilepsy-associated genes across human tissues, developmental stages, and different brain cell types. We group genes into 3 broad categories based on their associated phenotypes: core epilepsy gene (CEG), where seizures are the core symptom, developmental and epileptic encephalopathy gene (DEEG) that are associated with neurodevelopmental delay induced by epileptic activity, and

seizure-related gene (SRG), which are characterized by developmental delay and gross brain malformations. We find that DEEGs are highly expressed within the CNS, while SRGs are most abundant in non-CNS tissues. DEEGs and CEGs exhibit highly dynamic expression in various brain regions across development, spiking during the prenatal to infancy transition. Lastly, the abundance of CEGs and SRGs is comparable within cellular subtypes in the brain, while the average expression level of DEEGs is significantly higher in GABAergic neurons and non-neuronal cells. Together, our analysis provides an overview of the expression pattern of epilepsy-associated genes with spatiotemporal resolution and establishes a broad expression-phenotype correlation in epilepsy.

Disclosures: W. Chi: None. **E. Kiskinis:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Program #/Poster #: PSTR189.22/D21

Topic: B.08. Epilepsy

Support: Leon Levy Foundation
NIH NINDS U54NS117170

Title: 2-dimensional and neural organoid patient-derived stem cell models of stxbp1-associated epilepsy

Authors: W. PARKER[1], C. WANG[2], T. JI[3], W. NIU[3], J. PARENT[3], M. D. UHLER[4], M. ROSS[2], ***C. A. PEARSON**[2];
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Abstract: STXBP1 encephalopathy causes epilepsy and often severe developmental delay. There are currently no effective treatments, and the mechanism of disease is debated. STXBP1 is a critical component of the SNARE complex and is necessary for presynaptic neurotransmitter release. Previous studies have demonstrated individual genetic variants impact protein function in a variety of ways. Through a battery of biochemical, immunohistochemical (IHC) and functional assays, we sought to characterize patient specific STXBP1 variants in 2D and 3D-directed neuronal differentiation models. Using patient-derived induced pluripotent stem cells (iPSCs), we used Dual Smad inhibition protocols to generate glutamatergic and GABAergic neurons with missense/frameshift and truncating mutations. Furthermore, using one of our patient-derived iPSC lines we generated glutamatergic iNeurons and cortical organoids to assess STXBP1 mutant phenotypes.
Our biochemical assays demonstrated diminished protein expression levels, decreased solubility and binding of STXBP1 variants with other components of the SNARE complex is ameliorated.

IHC analyses demonstrated significantly increased neuronal cell death, aggregation of STXBP1 protein and decreased incorporation of STXBP1 at synapses in patient-derived neurons. 3D cortical organoids generated with patient-derived iPSCs also demonstrate a severe neuronal cell death phenotype. Importantly, the phenotypes we have observed are recapitulated in all three differentiation models.
Functional analyses including Multielectrode Array (MEA) and calcium imaging assays demonstrate STXBP1 mutations resulted in reduced firing rate and a lack of synchronous network activity. Reduced firing rate is observed in glutamatergic and GABAergic neurons and this phenotype is rescued by treatment with the molecular chaperone 4-phenylbutyrate (PHB). We demonstrate a common mechanistic action across different categories of STXBP1 variants. Our results suggest that both haploinsufficiency and dominant-negative mechanisms contribute to STXBP1-associated disease. In addition, our data provide pre-clinical data supporting trials of PHB as therapy for children with STXBP1 encephalopathy.

Disclosures: W. Parker: None. **C. Wang:** None. **T. Ji:** None. **W. Niu:** None. **J. Parent:** None. **M.D. Uhler:** None. **M. Ross:** None. **C.A. Pearson:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.23/D22

Topic: B.08. Epilepsy

Support: AES Junior Investigator Award

Title: Machine learning, genome editing, and functional characterization to resolve variants of uncertain significance in TSC2

Authors: *C. G. BIAR, *C. PFEIFER, G. L. CARVILL, J. D. CALHOUN;
Ken and Ruth Davee Dept. of Neurol., Northwestern Univ., Chicago, IL

Abstract: Tuberous sclerosis (TS) is among the group of neurodevelopmental disorders called the mTORopathies. Characterized by benign tumors, drug-resistant epilepsy, and other cognitive manifestations, TS affects 1 in 6,000 individuals born in the United States. This condition is caused by loss-of-function (LoF) genetic variants in the TSC1/2 complex, which result in constitutive mTOR signaling. As a result, ongoing clinical trials are testing mTOR inhibitors as a precision therapy for individuals with TS. However, access to precise treatment requires a precise genetic diagnosis. In *TSC2* alone, more than 2,600 variants of uncertain significance (VUSs) have been documented in ClinVar—and this number is likely to increase as next-generation sequencing becomes increasingly affordable and accessible. To address the growing challenge presented by VUSs, there is a critical need to develop tools to resolve their functional impacts. Our long-term aims are to (1) develop a *TSC2*-specific machine learning (ML) algorithm for variant pathogenicity prediction and (2) establish a high-throughput functional

assay for *TSC2* VUS resolution. We have developed a ML model which utilizes ~40 features associated with variants in *TSC2*, including features related to evolutionary conservation and protein structure. We have also validated a functional assay whereby phosphorylation of S6 (P-S6), a well-characterized biomarker of mTOR pathway activity, distinguishes pathogenic from reference alleles. As a proof of principle, we used CRISPR/Cas9 genome editing to knockout *TSC2* in HAP1 cells. We then pooled $TSC2^{KO}$ and $TSC2^{WT}$ cells and tested whether FACS sorting by P-S6 level would be sufficient to enrich for cells with TSC2 LoF. Indeed, we observed that cells with high S6 phosphorylation were enriched for $TSC2^{KO}$ alleles. We next tested a known pathogenic missense variant, TSC2 p.Arg611Gln. Similarly, cells with constitutive mTOR signaling (high P-S6) were enriched for TSC2 p.Arg611Gln relative to unsorted cells or cells with low P-S6. Based on this and our previous work on mTORopathy-associated variants in *SZT2*, we conclude that sorting based on P-S6 labeling distinguishes LoF variants from WT. We are now adapting this approach to incorporate prime editing-mediated saturation genome editing, increasing throughput to hundreds of *TSC2* variants. This data will be used to test the validity of our ML pathogenicity predictions and to refine the performance of this classifier. This gene-specific workflow for improving the rate of VUS resolution is readily adapted to perform in other mTORopathy genes, such as *NPRL2*, *MTOR*, and *DEPDC5*.

Disclosures: **C.G. Biar:** None. **C. Pfeifer:** None. **G.L. Carvill:** None. **J.D. Calhoun:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.24/D23

Topic: B.08. Epilepsy

Support: NIH R01 AA011147

Title: Development of novel models to elucidate the role of ALDH4A1 deficiency on brain physiology

Authors: *B. R. KRAEMER, D. MOCHLY-ROSEN;
Chem. & Systems Biol., Stanford Univ., Stanford, CA

Abstract: Aldehyde dehydrogenases (ALDH) are a family of 19 $NAD(P)^+$-dependent human enzymes responsible for oxidizing toxic aldehydic species into carboxylates. ALDH4A1 is a mitochondrial, NAD^+-dependent, dimeric enzyme and oxidizes the toxic aldehyde glutamic-γ-semialdehyde to glutamate. ALDH4A1 lies at the nexus of proline metabolism, arginine metabolism, the TCA cycle, and affects mitochondrial health. The substrate of ALDH4A1, GSA, inactivates vitamin B6, an essential cofactor for amino acid and neurotransmitter biosynthesis. ALDH4A1 is highly expressed in the brain, and mutations of ALDH4A1 cause the severe pediatric disease Hyperprolinemia Type II, which results in seizures, though the precise mechanism of ALDH4A1 pathology is poorly understood. We have developed a novel cell

culture and zebrafish model of ALDH4A1 deficiency to characterize the human disease. Human induced pluripotent stem cells (iPSCs) were edited using CRISPR to knock-in a disease-causing variant (Ser352Leu). Cells were differentiated into neural stem cells, neurons, and astrocytes. Proline concentration was measured, and viability assays were performed to determine cell type specific effects. The S352L astrocytes exhibited the hallmark characteristic of the human disease, elevated proline levels, whereas neurons did not show changes in proline concentration. The S352L iPSCs were less viable upon treatment with GSA than the wildtype. Additionally, the S352L variant protein was much less stable than the wildtype although RNA expression was equivalent. Zebrafish larvae were treated with GSA to induce epileptiform activity *in vivo*. Larvae were also edited using CRISPR to knock-out ALDH4A1. Zebrafish larvae treated with GSA exhibited epileptiform activity and had greater *cfos* expression. The F_0 knock-out model displayed greater bursting activity, resembling the human seizure phenotype. Together, we have developed the first cell culture model of Hyperprolinemia Type II that can be used to study various cell types while maintaining the same genetic background. Our results indicate that ALDH4A1 pathology is mediated in a cell-specific manner, mainly astrocytes, resulting in the CNS-related pathologies. In addition, our *in vivo* zebrafish larvae model of Hyperprolinemia Type II provides a high-throughput assay to screen for chemicals and genes that modulate the human disease. These in culture and *in vivo* models will provide further insights into the effects of ALDH4A1 deficiency on physiology and provide a platform to test potential therapeutic agents to treat ALDH4A1 deficiency for a variety of neurological diseases, such as Hyperprolinemia Type II.

Disclosures: B.R. Kraemer: None. **D. Mochly-Rosen:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

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Topic: B.08. Epilepsy

Support: NINDS Grant R01-NS096976
NINDS Grant U54-N3117170

Title: A novel machine learning-based approach to seizure detection in larval zebrafish

Authors: *P. WHYTE-FAGUNDES[1], J. EFROMSON[2], A. VANCE[3], S. CARPENTER[2], A. CARROLL[3], M. HARFOUCHE[4], S. C. BARABAN[5];
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Abstract: Epilepsy is a major neurological disorder defined by unprovoked recurrent seizures. Treatment options often remain inadequate, and preclinical translational research falls short of

capturing the complexity and heterogeneity of the disorder. Clinically, behavioral seizure manifestations are an easily recognizable feature of epilepsy, however, they are objectively rated by clinicians. Similarly, traditional methods for evaluation of preclinical models also rely on a subjective “Racine” behavioral seizure scale (initially established in rodents over 60 years ago). Both strategies are susceptible to observer bias. To address this problem, we developed a machine learning algorithm to automate detection and quantification of seizure behavior in zebrafish; a preclinical vertebrate model compatible with high-throughput drug discovery. We collated a repertoire of spontaneous abnormal and frank convulsive behaviors compiled from monitoring 1200+ freely moving zebrafish larvae placed individually in a 96-well plate format. Pentylenetetrazol (PTZ) was used to acutely evoke seizures at varying stages of development (3, 5 and 7 days old); video images were captured using a high-resolution multi-camera array microscope (MCAM, developed by Ramona Optics) at frame rates greater than 160 Hz. PTZ-evoked seizures were detectable by quantifying changes in larval movement over time (termed a motion index (MI)), with greater MI values invariably associated with convulsive behavior. Coupling skeletal multi-point tracking of individual larvae with custom unsupervised machine learning algorithms, designed to automate identification of unique behavioral categories, we established a novel behavioral seizure scale. This algorithm detected the range of normal swim behaviors through full body convulsive events that are followed by prolonged loss of posture episodes. The clustering algorithm applied, utilized egocentrically aligned values of each individual larvae, to uncover clusters of varying seizure behaviors. These were automatically isolated on a frame-by-frame basis to track the progression of seizure activities. Implementing machine learning tools will accelerate (i) deep computational phenotyping of zebrafish epilepsy models and (ii) facilitate large-scale objective and reproducible screening of candidate antiseizure medications.

Disclosures: P. Whyte-Fagundes: None. **J. Efromson:** None. **A. Vance:** None. **S. Carpenter:** None. **A. Carroll:** None. **M. Harfouche:** None. **S.C. Baraban:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

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Topic: B.08. Epilepsy

Support: NINDS K08NS118107 (CMM)
CURE Taking Flight Award (CMM)
Epilepsy Study Consortium (CMM)

Title: Machine-learning enables high-throughput, low-replicate reverse genetic screen for novel anti-seizure targets in larval zebrafish

Authors: *C. M. MCGRAW[1], B. ROBENS[2], C. BAKER[3], C. M. LACOURSIERE[5], A. PODURI[4];

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Abstract: Rationale. New anti-seizure medications (ASMs) with better efficacy and tolerability are needed, but efforts to identify new anti-seizure targets have been limited. *We hypothesize that gene knock-outs that confer resistance to proconvulsants by loss-of-function in animals may suggest candidates for new ASM development*. Zebrafish are a model of chemical and genetic seizures. To facilitate zebrafish screens, we developed a method using machine learning to detect anti-seizure responses using calcium fluorescence. To identify novel anti-seizure targets, we propose a reverse genetic screen using the published MIC-Drop approach to deliver multiple sgRNA and Cas9 RNPs in oil droplets from a library of presynaptic targets. **Methods.** Calcium fluorescence. Calcium fluorescence from unrestrained larval zebrafish expressing neuronal genetically encoded calcium indicator (*elavl3::Gcamp6s*) was recorded using the Hamamatsu FDSS7000EX fluorescent plate reader. Multiple per-fish and per-event statistics including fish movement and fluorescence changes are extracted (MATLAB). An elastic net logistic classifier was trained (N>4000 events, N=63 fish; pre- and post-15mM PTZ treatment) using 70:30 train:test split and 10-fold cross-validation. Reverse genetic screen**.** Presynaptic genes with high human CNS expression / enrichment and known zebrafish orthologs are targeted by 4 sgRNA per gene (310 genes; N=1195 sgRNA total). Injected F0 CRISPant knock-out fish (N=12 fish per gene) are assessed for morphology; locomotor activity; and seizure-like activity (spontaneous and after PTZ). Fish with isolated reduction in PTZ response are prioritized, and gene targets identified by DNA barcodes. **Results.** The logistic classifier detects seizure-like events with high accuracy (AUC-ROC 0.98). Seizure event rate increases as a dose-response to proconvulsant (PTZ) and falls as a dose-response to anti-seizure drug treatment. Bootstrap simulation (5000 resamples) suggests anti-seizure responses can be detected with N=8 replicates based on robust strictly standardized mean difference (RSSMD) thresholds (brief VPA, RSSMD <= -0.82, TPR 92.2%; prolonged TGB, RSSMD<= -0.76, TPR 91.2%) while maintaining 5% false positive rate. In a test screen, the anti-seizure effect of 4 out of 5 known ASMs was detected at a single concentration (200uM) based on N=4-6 replicates. **Conclusions.** Our approach is suitable for detecting novel anti-seizure compounds or gene-specific changes in proconvulsant response. Phenotyping MIC-Drop injected F0 knock-out larvae may identify novel presynaptic targets whose loss-of-function confers seizure resistance similar to ASMs.

Disclosures: C.M. McGraw: None. **B. Robens:** None. **C. Baker:** None. **C.M. LaCoursiere:** None. **A. Poduri:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.27/D26

Topic: B.08. Epilepsy

Title: Seizures in a bang-sensitive mutant impact some forms of learning in a Drosophila larval associative learning model

Authors: S. CHO[1], *E. R. REYNOLDS[2];
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Abstract: The seizures associated with epilepsy may impact learning and memory and are associated with early cognitive decline, however the mechanism of how the seizures themselves lead to the deficiency is not clear (Duncan et al., 2006; Palop et al. 2009; Vingerhoets, 2006). Drosophila has been used effectively as a model for human epilepsy by using mutant flies that have seizure phenotypes (Fisher et al 2023). In this study, we tested whether seizures induced in Drosophila *para*^{bss} mutants, a mutation in the fly sodium channel gene, affect learning. We used both rewarding and aversive stimuli to test associative learning in Drosophila larvae, similar to the methods developed by Apostolopoulou et al. (2010) and Pauls et al. (2010). Third instar larvae were collected from CS (wildtype), *dunce* (a mutant that doesn't learn), and *para*^{bss} lightly laid bottles. For the rewarding stimulus task, larvae were trained 3x by pairing a neutral odor with a reward (sugar). The larvae were then tested for movement towards the odor without the reward. For the aversive learning task, a brief shock was paired with the reward as training and then movement away from the reward was determined. We found that we could demonstrate associative learning using larva and that *para*^{bss} learned well as compared to wildtype using both rewarding and aversive stimulus tasks. To test the impact of seizure, the three groups of larvae were exposed to the cold between the training and testing trials, which cause the *para*^{bss} mutant larvae to seize. Seizure in the mutant flies disrupted the rewarding associative learning but not the aversive learning task. Similar to results we obtained previously in an adult courtship assay, seizures in *para*^{bss} mutants can disrupt learning but may impact only some types of learning tasks.

Disclosures: S. Cho: None. **E.R. Reynolds:** None.

Poster

PSTR189. Epilepsy: Cellular and Molecular Mechanisms in Animal Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.28/D27

Topic: C.01. Brain Wellness and Aging

Title: Planaria as a Model for Understanding Alcohol-Induced Seizure Susceptibility

Authors: *H. IBRAHIM, B. HUANG, R. ARPAIO, M. SCOTTO, S. GUARIGLIA;
New York State Inst. for Basic Res., Staten Island, NY

Abstract: Alcohol use disorder is a significant public health issue associated with various physical and mental complications. Chronic alcohol exposure is known to increase seizure risk. Animal models, particularly ethanol (EtOH) exposure, have been widely used to study alcoholism effects. Chronic EtOH exposure induces brain and behavioral changes, affecting

neurotransmitter systems (glutamatergic, GABAergic, dopaminergic, and serotonergic). Our study aimed to determine if chronic alcohol exposure heightens seizure susceptibility using bicuculline, a GABAa receptor inhibitor. We conducted two experimental conditions in planaria. In the first condition, planaria were exposed to 0.1% EtOH for seven days, followed by testing seizure-like activity after exposure to increasing bicuculline concentrations (0 uM to 100 uM) for three minutes in an open field. In the second condition, planaria were exposed to EtOH for 14 days, experienced a day of withdrawal, and then received increasing bicuculline concentrations (2 uM, 20 uM, and 200 uM) for ten minutes in an open field. Our results showed that chronic EtOH exposure increased seizure-like activity susceptibility in planaria after withdrawal. This suggests that (1) GABAergic neurotransmission plays a role in the mechanism of chronic alcoholism and seizure susceptibility, and (2) planaria can serve as a high throughput model for alcohol toxicity and therapeutic testing. In summary, our study demonstrates the relevance of chronic alcohol exposure in seizure susceptibility, implicating the involvement of GABAergic neurotransmission. Planaria offer a valuable model for studying alcohol toxicity and testing potential therapies in a high throughput manner.

Disclosures: H. Ibrahim: None. **B. Huang:** None. **R. Arpaio:** None. **M. Scotto:** None. **S. Guariglia:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR190.01/D28

Topic: B.08. Epilepsy

Support: NOVO Nordisk Foundation (#81536)

Title: Reduction in radioligand binding to Monoacylglycerol lipase (MAGL) in brains from patients with treatment resistant temporal lobe epilepsy and brains from a mouse model of the same disease

Authors: *J. D. MIKKELSEN[1,2,3], S. S. ARIPAKA[2], B. A. PAZARLAR[2], L. H. PINBORG[2], F. GASTAMBIDE[3], B. BANG-ANDERSEN[3], J. F. BASTLUND[3];
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Abstract: Monoacylglycerol lipase (MAGL) is a cytosolic serine hydrolase that cleaves 2- and 1-monoacylglycerols into fatty acids and glycerol and is a potential novel drug target for treatment of numerous CNS disorders. Several MAGL inhibitors have been developed, some of which show anti-epileptic activity, probably via its inhibition of the degradation of the endocannabinoid, 2-arachidonylglycerol (2-AG). Radioligands such as [^{18}F]T-401 have been developed to identify MAGL binding sites in the brain and to determine occupancies and kinetics in drug development. Here we have further validated the binding properties of T-401 using

[^{3}H]T-401 in the human cortex obtained from patients with temporal lobe epilepsy (n = 19; temporal cortical samples resected under neurosurgical operations) and from brains from mice with chronic reoccurring seizures (brains obtained 44 weeks after unilateral intra-hippocampal injection of kainic acid). Specific and saturable binding of [^{3}H]T-401 was detected mostly in the cortical grey matter. Significant binding was also detected in the subcortical white displaying the same binding kinetics. The level of binding in the two compartments was strongly correlated. Saturation experiments revealed a Kd around 4 nM for human temporal cortex, and 7 nM for mouse brain, and full saturation around 30 nM. The binding could be completely blocked with the cold ligand and with another structurally different MAGL inhibitor, ABD-1970. The level of [^{3}H]T-401 binding in the human temporal cortex was highly variable among patients. Even it varied about 4-fold, it was not correlated to either epilepsy duration or the age of the patients. In the epileptic mouse brain, a significant reduction was observed bilaterally in the hippocampus, as well as in other cortical regions, including the temporal cortex. There was no correlation between the number of hippocampal discharges in the epileptic animals and [^{3}H]T-401 binding levels. These data support the presence of MAGL in neuronal and non-neuronal cells, and indicate that MAGL levels in brains of patients with epilepsy and mouse model of epilepsy is reduced not only in the epileptic zone in the hippocampus, but more widespread in the brain.

Disclosures: **J.D. Mikkelsen:** None. **S.S. Aripaka:** None. **B.A. Pazarlar:** None. **L.H. Pinborg:** None. **F. Gastambide:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **B. Bang-Andersen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **J.F. Bastlund:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.02/D29

Topic: A.02. Postnatal Neurogenesis

Title: Chronic administration of cannabidiol reduces LPS-induced inflammation and kainic acid-induced seizures in immature rats.

Authors: *O. GARCÍA[1,2], A. VEGA GARCIA[3], S. MEZA TOLEDO[1], S. A. OROZCO SUÁREZ[3];
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Abstract: Aim. To evaluate the anti-inflammatory effect of cannabidiol on the expression of cyclooxygenase-2 (COX-2), interleukin 1-β (IL 1-β) and tumor necrosis factor α (TNF-α) induced by administration of lipopolysaccharide (LPS) and on seizure susceptibility in immature rats. Methodology. Male rats, Sprague Dawley strain, 10 days postnatal age, were used, an LPS dose of 1 mg/kg i.p/ was administered for 5 days with a n=40, the following groups were made: Saline (SHAM) (0.9 % NaCl v.o.), Celecoxib (CLBX) 20 mg/kg/v.o. and Cannabidiol (CBD) 20

mg/kg/v.o. They were administered 2h post LPS, every 24h for 5 days, at 14PN seizure susceptibility was assessed with a subconvulsive dose of kainic acid (AK), i.p. 1.5mg/kg. The protein levels of COX-2, IL 1-β and TNF-α in hippocampal tissue were analyzed by Western blot at 6, 12 and 24 h post AK. Results. The experimental CBD group showed a significant decrease in the expression of COX-2 and the proinflammatory cytokines IL 1-β and TNF-α, similar to the CLBX group at 6 ($p<0.001$), 12 ($p<0.001$) and 24($p <0.001$) h compared to the LPS group, with a maximum peak at 12 hours. CBD increased seizure activity latencies with 60% of rats presenting severe seizures and 15% s*tatus epilepticus*, similar to the CLBX group with 80% severe seizures and an SE of 25%, compared to the CLBX group treated with LPS (100% severe crises and 80% SE). Conclusion. Treatment with CBD showed a neuroprotective effect on seizure susceptibility in the immature stage, associated with a decrease in COX-2 and proinflammatory cytokines (IL 1-β and TNF-α), which gives CBD an anti-inflammatory effect.

Disclosures: O. García: None. **A. Vega garcia:** None. **S. Meza Toledo:** None. **S.A. Orozco Suárez:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.03/D30

Topic: B.08. Epilepsy

Title: Cb2 activation delays epileptogenesis and protects cognitive functions without resolving neuroinflammation following status epilepticus induced in rat pups

Authors: *L. BEZIN, W. GRABON, B. GEORGES, J. BODENNEC, V. BLOT, S. RHEIMS, A. BELMEGUENAI;
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Abstract: Epileptogenesis is the neurobiological process by which epilepsy develops. It most often affects a healthy brain and occurs following severe brain damage. One of the key mechanisms underlying this pathological conversion is the subsequent neuroinflammation, mainly supported by microglial cell activation and extravasation of circulating monocytes. Agonists of the cannabinoid receptor type 2 (CB2), expressed in vivo by monocytes and microglia, i.e., myeloid cells, are known to mediate potent anti-inflammatory and neuroprotective effects. Here, we evaluated whether GP1a, a CB2-specific ligand administered following pilocarpine-induced *status epilepticus* (Pilo-SE) in 21-old-day male rat pups, counteracted epileptogenesis and underlying inflammatory processes and reduced the severity of cognitive impairments. We first found that CB2-mRNA level is increased in the hippocampus by 50% and 100% compared to controls at 1 day and 9 days post-Pilo-SE. At 1 day, CB2-mRNA level remained stable in resident microglia, meaning that the tissue increase observed is solely due to the massive monocyte infiltration. GP1a was administered at the dose of 3 mg/kg i.p. every other day for 2 weeks, starting 2 hours after SE onset. We provide evidence that the

establishment of memory impairments and their underlying molecular and cellular mechanisms can be thwarted by GP1a given during the early phase of epileptogenesis. We also show that the development of handling-induced seizures can be delayed and their number lessened by GP1a. Interestingly, these beneficial effects do not appear to be underpinned by a decrease in the inflammatory peak following Pilo-SE. In contrast to what has been previously described in other neuroinflammatory contexts, GP1a did not inhibit but rather potentiated monocyte extravasation into the brain. Investigations at cellular level by flow cytometry showed that GP1a did not alter the pro-inflammatory or neuroprotective phenotype of microglia and infiltrating monocytes. We also show that infiltrating monocytes express less TNFa mRNA and more IL10 mRNA than microglia, and are the only cells present in the brain to express arginase 1 mRNA, a prototypic marker of the neuroprotective/M2 phenotype of myeloid cells. Overall, our study highlights the promising therapeutic potential of drugs targeting CB2 during epileptogenesis, by promoting extravasation of neuroprotective monocytes.

Disclosures: **L. Bezin:** None. **W. Grabon:** None. **B. Georges:** None. **J. Bodennec:** None. **V. Blot:** None. **S. Rheims:** None. **A. Belmeguenai:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.04/D31

Topic: B.08. Epilepsy

Support: R01 NS126594
R56 NS099586

Title: Cannabidiol does not reduce seizure count in a Gria4 mouse model of absence seizures

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[1]Pharmacol., [2]Univ. of Virginia Sch. of Med., Charlottesville, VA; [3]Univ. of Virginia, Charlottesville, VA

Abstract: Absence seizures almost exclusively manifest in children and freedom from treatment failure rates are unacceptably poor. While evidence mounts supporting the use of cannabinoids to treat epilepsy, few studies have explored their performance in absence models. We explored the performance of cannabidiol (CBD) compared to ethosuximide and saline in Gria4spkw1 knockout CH3/HeJ mice (Jackson Laboratories), a model of absence seizures. Nine mice, ages 6-10 weeks, were randomly assigned to treatments of saline, ethosuximide (200 mg/kg), and CBD (100 mg/kg). All three compounds were tested in each mouse with a 24-hr washout period between tests. Compounds were administered via i.p. injection in a volume of 0.01 mL/g body weight following a 90-minute control period that measured spontaneous seizure rate. Seizures, defined as EEG waves exhibiting spike-wave discharge patterns with amplitude increases from baseline, were counted blindly using MATLAB-based seizure identification software and

manually validated for 90 minutes following injection and compared to pre-injection counts. Compared to saline controls, CBD failed to significantly reduce seizure counts (Mean= 11.1, SD= 37.8, p= 0.73, paired t-test). Conversely, ethosuximide produced a significant reduction in seizure count (Mean= -33.6, SD= 20.6), achieving significance relative to saline controls (p=0.002, paired t-test). Our results indicate that ethosuximide, but not CBD, reduces seizure counts in the Gria4spkw1 knockout model of absence epilepsy within 90 minutes of administration. Ethosuximide administration is expected to reduce seizure counts in an absence model and served as a positive control for the experiment. Additional dosing strengths of both CBD and ethosuximide should be assessed to establish a dose-response relationship in this model. Additional cannabinoids (cannabidivarin, cannabigerol) may also be evaluated in this model, as each cannabinoid demonstrates unique pharmacological properties. Nonetheless, we currently conclude that CBD is not an effective treatment for absence epilepsy.

Disclosures: **C. Vavrik:** None. **B. Goerl:** None. **M. Beenhakker:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.05/D32

Topic: B.08. Epilepsy

Support: RO1 NS040337
UVA Brain Institute

Title: β-hydroxybutyrate reduces neuronal excitability and seizures

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Abstract: The Ketogenic Diet (KD), a high-fat, low-carbohydrate diet, is a treatment option for adult and pediatric patients with epilepsy. The therapeutic effect of KD can result from the production of ketone bodies. β-Hydroxybutyrate (β-HB) is the primary ketone generated by KD, but there is debate about whether it exerts an anti-seizure effect and reduces neuronal excitability. In this study, we investigate the anti-seizure effect of β-HB on the most extreme form of a seizure, status epilepticus (SE), *in vivo* and its impact on neuronal excitability *in vitro*. We induced SE in 7-8 weeks old C57Bl/6 mice using Continuous Hippocampal Stimulation protocol. We injected either β-HB (1g/kg) or saline (control) intraperitoneally, 15 minutes after stimulation, in animals experiencing self-sustaining SE. We evaluated seizure duration and severity using video-EEG recordings. β-HB treatment significantly reduced SE duration compared to saline-injected mice (n=8 each, P<0.05; Kaplan-Meier survival comparison). To study the effect of β-HB treatment on the excited neuronal network *in vitro*, we injected CaMKII-Cre driven GCaMP7-AAV9 into the dorsal and ventral CA1 hippocampus of 4-6 weeks

old c57BL/6 mice. We studied GCaMP7-transfected CA1 pyramidal neurons, excited by elevating extracellular potassium, under the wide-field microscope before and after perfusing with β-HB. β-HB significantly reduced the fluorescence intensity of CA1 pyramidal neurons compared to the baseline fluorescence (%ΔF/F Baseline vs. β-HB 15.91 and -7.874%, n=3, P<0.0001; Brown-Forsythe and Welch ANOVA test), and the β-HB washout reversed its effect (%ΔF/F β-HB vs washout -7.874 and 8.034%; n=3, P<0.0001; Brown-Forsythe and Welch ANOVA test). We assessed the β-HB effect on active and passive neuronal membrane properties of CA1 neurons and on spontaneous excitatory postsynaptic currents (sEPSCs) and miniature EPSCs (mEPSCs) using patch-clamp electrophysiology. β-HB hyperpolarized the membrane potential of CA1 pyramidal neurons (n=14 cells; p<0.01, paired *t-test*). β-HB perfusion reduced the sEPSC frequency (n=8, P<0.0001; Kolmogorov-Smirnov test) and mEPSC frequency (n=8, P<0.0001; Kolmogorov-Smirnov test). β-HB shows promise as an anti-seizure agent against severe seizures, such as SE. β-HB reduces neuronal excitability and reduces the glutamate release from the presynaptic terminals.

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Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.06/D33

Topic: B.08. Epilepsy

Support: CMRPG3K1021
MOST 110-2314-B-182-055

Title: The anti-epileptogenic properties of 2-butyloctanoic acid in a rodent model of chronic epilepsy

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Abstract: Epilepsy is a prevalent chronic neurological disorder that currently lacks a curative treatment. The available therapies primarily serve as a symptomatic treatment to mitigate the frequency and severity of seizures, but do not target epileptogenesis or provide disease-modifying effects to prevent the development of epilepsy or alter its course and progression. There is a critical unmet need in epilepsy to seek a promising therapy to modify the epileptogenic process in the development of epilepsy. 2-butyloctanoic acid (2-BOA), a novel branched derivative of medium-chain fatty acids, has been suggested to have significant potency in suppressing epileptiform discharges *in Vitro*. To investigate the anti-epileptogenic properties of 2-BOA, we used a rat model of chronic epilepsy to determine its ability to suppress the

progression of epileptogenesis and to evaluate the neuroprotective and disease-modifying potentials in the development of epilepsy.Adult male Sprague-Dawley rats underwent stereotactic surgery for the implantation of a long-term video EEG/LFP telemetry recording system and intracerebral drug infusion system. A rat model of chronic mesial temporal lobe epilepsy was established by intra-amygdala microinjection of kainic acid (K.A.) to induced status epilepticus (SE), followed by a latent period and subsequent development of chronic epilepsy. One hour after K.A. induction, either 2-BOA or 0.9% saline was intraventricularly infused into brain and continued for one month. There was no significant difference in seizure severity and duration of SE between rats treated with slow infusion of 2-BOA and 0.9% saline, both electrographically and behaviorally. 2-BOA administration dramatically abolished the late spontaneous seizures during the one-month recordings after SE. Histological findings revealed that 2-BOA treatment significantly inhibited mossy fiber sprouting and alleviated hippocampal atrophy implicating its anti-epileptogenic properties. The treatment with 2-BOA significantly reduced caspase-3 expression in the CA1 region and TUNEL counts in the CA3 region, indicating that 2-BOA treatment effectively ameliorated neurodegeneration and apoptosis. Our study demonstrated 2-BOA effectively inhibits the development of spontaneous recurrent seizures with the presence of anti-epileptogenic and neuroprotective effects, suggesting its disease-modifying potentials.

Disclosures: **W. Chang:** None. **P. Kao:** None. **B. Chang:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.07/D34

Topic: B.08. Epilepsy

Support: NINDS [U01 NS074926]
Interagency Agreement AOD21005-001 00000

Title: Evaluation of Brivaracetam Alone or as an Adjunct Therapy Against Cholinergic-Induced Status Epilepticus in Rats

Authors: *D. NGUYEN[1], M. F. STONE[1], C. R. SCHULTZ[1], S. ORTA[1], M. D. FURTADO[2], J. NIQUET[3], C. G. WASTERLAIN[4], L. A. LUMLEY[1];
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Abstract: Status epilepticus (SE) is a life-threatening development of self-sustaining seizures which requires immediate response to effectively control. Benzodiazepines are the standard first line treatment against SE, however when treatment is delayed, internalization of $GABA_A$ receptors occurs over time and causes benzodiazepine pharmacoresistance to develop. The

addition of adjunct antiseizure medications (ASMs) is essential to ensuring protection against SE-related pathologies in models of benzodiazepine-refractory SE. Preclinical models have shown effective, but incomplete protection when NMDA receptor antagonist ketamine is used as an adjunct therapy to benzodiazepines. Brivaracetam is a recently FDA-approved ASM to treat partial onset seizures in pediatric and adult patients as a monotherapy or adjunct therapy. The current study compared delayed administration of benzodiazepine midazolam to brivaracetam monotherapy, brivaracetam-midazolam dual therapy, and brivaracetam-ketamine-midazolam triple therapy on multiple metrics of protection such as seizure severity, epileptogenesis, neuronal loss and neuroinflammation in a model of cholinergic-induced SE. Adult male rats were surgically implanted under anesthesia with subcutaneous telemetry transmitters for continuous monitoring of electroencephalographic (EEG) activity. Rats were exposed to a seizure inducing dose of organophosphorus chemical and treated with atropine sulfate and an oxime 1 min later. Through telemetry transmitters, seizure onset was identified in real time, and mono- or combination therapies were administered 40 min later. Epileptogenesis was identified by the onset of spontaneous recurrent seizures (SRS) between exposure and the 14-day study endpoint. Brain tissue was collected and processed at the study endpoint for markers of neuronal loss and neuroinflammation. While high survival rates were seen with brivaracetam monotherapies, with 100% survival, protection from SRS development, seizure severity, and neuropathology was not seen until the ASM was used as an adjunct to ketamine and midazolam. Similar protective effects were seen in triple therapy combinations where brivaracetam was administered at 10 or 30 mg/kg. Although the combination therapies with brivaracetam were not as effective on reduction of seizure severity and brain damage as our previously published reports on combinations that included phenobarbital or valproate, the current study reports potential for brivaracetam to improve outcome compared to midazolam monotherapy. Further research is needed to identify the most optimal drug combinations against cholinergic-induced SE.

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Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.08/D35

Topic: B.08. Epilepsy

Support: CNSF 32111530119
CNSF 81971204

Title: Anti-epilepsy effect of ZT-1a by enhancing potassium chloride cotransporter-2 functiontransporter function

Authors: J. CAI[1], X. LIU[2], J. ZHANG[3], ***Y. WANG**[4];
[1]Fudan Uni, Shanghai, China; [2]Zhongshan Hosp., Shanghai, China; [3]Exeter Univ., Exeter, United Kingdom; [4]Fudan Univ., Shanghai, China

Abstract: Epilepsy is a chronic neurological disorder featuring recurrent and unprovoked seizures, which is characterized by abnormal, synchronous, high-frequency neuronal discharges within the central nervous system. GABA and GABA receptors are one of the main inhibitory systems in adult CNS, and play an important role in epileptogenesis. $GABA_AR$-mediated inhibition depends on the maintenance of the low intracellular [Cl^-] concentration, which is mainly regulated by K^+-Cl^- cotransporter-2 (KCC2) in mature neurons. Previous studies have shown that the transport function of KCC2 is regulated by WNK-SPAK-KCC2 signaling pathway during neuronal development and this pathway can be activated under pathological states. ZT-1a is a selective SPAK inhibitor, supposed to activate KCC2-mediated Cl^- extrusion. This study aims to explore whether ZT-1a can activate KCC2-mediated Cl^- extrusion through inhibiting WNK-SPAK-KCC2 signaling pathway and whether ZT-1a can interrupt the progression from acute seizures to epileptogenesis. We applied ZT-1a in either *in vivo*, pentylenetetrazol and pilocarpine models or *in vitro* cyclothiazide-induced cultured hippocampal neurone model. The changes of correlated signaling pathway proteins and $GABA_AR$-mediated inhibition were quantitatively or qualitatively investigated by a series of experiment such as Western Blots, electrophysiology recordings, immunostaining, etc. to provide theoretical basis for the potential therapeutic effect of ZT-1a. We discovered that ZT-1a, (1) attenuated CTZ-induced epileptiform bursting activities in primary cultured hippocampal neurons, (2) decreased the susceptibility to PTZ-induced seizures, (3) alleviated pilocarpine-induced chronic spontaneous seizures, and (4) the suppressive effect of ZT-1a on epilepsy attributed to the inhibiting of the WNK-SPAK-KCC2 signaling pathway. These results demonstrated that zt-1a inhibition of WNK-SPAK pathway capable of preventing KCC2 deficit related epileptogenesis. In conclusion, WNK-SPAK inhibitor ZT-1a may serve as a lead compound for future anti-seizure drug development .

Disclosures: **J. Cai:** None. **X. Liu:** None. **J. Zhang:** None. **Y. Wang:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.09/D37

Topic: B.08. Epilepsy

Title: Gintonin, a Panax ginseng-derived LPA receptor ligand, attenuates kainic acid-induced seizures and neuronal cell death in the hippocampus via anti-inflammatory and anti-oxidant activities

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Abstract: Background: Gintonin (GT), a Panax ginseng-derived lysophosphatidic acid receptor (LPAR) ligand, has positive effects in cultured or animal models for Parkinson's disease, Huntington's disease, and so on. However, the potential therapeutic value of GT in treating epilepsy has not yet been reported.
Methods: Effects of GT on epileptic seizure (seizure) in kainic acid [KA, 55mg/kg, intraperitoneal (i.p.)]-induced model of mice, excitotoxic (hippocampal) cell death in KA [0.2 μg, intracerebroventricular (i.c.v.)]-induced model of mice, and levels of proinflammatory mediators in lipopolysaccharide (LPS)-induced BV2 cells were investigated.
Results: An i.p. injection of KA into mice produced typical seizure. However, it was significantly alleviated by oral administration of GT in a dose-dependent manner. An i.c.v. injection of KA produced typical hippocampal cell death, whereas it was significantly ameliorated by administration of GT, which was related to reduced levels of neuroglial (microglia and astrocyte) activation and proinflammatory cytokines/enzymes expression as well as increased level of the Nrf2-antioxidant response via the upregulation of LPAR 1/3 in the hippocampus. However, these positive effects of GT were neutralized by an i.p. injection of Ki16425, an antagonist of LPA1-3. GT also reduced protein expression level of inducible nitric-oxide synthase, a representative proinflammatory enzyme, in LPS-induced BV2 cells. Treatment with conditioned medium clearly reduced cultured HT-22 cell death.
Conclusion: Taken together, these results suggest that GT may suppress KA-induced seizures and excitotoxic events in the hippocampus through its anti-inflammatory and antioxidant activities by activating LPA signaling. Thus, GT has a therapeutic potential to treat epilepsy.

Disclosures: Y. Ha: None. **J. Choi:** None. **T. Kwon:** None. **H. Jo:** None. **I. Cho:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.10/D38

Topic: B.08. Epilepsy

Support: Biocodex

Title: Stiripentol efficacy against lethal audiogenic seizures in the LAGS+ selected mouse line used as a model of SUDEP

Authors: *B. MARTIN, G. DIEUSET, A. BIRABEN;
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is a dramatic outcome of epileptic patients, with a prevalence of 1/1000 person-years. Stiripentol (STP, Diacomit©) is an antiseizure

medication indicated for Dravet syndrome, a rare developmental and epileptic encephalopathy with high mortality rate (15/1000), in which SUDEP accounts for about 2/3. Here, we used a new genetic mouse model of SUDEP, the lethal audiogenic seizure line (LAGS+), to evaluate the efficacy of STP to reduce fatal seizures. Precisely this model developed by our lab, based on a directional genetic selection on phenotypic criteria, allows to dissociate indirect and direct anti-lethal effect. This selected line shows lethal hyper-sensitivity to audiogenic stimulation following presentation of a white noise sound stimulus. Directional selection was initiated from a four-way cross derived from the audiogenic prone strains DBA/1J, DBA/2J, BALB/cJ and 129/SvTer. After 9 generations of selection, LAGS+ mice showed a 99% rate of tonic seizures associated with a 99% lethality rate. Here, male and female LAGS+ mice at S9 generation received a single dose of STP (from 0 to 1000 mg/kg i.p.) 30 min before audiogenic stimulation. Each mouse was evaluated once, during a maximum of 60 sec, and the following patterns were scored: wild running, clonic seizures, tonic-clonic seizures, non-lethal tonic seizures, lethal tonic seizures. Regarding an indirect effect on lethality (or a reduction of the seizure susceptibility), STP has shown an anti-seizure effect from the dose of 100 mg/kg. This effect increased in a dose-dependent manner until the maximum dose of 1000 mg/kg, at which no mice had seizures. Regarding a direct effect on lethality (or an inhibition of the lethality in tonic seizures), we have observed that STP can reduce the lethality following a tonic seizure, when doses ranged between 100 and 200 mg/kg. In comparison, LAGS+ mice treated with vehicle all died from a tonic seizure. The major advantage of our new mouse model to study SUDEP lies on its robustness in presenting a lethal tonic seizure (>99%) following audiogenic stimulation. In conclusion, STP has shown that, depending on the dose range, it can reduce the lethality of tonic seizures, and at higher doses, exhibits anticonvulsant effects. Surprisingly, we did not observe any inhibition of lethality following tonic seizures at higher doses (>200 mg/kg). This result suggests that the direct and indirect effects of STP against lethality represent two independent mechanisms, and that STP is a suitable drug to prevent SUDEP. To our knowledge, this is the first time that a direct effect against mortality has been observed with an antiseizure medication.

Disclosures: **B. Martin:** 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.; Biocodex. **G. Dieuset:** None. **A. Biraben:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.11/D39

Topic: B.08. Epilepsy

Support: NS109668
NS112788
NS113955

Title: Riluzole and SKA-378 greatly reduce acute neural excitotoxic injury and neuroinflammation in the kainic acid rat model of human temporal lobe epilepsy

Authors: *T. KYLLO[1], L. SINGH[2], H. WULFF[2], J. ERICKSON[3];
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Abstract: Kainic acid (KA) evoked status-epilepticus (SE) in rats leads to excitotoxic neuronal cell death and increased microglia activation and astrocyte gliosis in the hippocampal CA1-4 regions. We have previously shown that systemic administration of riluzole or novel naphthalenyl substituted aminothiazole derivatives such as SKA-378 are neuroprotective in the hippocampus and other limbic regions (e.g., entorhinal/pyriform cortex, amygdala) at 3 days post KA-induced SE. Here, we tested the hypothesis that riluzole and SKA-378 protect hippocampal CA1-4 neurons at 7 and 14 days following KA-induced SE in adult male Sprague Dawley rats. Riluzole (10 mg/kg) and SKA-378 (30 mg/kg) were administered 1 h after KA-induced SE, and neural injury was assessed by fluorojade C labeling and neuroprotection was assessed by NeuN staining by IHC. KA-induced SE also is known to stimulate an inflammatory response in the brain. Microglial activation was assessed by Iba-1 and ED-1 labeling and gliosis was determined by GFAP and vimentin labeling by IHC. Immunohistochemical and Western blot analysis indicate that riluzole and SKA-378 administration reduces acute neuroinflammation that normally occurs from KA-induced SE at 7 and 14 d post-SE. Thus, riluzole and SKA-378 are compounds that attenuate acute neural hippocampal injury and inflammation at 3, 7, and 14 days following KA-induced SE. These results support the concept that acute limbic neural injury drives the inflammatory response after KA-induced SE. Our results predict that riluzole and SKA-378 may be anti-epileptogenic compounds that may help prevent the development of epilepsy in the KA model of TLE.

Disclosures: **T. Kyllo:** None. **L. Singh:** None. **H. Wulff:** None. **J. Erickson:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.12/D40

Topic: B.08. Epilepsy

Support: HORIZON-WIDERA-2021-ACCESS-03 [Grant Nr 101078981-GEMSTONE]

Title: Atipamezole suppressed dexmedetomidine-induced absence status epilepticus-like events

Authors: *M. YAVUZ[1], F. ONAT[2,3];
[1]Fac. of Pharmacy, Dept. of Pharmacol., [2]Dept. of Med. Pharmacology, Fac. of Med., [3]Inst. of Neurosciences, Acibadem Mehmet Ali Aydinlar Univ., Istanbul, Turkey

Abstract: Previously we have shown that alpha-2a adrenergic receptor antagonist atipamezole suppresses spike-and-wave discharges (SWDs) in genetic absence epilepsy rats from Strasbourg (GAERS) (Yavuz et al., 2020), and the antagonist dexmedetomidine induces absence status epilepticus-like events with accompanying behavioural arrest. Dexmedetomidine induces a two-phase of SWD to/sleep and sleep to/ absence status-like events transition and finally sustains prolonged absence status-like events after sleep in the second phase (Yavuz et al, 2022). The main purpose of this study was to evaluate if atipamezole can be an efficient anti-absence status medication in terms of suppressing prolonged seizures but not causing sedation or sleep. For this purpose, adult male GAERS rats were stereotaxically implanted with a guide cannula unilaterally into the right ventricle and bilaterally cortical electrodes over the frontoparietal cortex for EEG recordings. After recovery for one week and following 30 min basal recording, 2.5 µg dose of dexmedetomidine was injected through an intracerebroventricular route with simultaneous video recordings. Following anesthesia-sleep induced by dexmedetomidine, when the initial absence status-like events were triggered, diazepam (5 mg/kg) or atipamezole (1 mg/kg) were injected through the intraperitoneal route. Their effect on the prolonged seizure activities and wakefulness of the animals was evaluated in comparison to baseline EEGs of GAERS. Data are given as mean±SEM, and analysis was performed using two-way ANOVA followed by Tukey's multiple comparisons test. Atipamezole and diazepam both suppressed the prolonged seizure activities for 120 hours and no activity above 1 min has been observed in the EEGs following both injections ($p<0,05$). SWDs with a duration of 10-16 sec still occurred with atipamezole. The mean duration at the 140^{th} min of SWDs or prolonged seizures in groups were: Baseline: (14.2±1.8; n =4), Atipamezole: (15.2±3.8; n =4), Diazepam: (62.0±24.7; n =4) ($p<0,05$). These results indicate that a selective alpha-2a adrenergic receptor blockade stop absence status-like events without modifying SWDs. In comparison to diazepam, it is more advantageous in recovering sedation and sleep since the animals were awake. Atipamezole has the potential to be used as an anti-absence status agent in addition to an anti-seizure medication and can be superior to other agents currently used in clinics specifically for absence statuses or NCSEs.
Supported by HORIZON-WIDERA-2021-ACCESS-03 [Grant Nr 101078981-GEMSTONE].
1-Yavuz M, et al., Epilepsia 2020, 61, 2825-2835.2-Yavuz M, et al., Authorea, 2022, 8, 1046.

Disclosures: **M. Yavuz:** None. **F. Onat:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.13

Topic: B.08. Epilepsy

Support: Israel Science Foundation (Grant 1976/20)

Title: Cns-targeted antioxidant gene therapy suppresses the development of epilepsy

Authors: A. SAADI[1], P. K. SINGH[1], M. SHERMAN[1], A. SNOWBALL[2], A. LIEB[3], ***T. SHEKH-AHMAD**[1];
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Abstract: Epilepsy is a common neurological disorder affecting 1% of the global population, significantly burdening patients and society. Although many epilepsies are acquired following brain injury, available treatments only alleviate symptoms, and no effective prophylaxis or cure exists. Accumulating evidence suggests that oxidative stress plays a critical role in the development of seizures and epilepsy and that pharmacological targeting of oxidative stress can prevent spontaneous seizures. However, non-specific antioxidant therapies may disrupt the physiological balance of oxidants/antioxidants, highlighting the need for targeted interventions. Here, we used AAV vectors to drive the expression of the Nrf2-encoding gene, which promotes the endogenous antioxidant systems, under the control of CaMKIIa, a constitutive, cell-type-specific promoter for targeting excitatory neurons. We demonstrated that our AAV-CaMKIIa-Nrf2 vectors were selectively expressed in neurons and showed minimal expression in other cell types. Furthermore, our AAV-CaMKIIa-Nrf2 vector significantly decreased neuronal cell death induced by kainic acid-SE in the hippocampus. When injected prior to KA-SE, our AAV-CaMKIIa-Nrf2 vector dramatically reduced seizure frequency over 12 weeks and significantly decreased the total number of seizures compared to control rats. Additionally, 50% of animals remained seizure-free for 12 weeks after SE induction, and only 20% of animals became epileptic after treatment with our AAV-CaMKIIa-Nrf2 vector compared to 100% of animals in the control group. Our cell type-specific approach for targeted delivery of antioxidant therapies offers a promising strategy for combating oxidative stress following brain injury, preventing or modifying the development of epilepsy, while preserving the critical balance of oxidants/antioxidants in non-affected cells.

Disclosures: A. Saadi: None. **P.K. Singh:** None. **M. Sherman:** None. **A. Snowball:** None. **A. Lieb:** None. **T. Shekh-Ahmad:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.14/Web Only

Topic: B.08. Epilepsy

Support: Section of Postgraduated Studies and Research, Academic Secretary of National polytechnic Institute, México

Title: Effect of nitric oxide on glutamic acid decarboxylase activity, an in vitro study

Authors: *L. A. VEGA RASGADO[1], K. COSS SANCHEZ[2];
[1]BIOQUÍMICA, [2]ESCUELA NACIONAL DE CIENCIAS BIOLÓGICAS, INSTITUTO POLITÉCNICO NACIONAL, CDMX, Mexico

Abstract: Nitric oxide (NO) is a neurotransmitter/neuromodulator synthesized in a reaction catalyzed by Nitric Oxide Synthase (NOS), enzyme which presents 3 different isoforms: endothelial (e-NOS), neuronal (n-NOS) and inducible (i-NOS). NO participation in epilepsy is widely demonstrated, but its role in epileptogenesis is still a matter of controversy, with reports showing either pro or anticonvulsant effects. Previous results lead us to propose that a possible explanation of these paradoxical properties could be on the NOS isoform involved, which may present even opposite effects. Since main effects of NO in SNC are mediated through NMDA glutamate receptors and/or gamma aminobutyric acid (GABA) release and considering the key role of glutamic acid decarboxylase (DAG) in the metabolism of both neurotransmitters, the effect of NO on DAG activity was investigated. With this aim the effects of different concentrations and times of treatment of Nω-Nitro-L-arginine (L-NAME), 7-Nitroindazole (7-NI) and S-methylisothiourea hemisulfate (SMT), specific inhibitors of eNOS, nNOS and iNOS respectively, on DAG activity from mouse brain (CD1 strain, 20-25 g of body mass) were studied employing a spectrophotometric method described by Sasaki et al (Eur. J. Pharmacol. 367, 165-173, 1999). Experiments were conducted accordingly with the Helsinki Guide for Laboratory animals (Results $\pm$ SEM, $n \geq 4$, $p < 0.05$). Effects of different drugs depend on concentration and time of treatment. L-NAME decreased GAD activity at all concentrations tested, specially at short times of treatment (10 and 20 minutes), whereas 7-NI increased it to diminish slowly after 20 minutes of treatment (except for $1X10^{-5}$ M). SMT raised DAG activity at $1 X 10^{-5}$ M at all times of treatment essayed, but no effect or diminished enzymatic activity as concentration increased. Together, results suggest that NO convulsant or anticonvulsant properties are related to its effect on GAD activity, which depend on isoform involved, concentration and time of treatment. Apparently eNO seems to present anticonvulsant properties, whereas nNO and iNO have proconvulsant effects. These results contribute to understand the role of NO in epileptogenesis and represent a base to the development of new antiepileptic drugs.

Disclosures: L.A. Vega rasgado: None. **K. Coss sanchez:** None.

Poster

PSTR190. Antiepileptic Therapies: Pharmacology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.15/D41

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

Title: An integrated approach for early in vitro seizure prediction utilising hiPSC neurons and human ion channel assays

Authors: *R. ROBERTS[1,2], K. ROCKLEY[1], M. MORTON[1];
[1]Apconix, Macclesfield, United Kingdom; [2]Biosci., Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Seizure liability remains a significant cause of attrition throughout drug development. Advances in stem cell biology coupled with an increased understanding of the role of ion channels in seizure offer an opportunity for a new paradigm in screening. We assessed the activity of 15 pro-seizurogenic compounds (7 CNS active therapies, 4 GABA receptor antagonists and 4 other reported seizurogenic compounds) using automated electrophysiology against a panel of 14 ion channels (Nav1.1, Nav1.2, Nav1.6, Kv7.2/7.3, Kv7.3/7.5, Kv1.1, Kv4.2, KCa4.1, Kv2.1, Kv3.1, KCa1.1, GABA $\alpha_1\beta_2\gamma_2$, nicotinic $\alpha_4\beta_2$, NMDA 1/2A) (Figure 1). These were selected based on linkage to seizure in genetic/pharmacological studies. Fourteen compounds demonstrated at least one "hit" against the seizure panel and 11 compounds inhibited two or more ion channels. Next, we assessed the impact of the 15 compounds on electrical signalling using human induced pluripotent stem cell (hiPSC) neurons in microelectrode array (MEA). The CNS active therapies (amoxapine, bupropion, chlorpromazine, clozapine, diphenhydramine, paroxetine, quetiapine) all caused characteristic changes to electrical activity in key parameters indicative of seizure such as network burst frequency and duration. The GABA antagonist picrotoxin increased all parameters, but the antibiotics amoxicillin and enoxacin only showed minimal changes. Acetaminophen, included as a negative control, caused no changes in any of the parameters assessed. Overall, pro-seizurogenic compounds showed a distinct fingerprint in the ion channel/MEA panel. These studies highlight the potential utility of an integrated in vitro approach for early seizure prediction to provide mechanistic information and to support optimal drug design in early development, saving time and resources. Furthermore, this model offers great opportunity for further development towards discovery of novel anti-epileptics.

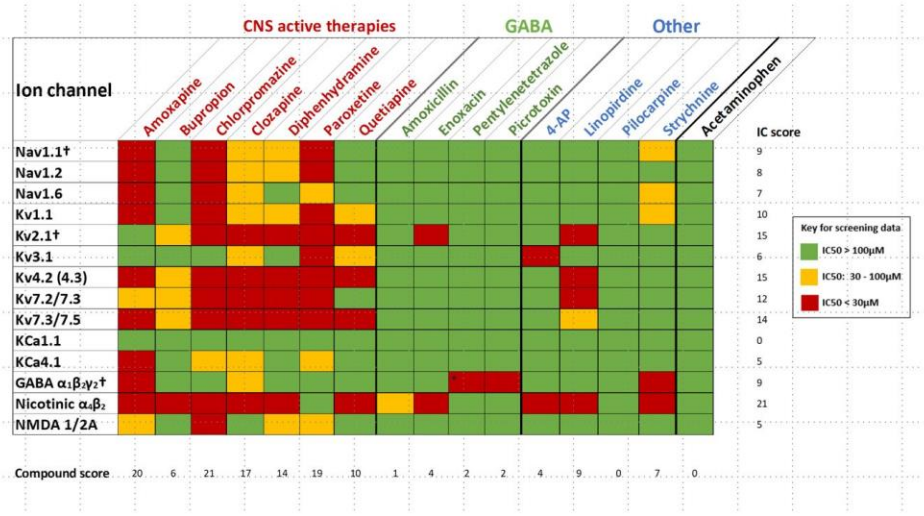

Figure 1. Activity of 15 CNS active therapies, GABA receptor antagonists and other reported seizurogenic compounds at 14 ion channels implicated in seizure. Key depicts highly active (IC_{50}<30mM red), moderately active (IC_{50} 30-100mM orange) and inactive active (IC_{50}>100mM green) compounds. Right-hand column: cumulative ranking of each ion channel for its potential association with seizure based and bottom row: cumulative ranking of each compound for its activity at ion channels based on a value of 2, 1 or 0 for high, intermediate and low inhibition hits, respectively.

Disclosures: **R. Roberts:** A. Employment/Salary (full or part-time):; ApconiX. **K. Rockley:** A. Employment/Salary (full or part-time):; ApconiX. **M. Morton:** A. Employment/Salary (full or part-time):; ApconiX.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.01/D42

Topic: B.09. Glial Mechanisms

Support: R01NS076815
R01MH113535
R01AG058621
startup funds from UT Health San Antonio, Joe R. & Teresa Lozano Long School of Medicine

Title: Single-cell transcriptomics analysis reveals enhanced glial immunity in mice deficient in monoacylglycerol lipase

Authors: *D. ZHU, J. ZHANG, J. HASHEM, F. GAO, C. CHEN;
UT Hlth. San Antonio, San Antonio, TX

Abstract: Inflammation has been recognized as a common mechanism of disease, and neuroinflammation is considered the root cause of neurodegenerative diseases. Previous studies have provided evidence that inactivation of monoacylglycerol lipase (MAGL) alleviates neuropathology and inhibits neuroinflammation by blocking the degradation of 2-arachidonoylglycerol in animal models of neurodegenerative diseases. Nevertheless, the precise molecular mechanisms underlying MAGL inactivation in curbing neuroinflammation remain largely undisclosed. To address this, we conducted single-cell transcriptomic analysis of microglia and astrocytes in MAGL conditional knockout (KO) mice. We present compelling evidence that mice with cell type-specific MAGL knockout exhibit distinct gene expression profiles in the brain. Inactivating MAGL triggers substantial alterations in the expression of genes associated with immune responses and inflammation in microglia and astrocytes. Notably, the increased expression of chemokines in microglia is more pronounced in mice lacking MAGL in astrocytes. Additionally, MAGL KO mice display modified expression of genes involved in regulating other cellular functions and Wnt signaling, specifically in astrocytes. Our findings offer transcriptomic confirmation that the cell type-specific inactivation of MAGL induces varying expression patterns of immune-related genes and essential cellular pathways in microglia and astrocytes. The upregulation of immune/inflammatory genes suggests that the baseline levels of immune/inflammatory surveillance are enhanced in microglia and astrocytes, particularly in microglia, through the inhibition of 2-AG metabolism. These findings likely contribute to the observed anti-inflammatory and neuroprotective effects following MAGL inactivation in neurodegenerative diseases.

Disclosures: **D. Zhu:** None. **J. Zhang:** None. **J. Hashem:** None. **F. Gao:** None. **C. Chen:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.02/D43

Topic: B.09. Glial Mechanisms

Title: Reactive astrocytes exhibit distinct genomic and transcriptomic states depending on the temporal duration of stimuli

Authors: *E. J. HILL, C. SOJKA, M. M. SAMPSON, A. T. KING, S. A. SLOAN;
Dept. of Human Genet., Emory Univ., Atlanta, GA

Abstract: Astrocytes respond to external or inflammatory stimuli, often produced by traumatic injury, ischemia, or neurological disease. These responses include transcriptomic, morphologic, and functional changes that together comprise a reactive phenotype. While reactive astrocytes can be induced via multiple intrinsic and extrinsic signals, exposure to the microglial-secreted cytokines, TNF- α, Il-1α, and C1q (TIC), is a robust trigger of the reactive state in vitro. However, several important questions remain, including which genomic processes initiate and maintain the reactive state, how the temporal duration of stimuli affects astrocytes, and whether the process is reversible. To explore these questions, we used human cortical organoids (hCO), which recapitulate human cortical development, including the formation of quiescent astrocytes. We validated the reactive astrocyte response by exposing hCOs to TIC for 24 hours and performing bulk and single-cell RNA-seq. We then observed transcriptomic changes reflective of a reactive state. Next, we exposed hCOs to TIC for a time course spanning one day to three months, performing paired ATAC- and RNA-seq at each timepoint. These data reveal the existence of at least two distinct genomic and transcriptomic stages of reactivity — an acute phase (induced by TIC exposure for 1-7 days), and a chronic phase (induced by TIC exposure for 1-3 months). Both stages possess unique differentially accessible transcription factor binding motifs, coupled with distinct differential gene expression profiles, which suggest that the reactive responses in astrocytes are temporally plastic. Analysis of chronic reactive astrocytes also revealed increased genomic accessibility and upregulation of major histocompatibility complex (MHC) class II genes, which encode receptors that are typically only present on professional antigen-presenting cells. We confirmed MHC class II protein expression via immunostaining and fluorescence-activated cell sorting. To investigate reversibility of the reactive state, we allowed hCOs exposed to either acute or chronic TIC to undergo a period of withdrawal. Both acute and chronic reactive astrocytes returned to a quiescent transcriptomic state, implying that the reactive state is highly plastic in the absence of sustained insult. However, chronic reactive astrocytes retained higher expression levels of MHC class II genes, even after withdrawal for 55 days. In ongoing experiments, we are now testing whether MHC class II upregulation in astrocytes promotes an inflammatory state or anti-inflammatory response to cytokine stimulation.

Disclosures: **E.J. Hill:** None. **C. Sojka:** None. **M.M. Sampson:** None. **A.T. King:** None. **S.A. Sloan:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.03/D44

Topic: B.09. Glial Mechanisms

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
NIH F32NS087783
Larry L. Hillblom Postdoctoral Fellowship

Title: Transcriptomic analysis of white matter and cortical reactive astrocyte heterogeneity after stroke indicates astrocyte-induced angiogenesis is a region-specific mechanism of repair.

Authors: *A. J. GLEICHMAN[1], R. KAWAGUCHI[2], G. COPPOLA[1], M. V. SOFRONIEW[3], S. CARMICHAEL[3];
[1]Neurol., [2]UCLA, Los Angeles, CA; [3]UCLA Sch. Med., Los Angeles, CA

Abstract: Stroke is one of the most common causes of death and disability, with few treatment options. Astrocytes respond rapidly and in a gradated manner to stroke, yet relatively little is known about the details of these responses or the functional changes astrocytes undergo. In order to more fully understand astrocytic responses to stroke and to identify targets to promote repair, we identified morphologically and phenotypically distinct zones of reactive astrocytes post-stroke and used these zones to inform a transcriptomic analysis of astrocyte heterogeneity. We have performed these analyses using both white matter and cortical stroke models, clinically distinct forms of stroke with different astrocyte subpopulations, and are using these datasets to identify and manipulate astrocytic responses to promote post-stroke repair. Transcriptomic data suggest that the most reactive cortical astrocytes promote angiogenesis to a greater extent than reactive white matter astrocytes. Angiogenesis is associated with recovery after gray matter stroke, but its role in recovery after white matter stroke is unclear. We confirmed that the gene Lamc1 is upregulated specifically in reactive cortical astrocytes and developed a viral Crispr-based approach to knock out this gene in astrocytes in the periinfarct cortex. This led to a decrease in angiogenesis specifically of larger-caliber vessels in the periinfarct 7 days post-stroke, while capillary angiogenesis was unaffected. We then virally overexpressed Lamc1 in peri-infarct white matter astrocytes and found an increase specifically in larger-caliber vessels in the peri-infarct white matter 7 days post-stroke. These vessels improve white matter stroke recovery, speeding behavioral recovery and leading to a smaller scar and denervated region Together, these results suggest astrocytic subpopulations response differently even to similar injuries, and that these differences, particularly pro-angiogenic factors, may be leveraged to promote region-specific repair.

Disclosures: A.J. Gleichman: None. **R. Kawaguchi:** None. **G. Coppola:** None. **M.V. Sofroniew:** None. **S. Carmichael:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.04/D45

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01NS110694

Title: Amelioration of reactive astrocyte transcriptomic signatures by haploinsufficiency of casein kinase alpha-prime (CK2)

Authors: *R. PELZEL, T. BROWN, J. VANTREECK, R. GOMEZ-PASTOR;
Univ. of Minnesota Grad. Program In Neurosci., Minneapolis, MN

Abstract: Amelioration of reactive astrocyte transcriptomic signatures by haploinsufficiency of casein kinase alpha-prime (CK2)
Authors**Ross Pelzel**, Taylor Brown, Mackenzie Thayer, Jillian VanTreek, Rocio Gomez-Pastor
AbstractHuntington's Disease (HD) is an adult-onset progressive motor disease caused by a PolyQ (CAG) repeat expansion in the Huntingtin gene (HTT), leading to the selective degeneration of striatal medium-spiny neurons (MSNs). Another well characterized hallmark of HD is reactive astrogliosis, which results in increased density of astrocytic processes as well as astrocyte dysfunction, which is a key factor in disease onset and progression. Interestingly, when the transcriptomic profile of various HD mouse models was analyzed, no robust differences in inflammatory RNA signatures were found in these disease models versus wild-type mice. However, a subset of genes has been found to be altered in HD mouse models that relate to astrocyte dysfunction, known as the 'HD-associated astrocyte molecular signature. Importantly, we have identified that haploinsufficiency of the catalytic subunit of casein kinase 2 (CK2), which has been reported to ameliorate pathology of HD in HD mice, ameliorated transcriptional changes of seven genes in the top 15 most dysregulated genes in the HD-associated astrocyte molecular signature. However, immunofluorescence experiments in the lab have revealed that haploinsufficiency of CK2 does not have an effect on number of astrocytes expressing glial fibrillary acidic protein (GFAP), a common marker for reactive astrocytes, or the relative intensity of GFAP immunoreactivity. Based on these results, multiple experiments are being performed in the lab in order to decipher how CK2 ' haploinsufficiency impacts astrocyte function and whether transcriptional changes seen in these astrocytes translate to disease modifying functions.

Disclosures: R. Pelzel: None. **T. Brown:** None. **J. VanTreeck:** None. **R. Gomez-Pastor:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.05/D46

Topic: B.09. Glial Mechanisms

Support: Louisiana Board of Regents
UL Lafayette, GSO

Title: Transcriptomics of the amygdala in a non-human primate model of self-injurious behavior

Authors: *J. BARUA[1], M. JACKSON[2], B. FORET[3], E. ROMERO[4], D. HASSELSCHWERT[4], J. FONTENOT[4], J. YOUNG BROOKS[5], F. VILLINGER[4], K. M. SMITH[2];
[1]Univ. of Louisiana, Lafayette, LAFAYETTE, LA; [2]Biol., Univ. of Louisiana At Lafayette, Lafayette, LA; [3]Biol., Univ. of Louisiana Lafayette, Lafayette, LA; [4]New Iberia Res. Ctr., New Iberia, LA; [5]Biol., Univ. of Louisiana at Lafayette, Lafayette, LA

Abstract: Self-Injurious Behavior (SIB), also known as Non Suicidal Self-Injury (NSSI), is the destruction of one's own body tissues without suicidal intent. It affects both humans and non-human primates and it has the potential to lead to suicide in both humans and non-human primates. Self-Injury is performed to alleviate emotional distress and is directly linked to the limbic system, particularly the amygdala region and reward circuit, which includes the Ventral Tegmental Area (VTA) and Nucleus Accumbens (NA). To fully comprehend the neurobiology of SIB, we are utilizing Rhesus Macaque (*Macaca mulatta*) brain samples provided by the New Iberia Research Centre (NIRC). These animals experienced modest to moderate wounding and SIB, neither of which was alleviated by treatment with Diazepam and increased environmental enrichment. The control animals were sound and euthanized according to the terminal research protocol. Previous studies showed the increased tendency of SIB in males than females at NIRC where the percentage is 1% due to robust behavioral enrichment program. This is especially modest compared to other primate facilities, where Self-Injury rates can reach up to 14%. It is thought that NSSI can be addictive by stimulating endogenous opioids in the reward circuit and amygdala. We have previously analyzed endogenous opioids and discovered a significant increase in the expression of Mu opioids receptor (MOR) mRNA in the amygdala of primates with SIB, as measured by quantitative reverse transcription (qRT-PCR). Here, we compared the transcriptome amygdala of primates with SIB (12 males and 4 females with SIB) to the amygdala of monkeys without a history of SIB (5 males and 4 females) via RNAseq studies. We performed gene sequencing experiments and used the DAVID and PANTHER bioinformatics tools to identify the 26 Differentially Expressed Genes (DEG) mainly based on log2 fold change and adjusted P-value. The genes Vimentin (VIM), HSD11B1, CD44, RARRES2, MB, MIR675, and HBA1 are among the Differentially Expressed Genes. For Immunohistochemistry (IHC) investigations, we have collected an additional five brains from control males and fifteen PFA-fixed brains from male primates with SIB. Since we hypothesize that self-injury is an inflammatory condition of the brain and that Vimentin is likely to be expressed as a result of overreacting astrocytes, we are conducting IHC studies with the Vimentin antibody in conjunction with unbiased stereological cell counts of Vimentin-positive cells. In addition, we

will conduct IHC combined unbiased stereological cell counts for MOR+ cells and immunoblot confirmation of MOR mRNA and Vimentin data in the amygdala.

Disclosures: **J. Barua:** None. **M. Jackson:** None. **B. Foret:** None. **E. Romero:** None. **D. Hasselschwert:** None. **J. Fontenot:** None. **J. Young Brooks:** None. **F. Villinger:** None. **K.M. Smith:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.06/D47

Topic: B.09. Glial Mechanisms

Title: Serum-free human iPSC-derived astrocytes as a platform for modeling astrocyte activation in injury and disease

Authors: S. A. BURTON, R. BRADLEY, R. LEWIS, C. HOGAN, C. CARLSON, ***S. SCHACHTELE**, S. HILCOVE, J. LIU;
FUJIFILM Cell. Dynamics, Madison, WI

Abstract: Incorporation of induced pluripotent stem cell (iPSC)-derived human astrocytes into co-cultures with neurons and/or microglia (‘brain-in-a-dish’) or pericytes and endothelial cells (blood brain barrier) have been instrumental in next generation disease models and drug discovery platforms. Astrocytes are a critical mediator of central nervous system (CNS) responses to insults and injury, displaying both proinflammatory and anti-inflammatory actions mediated in part through secretion of cytokines and chemokines. Astrocyte-microglia crosstalk is an especially important and widely studied aspect of CNS response to injury. Historical protocols for culturing primary and iPSC-derived astrocytes require serum-containing medium, which have undefined components that can impact astrocyte proliferation and activation. In this study, we profile the secretome of iPSC-derived astrocytes (iCell Astrocytes 2.0), differentiated and cultured using serum-free medium, under basal conditions and following stimulation. We show by use of Luminex array that these iPSC-derived astrocytes secrete a variety of factors in response to stimulation and that the activation profile varies by stimulation method (i.e., LPS, IL1α, IFNγ). Additionally, the astrocytes display low basal expression of cytokines, suggesting serum-free differentiated astrocytes are quiescent at rest and respond to injury with robust and physiologically relevant responses. We also identify multiple developmentally important factors whose secretion from serum-free astrocytes is not affected by cytokine stimulation. To evaluate microglia crosstalk, we evaluated the astrocyte secretome following exposure to iPSC-derived microglia conditioned medium. Taken together, these data demonstrate the unique utility of serum-free, human iPSC-derived astrocytes in modeling neural injury and neurodegenerative disease.

Disclosures: **S.A. Burton:** None. **R. Bradley:** None. **R. Lewis:** None. **C. Hogan:** None. **C. Carlson:** None. **S. Schachtele:** None. **S. Hilcove:** None. **J. liu:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.07/D48

Topic: B.09. Glial Mechanisms

Support: Simons Foundation Autism Research Initiative (SFARI)
R01MH085802

Title: Impaired astrocytic signaling contributes to the synaptic deficits seen in Rett Syndrome

Authors: *P. OJHA[1], M. SUR[2];
[1]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [2]Dept. of Brain and Cognitive Sci., MIT Grad. Brain and Cognitive Sci., Cambridge, MA

Abstract: Rett syndrome (RTT) is a devastating neurodevelopmental disorder that is caused by mutations in the X-linked gene, methyl-CpG binding protein 2 (*MECP2*). Astrocytic loss of MeCP2 has been implicated in diminished neurite outgrowth seen in Rett syndrome. Restoration of MeCP2 specifically in astrocytes rescues the behavioral deficits seen in RTT. However, the mechanisms underlying regulation of astrocytic function by MeCP2 are unclear. We hypothesized that knockdown (KD) of MeCP2 leads to abnormal secretion of synaptogenic proteins from astrocytes which leads to impaired synaptogenesis and activity-dependent synaptic plasticity in RTT. To test this hypothesis, primary cortical neurons and astrocytes from P0 mice were co-cultured and the effects of knocking down MeCP2 specifically in astrocytes were examined. We observed that wild-type (WT) neurons cultured with MeCP2-KD astrocytes had reduced synaptogenesis comparable to MeCP2-KD neurons cultured with MeCP2-KD astrocytes. This decrease was rescued when MeCP2-KD neurons were cultured with WT astrocytes. Interestingly, this decrease in synaptogenesis was seen only in excitatory synapses (colocalized PSD-95/ Bassoon punctae) and not in inhibitory synapses (colocalized Gephyrin/Bassoon punctae). To elucidate the mechanism by which loss of MeCP2 in astrocytes causes this decrease, a qPCR screen of all the known astrocyte-secreted synaptogenic molecules in mutant astrocytes was done. We observed that Ephrin A3 (EphA3) was highly upregulated in mutant astrocytes. Interestingly, EphA3 in astrocytes interacts with EphA4 receptors on neurons to regulate the levels of synaptic AMPA receptors and glutamate transporters. It has also been shown to regulate hippocampal dendritic spine morphology. Thus, we predict that overexpressing EphA3 in WT astrocytes will partially recapitulate the synaptic deficits seen in neurons cocultured with MeCP2-KD astrocytes. Some other genes that were dysregulated in mutant astrocytes include EphA2, EphB4, Nlgn-2 and 3, and thrombospondin. Most of these genes are involved in excitatory synapse formation which may underlie the decrease in the number of excitatory synapses observed earlier. The active peptide of insulin-like growth factor

1 [(1-3)IGF-1] which has been FDA-approved for treating RTT has been shown to increase the synaptic marker synapsin in neurons and activate PI3 kinase signaling in astrocytes. Currently, we are investigating whether (1-3)IGF-1 treatment of mutant astrocytes can rescue the levels of the altered synaptogenic proteins in astrocytes, pointing to additional mechanisms by which (1-3)IGF-1 exerts its therapeutic effects on neurons.

Disclosures: P. Ojha: None. **M. Sur:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.08/D49

Topic: B.09. Glial Mechanisms

Support: R01 NS051445
R01 NS105767

Title: Exploring the Role of Astrocyte System x_c^- in Excitatory/Inhibitory Balance

Authors: *S. O. SUTTON[1], S. J. HEWETT[2];
[1]Biology/Program in Neurosci., Syracuse Univ., Syracuse, NY; [2]Biology/Program in Neurosci., Syracuse Univ., SYRACUSE, NY

Abstract: An optimally functional brain requires both excitatory and inhibitory inputs that must be regulated and balanced. Mice globally devoid of System x_c^- (Sx_c^-)— a $[cystine]_i/[glutamate]_e$ exchanger whose substrate specific light chain is encoded by *SLC7a11*– demonstrate excitatory/inhibitory (E/I) imbalance. Whether this imbalance occurs when *SLC7a11* is conditionally inactivated in astrocytes only was addressed herein. Toward this end, the chemoconvulsant pentylenetetrazole (PTZ) was used to uncover *in vivo* excitability changes between astrocyte conditional Sx_c^- null mice [Sx_c^- AcKO: mGFAP-Cre-SLC7a11$^{fl/fl}$] and their wild-type littermates [WT: mGFAP-Cre-SLC7a11$^{+/+}$]. Both male and female mice were used to account for sex differences. Twelve- to 14-week-old mice received a single intraperitoneal injection of PTZ containing either 25, 30, 39, 48, or 58 mg/kg body weight. Mice were observed for 30 min following dosing and their seizure behavior scored using a 5-point scale: 0 = no behavioral change, 1 = hypomobility, 2 = myoclonus, 3 = generalized convulsion with righting reflex and 4 = generalized convulsion without righting reflex. Additionally, the percentage of mice convulsing at each dose was calculated by dividing the number of animals with a seizure score ≥ 3 by the total number of animals dosed. Dose response curves (DRCs) for seizure severity and percentage convulsing were fitted using modified Hill Plot equations and ED_{50s} for each were calculated using the four-parameter logistic equation and the Find ECanything with Robust regression equation, respectively (GraphPad Prism). We found that female Sx_c^- AcKO mice had a lower sensitivity to PTZ as demonstrated by an increase in the effective dose of PTZ needed to elicit a seizure severity score of 2 [ED_{50}: WT = 32.98 ± 2.97 and Sx_c^- AcKO = 38.77 ±

1.49, $p = 0.091$], an effect not seen in males [ED_{50}: WT = 39.54 ± 2.02 and Sx_c^- AcKO 41.74 ± 1.65, $p = 0.397$]. Likewise, the effective dose of PTZ that elicits a convulsive seizure in 50% of all mice dosed is notably higher in female, but not male, Sx_c^- AcKO mice as compared to their sex and age-matched wild-type littermates [Female ED_{50s}: WT = 37.24 (1.45-1.80); Sx_c^- AcKO = 43.81 (1.64-1.65), $p = 0.085$; Male ED_{50s}: WT = 44.16 (1.56-1.78); Sx_c^- AcKO 46.15 (1.61-1.71), $p = 0.561$]. These results suggest that astrocyte Sx_c^- signaling contributes to seizure susceptibility in female, but not male, mice fostering the idea that maintenance of the acute convulsive seizure threshold by astrocyte Sx_c^- is sexually dimorphic.

Disclosures: S.O. Sutton: None. **S.J. Hewett:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.09/D50

Topic: B.09. Glial Mechanisms

Support: Academy of Finland Grant 334525
Sigrid Juselius Foundation

Title: Astrocytes regulate neuronal network burst frequency through NMDA receptors species and donor-specifically

Authors: *N. RÄSÄNEN[1], J. TIIHONEN[2], M. KOSKUVI[2], Š. LEHTONEN[3], O. PIETILÄINEN[1], J. KOISTINAHO[1];
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Abstract: Development of synaptic activity depends on interactions between neurons and astrocytes. Although astrocytes have known roles in regulating synaptic function and malfunction, the use of human or donor-specific astrocytes in disease models is still rare. Rodent astrocytes are routinely used to enhance neuronal activity in cell cultures, but less is known about how human astrocytes influence neuronal activity. To illuminate the human characteristics of the functional maturation of neurons, we compared human induced pluripotent stem cell (hiPSC) - derived neuron-astrocyte co-cultures (n = 6 cell lines) to cultures of hiPSC-derived neurons and rat astrocytes (E18). We monitored the functional development of these cultures on microelectrode array and investigated the role of NMDA and AMPA receptors on neuronal activity using antagonists specific for each receptor type (D-AP5 and NBQX). We also applied GABA to study the presence of functional GABA receptors. Furthermore, we utilized hiPSC-derived neurons and astrocytes from 3 twin pairs discordant for schizophrenia and 4 controls to set up the co-culture system to model disease-related functional alteration in neurons. By 5 weeks of differentiation, hiPSC-derived co-cultures developed network bursting activity comparable to mixed-species co-cultures. At this stage, both culture types expressed functional

NMDA receptors and inhibitory response to GABA. However, the hiPSC-derived cultures displayed a greater decrease in network burst frequency (NBF, mean 72.0% decrease, SD 16.6) in response to NMDA receptor blockage than the mixed-species cultures (mean 12.6% decrease, SD 19.6). We also observed lower neuronal NBF in co-cultures derived from patients with schizophrenia (mean 0.030, SD 0.0090) than in cultures from unaffected individuals (mean 0.101, SD 0.053). The reduced NBF in the affected neurons was rescued by unaffected astrocytes that enhanced NMDA receptor activity in the cells. Our findings highlight the importance of a fully human co-culture system for studying the *in vitro* interactions of neurons and astrocytes.

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Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.10/Web Only

Topic: B.09. Glial Mechanisms

Support: PAPIIT Grant IN221023
APEC Grant INV-21-05
Presupuesto interno de la Facultad de Medicina UNAM
CONACYT fellowship 895582

Title: The activation of GABA receptors reduces VEGF-A secretion and promotes cell proliferation in Müller glial cells

Authors: *A. MEDINA ARELLANO[1], L. OCHOA DE LA PAZ[1,2], K. TOVAR-HERNÁNDEZ[2];
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Abstract: In glial cells, GABA receptors activation evokes cellular depolarization, which mediates extracellular Cl^- concentration and pH and activates the secretion of cytokines and growth factors that regulate metabolic and biochemical responses of neighboring neuronal and glial cells. In the retina, Müller glial cells (MGC) are the most abundant glial cells and perform several functions to maintain the homeostasis of this tissue. However, the study of GABA receptors expressed in Müller glial cells has not been extensively addressed. It is also known that MGC increases the secretion of VEGF-A during proliferative diabetic retinopathy (PDR). High concentrations of other molecules, including GABA, have been found in the vitreous of patients with PDR. However, the role these molecules may be playing in this pathology remains unknown. In this study, we used MGC primary cultures obtained from 5 to 7-day-old CD1 mice, which were exposed to GABA, muscimol, or baclofen for 48 hours, in the presence or absence of $GABA_A$ receptor ($GABA_AR$) antagonists (bicuculline, gabazine, TPMPA, picrotoxin) or $GABA_B$

receptor ($GABA_BR$) antagonist (CGP55845). The expression of VEGF-A mRNA and protein was quantified using RT-PCR and immunofluorescence, and its secretion was assessed by ELISA. Cell proliferation was evaluated using assays measuring DNA synthesis (BrdU), and total cell count (Countess-Invitrogen). The results indicate that GABA induces an increase in the percentage of cells overexpressing VEGF-A (VEGF-A+), without changes in the transcript, as well as a significant decrease in the secretion of this growth factor. These results are dose-dependent and showed significance at 100 µM. The increase in the percentage of VEGF-A+ cells is possibly associated with the observed increase in proliferation in the presence of GABA. Activation of $GABA_AR$ by muscimol reproduced similar effects to those observed with GABA, and the application of $GABA_AR$ antagonists blocked all these effects except for the reduction in VEGF-A secretion. The application of baclofen was unable to increase the percentage of VEGF-A+ cells, but it was able to decrease the release of VEGF-A. The application of GABA with CGP55845 was unable to reverse the decrease in VEGF-A release in Müller glial cell cultures. These findings suggest that GABA receptors play an important role in regulating the release of VEGF-A and the proliferation of Müller glial cells in the retina.

Disclosures: A. Medina Arellano: None. **L. Ochoa de la paz:** None. **K. Tovar-hernández:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.11/D51

Topic: B.09. Glial Mechanisms

Support: NIH (R01 NR019531)

Title: Methotrexate exposure alters glial transcriptional profiles and alters blood-brain-barrier permeability

Authors: *A. B. DAVIS, J. L. BOLLINGER, C. CANTELON, E. S. WOHLEB, T. REYES; Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Acute Lymphoblastic Leukemia (ALL) is one of the most prevalent cancers affecting children and adolescents. Survivorship has increased due to the success of chemotherapeutic protocols, involving drugs like methotrexate (MTX). While MTX has increased survival rates, it has also been linked to long term cognitive deficits which occur in 40% to 70% of survivors. Our previous studies indicate that MTX-induced neuroinflammatory responses in the prefrontal cortex (PFC) may be related to these deficits. To expand on this finding, we examined the effects of early-life cancer and chemotherapy on gene expression in astrocytes and microglia as well as blood-brain barrier (BBB) integrity in the PFC. To model pediatric cancer survival, C57BL/6 x DBA F1 mice were randomly assigned to an injection of mouse leukemic cells (L1210 cells) or saline on postnatal day 19 (P19). Starting on P21 the mice that received cancer began a 4-cycle

chemotherapy using MTX, vincristine, and leucovorin; the control group received saline injections. In one cohort, brains were collected (P34) and the frontal cortex was dissected out. Fluorescence activated cell sorting was used to isolate both cell types followed by transcriptional profiling using RNA-seq. In a separate cohort, the severity of BBB permeability was assessed using an injection of a fluorescently labeled small-molecule (cadaverine, 1,000 MW). Extravasation of the molecule was quantified in the PFC. Findings indicate significant transcriptomic alterations in astrocytes and microglia in a sex dependent manner. Female astrocytes had a total of 385 genes that were significantly altered, in comparison males had only 23 genes altered. Female microglia had 438 altered genes, whereas males had only 4. BBB permeability to cadaverine was increased in PFC of mice exposed to cancer+chemotherapy. Our findings indicate that early life cancer+chemotherapy dramatically alter the transcriptional profile of astrocytes and microglia in a sex-dependent manner. Further, blood brain barrier permeability is increased to small molecules. Future work will directly examine whether these glial and BBB changes contribute to cognitive deficits observed in this model of pediatric leukemia survival.

Disclosures: **A.B. Davis:** None. **J.L. Bollinger:** None. **C. Cantelon:** None. **E.S. Wohleb:** None. **T. Reyes:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.12/D52

Topic: B.09. Glial Mechanisms

Support: National Science and Technology Council (MOST 111-2320-B-A49-038), Taiwan()

Title: Astrocytic FKBP5 Deletion Attenuates Epileptic Seizure and Astrogliosis in a Temporal Lobe Epilepsy Mouse Model

Authors: *Y. KANG[1], Y.-L. GAN[2], Y.-J. HUANG[2], C.-C. HUNG[2], P.-C. HSU[2], Y.-H. LEE[2]; [2]Phisiol, [1]Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: The *Fkbp5* gene encodes FK506-binding protein 51 (FKBP51), a co-chaperone protein that negatively regulates the glucocorticoid receptor (GR) to maintain physiological homeostasis of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress. Overexpression of the *Fkbp5* gene indicates hyperactivity of the HPA axis, and has been reported to be highly associated with neuropsychiatric disorders. Previous transcriptomic studies identified that the increase of *Fkbp5* transcript is one of the markers for proinflammatory A1 astrocytes, a subtype of astrocytes that produce proinflammatory cytokines and cause neuronal damage. GFAP, a well-known reactive astrocyte marker, is also upregulated in A1 astrocytes. Excitotoxicity is the early phase pathophysiological event after brain injury such as epilepsy and

stroke, in which inflammatory astrogliosis was noted. Yet, the role of FKBP51 in excitotoxicity-induced astrogliosis remained unknown. Here, we established inducible astrocyte-specific *Fkbp5* conditional knockout mice (GLAST-Cre^{ERT}::*Fkbp5*$^{fl/fl}$, a*Fkbp5*-cKO) to investigate epileptic seizure and hippocampal astrogliosis in a kainic acid-induced temporal lobe epilepsy (TLE) mouse model. The deletion of astrocytic *Fkbp5* was induced by intraperitoneal injection of tamoxifen to generate a*Fkbp5*-cKO mice. The protein level of FKBP51 in the brain of a*Fkbp5*-cKO mice was lower compared to *Fkbp5*$^{fl/fl}$ mice 7 weeks after the tamoxifen induction. Locomotor activity and anxiety-like behaviors in the open field test and sucrose preference test all showed no differences between a*Fkbp5*-cKO and *Fkbp5*$^{fl/fl}$ mice. Intraperitoneal injection of excitotoxic kainic acid (KA) induced seizure activity, which was significantly lower in a*Fkbp5*-cKO mice compared to *Fkbp5*$^{fl/fl}$. Immunoreactivities of neuronal dendrite marker microtubule-associated protein 2 in the hippocampal CA3 and dentate gyrus were higher in the a*Fkbp5*-cKO than in *Fkbp5*$^{fl/fl}$ 7 days after KA injection. Hippocampal astrogliosis as indicated by GFAP immunostaining and immunoblotting showed less hypertrophy and lower protein levels in a*Fkbp5*-cKO compared to *Fkbp5*$^{fl/fl}$ after KA injection. Together, these results suggest that astrocytic FKBP51 plays important roles in the excitotoxicity-induced seizure, hippocampal neurotoxicity and astrogliosis. Grant: MOST 111-2320-B-A49-038, from National Science and Technology Council, Taiwan.

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Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.13/D53

Topic: B.09. Glial Mechanisms

Title: Let-7i regulation of pgrmc1 and bdnf expression in cortical astrocytes: implications for understanding the pathobiology of ischemic stroke

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Abstract: Stroke is a leading cause of death worldwide and often results in life-long disabilities that can profoundly impair day-to-day quality of life. Currently, recombinant tissue plasminogen activator (rtPA) is the only FDA-approved pharmacological treatment for ischemic stroke. However, due to its limited window of therapeutic opportunity, rtPA may not be applicable to all stroke patients. This underscores the critical need for better, safe, and more broadly applicable stroke treatments.Progesterone (P4) is a cholesterol-derived steroid hormone that is neuroprotective. These neuroprotective effects are mediated, at least in part, by brain-derived

neurotrophic factor (BDNF), which plays a role in enhancing synaptogenesis and neuronal survival. Previous studies from our lab demonstrated that the microRNA, let-7i, a non-coding RNA found to be upregulated in animal models of stroke, reduced PGRMC1 and BDNF expression in the peri-infarct tissue. Of note, administration of an antagomir against let-7i enhanced the neuroprotective properties of P4 when administered concurrently with the antagomir. Taken together, these data suggest that a combined treatment regimen of P4 with the let-7i antagomir has strong potential as a therapeutic for ischemic stroke. In an effort to further explore the mechanism(s) by which let-7i influences the protective effects of P4, we utilized an *in vitro* model of oxygen/glucose deprivation (OGD) to simulate the ischemic event in our animal model of stroke. Our data indicate that exposure to conditions of OGD results in significant astrocytic cell death. Further, this model of ischemia also showed a reduction in BDNF levels. In addition, overexpression of let-7i in astrocytes led to a reduction in PGRMC1, a key regulator of BDNF release. These data implicate PGRMC1 and BDNF as targets of let-7i following stroke, and importantly, implicate astrocytes as a potential important cellular target for interventions aimed at addressing the pathology associated with stroke and highlight the potential utility of the let-7i antagomir as a novel miRNA-based therapeutic.

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Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

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Topic: B.09. Glial Mechanisms

Support: This study was supported by a Grant-in-Aid for Scientific Research from the Japan Science and Technology Agency (JST) 22K09233.

Title: Neuroprotective effects of carbenoxolone against ischemia reperfusion injury: a comprehensive analysis

Authors: *Y. IKEUCHI, M. KOHTA, S. YAMASHITA, Y. YAMAGUCHI, A. FUJITA, T. SASAYAMA;
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Abstract: Introduction: Carbenoxolone (CBX), which is derived from licorice and is primarily used as a gap junction inhibitor, has been proposed to possess neuroprotective effects. Initially, these effects were attributed to the reduced glutamate released from the microglia to the neighboring tissues via gap junction inhibition. However, emerging reports suggest that CBX also induces heat shock protein (HSP) expression and activates the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (Akt) signaling pathway, thereby indicating that its neuroprotective mechanisms may extend beyond gap junction inhibition. We conducted an RNA sequencing

analysis using a rat model of middle cerebral artery occlusion/reperfusion (MCAO/R) with CBX administration to comprehensively understand the neuroprotective mechanism of CBX against cerebral ischemia. Methods: Nine-week-old male rats underwent the MCAO/R procedure. In the CBX group, CBX was administered three hours prior to initiating the MCAO/R protocol. Neurological outcomes were assessed 24 hours after MCAO/R, followed by euthanization, brain extraction, and Triphenyl tetrazolium chloride staining to measure the cerebral infarct volumes. Cortical tissue from the ischemic zone was obtained for Western blotting, RNA sequencing, and reverse transcription-polymerase chain reaction analyses. RNA sequencing data was compared in the rats subjected to MCAO/R with or without CBX administration with the corresponding control groups. Results: Rats in the CBX-administered and MCAO/R-treated group (CBX+ MCAO/R+) exhibited significantly reduced cerebral infarct volumes compared to their non-CBX-treated counterparts (CBX- MCAO/R+). Western blotting revealed increased Akt expression in the cortical region of the CBX+ MCAO/R+ rats. RNA sequencing analysis identified differential expression of genes, such as Ntrk1 and Dnajc6, in response to CBX administration in the MCAO/R+ rats. These genes demonstrated the most robust expression in the CBX+ MCAO/R+ rats compared with the other groups. Conclusion: The upregulated genes identified through RNA sequencing analysis were associated with a known mechanism of CBX action. Ntrk1, which encodes TrkA, activates the PI3K-Akt pathway upon binding to Nerve growth factor, thereby exerting neuroprotective effects. Dnajc6 encodes HSP40, which triggers HSP70 induction and contributes to neuroprotection. These genes may function upstream within the established mechanisms of CBX action.

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Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: B.09. Glial Mechanisms

Support: NINDS/NIA grant 1R01NS118569
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Title: Dementia-associated TDP-43 pathology and immune signaling disrupt astrocyte nucleocytoplasmic dynamics via stress granules

Authors: *C. ZHOU[1,2,4], T. S. ZIMMER[1,2], S. E. JACKVONY[1,2,3], D. BARNETT[1,2,3], A. L. ORR[1,2,3], A. G. ORR[1,2,3,4];
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Cornell Med., New York, NY; [4]Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD-PhD Program, New York, NY

Abstract: Transactivating response region DNA-binding protein 43 (TDP-43) pathology is prevalent in several neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and Alzheimer's disease (AD). TDP-43 pathology and other proteinopathies are known to occur in neurons and glial cells and have been linked to neurocognitive and behavioral deficits. In neurons, TDP-43 dysregulation and other proteinopathies induce changes in nucleocytoplasmic dynamics, which may contribute to disease pathogenesis. However, nucleocytoplasmic changes in glial cells remain undefined. Although astrocytes are responsive to dementia-linked factors and undergo various phenotypic and functional changes in disease, including TDP-43 dysregulation and altered neuroimmune signaling, the effects of TDP-43 pathology and immune factors on nucleocytoplasmic dynamics in astrocytes are mostly unknown. To test if and how astrocyte nucleocytoplasmic dynamics are altered in the presence of TDP-43 pathology and neuroimmune stimuli, we examined a) transgenic mouse-derived primary astrocytes and human iPSC-derived astrocytes with disease-associated TDP-43 mutations, and b) astrocytes treated with interleukin-1α (IL-1α), a cytokine implicated in neuroinflammation and neurodegenerative disorders. In both conditions, astrocytic TDP-43 pathology or acute stimulation with IL-1α altered astrocytic nuclear protein localization, nuclear envelope morphology, and nucleocytoplasmic protein shuttling in mouse and human astrocytes. Astrocytic nucleoporin NUP98 accumulated in the cytoplasm and colocalized with stress granules, causing distortions of the nuclear lamina and impairments in carrier-mediated nucleocytoplasmic protein transport. Treatment of astrocytes exhibiting TDP-43 pathology with an IL-1 receptor antagonist (IL-1Ra) reversed these nucleocytoplasmic changes. Moreover, inhibition of astrocytic stress granule formation prevented the nucleocytoplasmic changes, suggesting that disease-associated nucleocytoplasmic alterations are enabled by aberrant formation or persistence of stress granules. Together, our findings suggest that astrocytic nucleocytoplasmic functions are highly dynamic and sensitive to dementia-related factors and regulated by neuroimmune signaling and cell stress pathways. Thus, astrocytic dysfunction in dementia might be alleviated by targeting stress granule formation and nucleocytoplasmic dynamics.

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Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.16/D56

Topic: B.09. Glial Mechanisms

Title: Gliapharm compounds to rescue hypometabolism in alzheimer's disease: a novel therapeutic strategy

Authors: *C. FINSTERWALD[1], S. DIAS[1], F. JENCK[1], P. J. MAGISTRETTI[2,1], S. LENGACHER[1];
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Abstract: GliaPharm is a biotech company that develops innovative therapeutic strategies for Alzheimer's disease and related neurodegenerative conditions by targeting astrocytes to stimulate brain energy metabolism. Alzheimer's disease is associated with deficits in brain energy metabolism, as shown by decreased cerebral glucose uptake by 18F-Fluorodeoxyglucose (FDG) PET in patients early in the pathology (Cunnane et al., Nat Rev Drug Discov, 2020) and by increased circulating astrocyte-specific maker GFAP (Chatterjee et al., Transl Psychiatry, 2021). Targeting brain energy metabolism has recently concretely emerged as an innovative therapeutic strategy for Alzheimer's disease. Several preclinical and clinical trials that mostly consist in repurposing antidiabetic therapies, such as insulin-sensitizer agents, are currently ongoing (Cummings et al,. Alzheimers Dement (NY), 2023). GliaPharm has set up a drug discovery platform, Glia-X, to identify and develop drugs that modulate astrocyte functions. Specifically, Glia-X assesses astrocytic metabolic indicators such as glucose uptake, glycogen utilization, lactate production, mitochondrial activity and cellular redox status. Using its Glia-X platform, GliaPharm has performed high throughput screening (HTS) to identify small molecules that promote metabolic functions in astrocytes. Hits were next assessed in vitro and in vivo for efficacy, selectivity and safety, which led to the selection of drug candidates. Here, we show data about GliaPharm's drug candidate GP-101 that has been identified and optimized through the Glia-X platform. First, our data indicate that GP-001 increases glucose uptake, glycogen mobilization and lactate release in astrocytes from mouse and human origins, indicating stimulation of the Astrocyte Neuron Lactate Shuttle (ANLS; Magistretti and Allaman Nat Rev Neurosci, 2018). Further, GP-101 exhibits a neuroprotective effect in astrocyte-neuron co-culture system. In vivo, we show that GP-101 penetrates the brain at active concentrations after oral administration, which leads to an increase in cerebral glucose and lactate levels, as measured with biosensors, as well as cerebral glucose uptake as measured by FDG-PET. Importantly, oral administration of GP-101 increases long-term memory in young and aged mice, as shown in Inhibitory Avoidance and Morris Water Maze memory tasks. Our data indicate that promoting brain energy metabolism by targeting astrocyte-mediated pathways has a positive impact on brain functions and has therapeutic potential for restoring and normalizing brain hypometabolic conditions in neurological diseases such as Alzheimer's disease.

Disclosures: C. Finsterwald: A. Employment/Salary (full or part-time):; GliaPharm. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaPharm. **S. Dias:** A. Employment/Salary (full or part-time):; GliaPharm. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaPharm. **F. Jenck:** A. Employment/Salary (full or part-time):; GliaPharm. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaPharm. **P.J. Magistretti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaPharm. **S. Lengacher:** A. Employment/Salary (full or part-time):; GliaPharm. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaPharm.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.17/D57

Topic: B.09. Glial Mechanisms

Support: T. Denny Sanford Endowed Pediatric Collaborative Research Fund

Title: Loss of Neuroprotective Effects in iPSC-derived Astrocytes from a Smith-Lemli-Opitz Syndrome Patient

Authors: *S. BAKER;
Mayo Clin., Jacksonville, FL

Abstract: Smith-Lemli-Optiz syndrome (SLOS) is an autosomal recessive disorder caused by mutations in the gene coding for 7-dehydrocholesterol reductase (DHCR7). SLOS patients have DHCR7 dysfunction, leading to an accumulation of the reactant 7-dehydrocholesterol and low levels of cholesterol. Astrocytes are largely responsible for synthesizing cholesterol and maintaining lipid homeostasis in the brain. Characterizing the defects of SLOS in astrocytes will inform our understanding of glial contribution in diseases with intellectual disability and thus, provide novel therapeutic targets. Throughout this study, we intend to understand how *DHCR7* mutations affect astrocyte function. We generated iPSCs derived from a SLOS patient with *DHCR7* c.C278T missense mutation and created an isogenic gene-corrected iPSC line using the CRISPR/Cas9 system. These iPSC lines were differentiated into astrocytes and cortical neurons We did immunocytochemical staining for astrocyte markers and measured synaptic activity using Micro-Electrode Array (MEA). We used lipidomic analysis to investigate the overall lipid metabolism and dysregulation of SLOS astrocytes. We verified the successful differentiation of iPSCs into astrocytes by immunocytochemistry, in which cells stain positive for S100β and GFAP. Analysis of proliferation through the EdU assay revealed that SLOS iPSC-derived astrocytes proliferated at nearly twice the rate of control astrocytes. Notably, SLOS iPSC-derived astrocytes showed signs of compromised abilities. SLOS iPSC-derived astrocytes co-cultured with healthy iPSC-derived neurons had impaired neuroprotective effects. Moreover, neurons co-cultured with SLOS iPSC-derived astrocytes had decreased frequency of spontaneous electrical potential spikes and burst firing in comparison to isogenic astrocyte co-culture. Lipidomic analysis revealed that total cholesterol and cholesteryl ester levels were significantly decreased in SLOS astrocytes, while fatty acyl chains, triacylglycerol, and phosphatidylglycerol levels were increased, alluding to a compensatory cellular mechanism. Our data illustrates that SLOS iPSC-derived astrocytes less efficiently mediate neuronal growth and functionality.We demonstrated that SLOS iPSC-derived astrocytes have a loss of neuroprotective effects. We will further investigate the biochemical mechanism linking *DHCR7* mutations in SLOS to the observed astrocytic dysfunction.

Disclosures: S. Baker: None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.18/D58

Topic: B.09. Glial Mechanisms

Support: COBRE Grant 5P20GM103653
RISE Grant R25GM122722
HBGI Title III Grant 10003317

Title: A look into the function of cortical astrocytes in the SMNΔ7 mouse model for SMA

Authors: *T. BAMFO[1], V. A. N. TALABATTULA[1], M. MOORE[1,2], J. SUN[1,3], M. HARRINGTON[3];
[1]Delaware State Univ., Dover, DE; [2]DIST Imaging Facility, Dover, DE; [3]Delaware Ctr. for Neurosci. Res., Dover, DE

Abstract: Spinal muscular atrophy (SMA) is a debilitating neurodegenerative disease caused by the deletion or mutation of the Survival Motor Neuron 1 (*SMN1*) gene, and has long been characterized by the loss of motor neurons in the spinal cord. However, more recent studies have not only shown pathological changes in the brain due to lack of SMN protein, but also indicate that restoration of this protein to SMN-deficient ($SMN^{-/-}$) motor neurons alone is ineffective in improving SMA-related symptoms. Such findings demonstrate that complete understanding of the pathological and molecular mechanisms of SMA must go beyond lower motor neurons to include non-neuronal cells and how these cells are impacted in the brain. In this study, we aim to characterize the physical and functional properties of cortical astrocytes derived from the SMNΔ7 mouse model. *In vitro* experiments were performed on brain tissue samples and cortical astrocytes cultured from SMNΔ7 mice. Wild-type (WT) and $SMN^{-/-}$ astrocytes were isolated and cultured from the cortex of neonatal mice of the SMNΔ7 mouse model on post-natal day (P)0. Brain tissue samples from P12 SMA mice in western blots showed reduced SMN protein expression in various brain regions, with levels comparable to those detected in the spinal cords. Western blots were also used to confirm SMN protein expression in the cortical astrocytes. We found that, in comparison to WT astrocytes, relative SMN expression levels are significantly reduced in $SMN^{-/-}$ astrocytes. To determine whether lack of SMN protein alters regulation of intrinsic cellular properties, we also examined differences in calcium signaling in the comparison of WT and $SMN^{-/-}$ cortical astrocytes. Results are compared to similar experiments done in spinal astrocytes where results suggested that the changes observed in intracellular calcium signaling of $SMN^{-/-}$ cortical astrocytes may negatively impact interaction between astrocytes and motor neurons[1]. The goal of this study is to provide a better understanding of how cortical astrocytes are affected by the pathology of SMA, as well as their role in supporting other neuronal cells in this SMA disease model. Such studies may aid in targeting new areas for further development of efficient therapeutic solutions for those diagnosed with SMA.
Reference(s):1.Zhou C, Feng Z, Ko CP. Defects in Motoneuron-Astrocyte Interactions in Spinal

Muscular Atrophy. J Neurosci. 2016 Feb 24;36(8):2543-53. doi: 10.1523/JNEUROSCI.3534-15.2016. PMID: 26911699; PMCID: PMC6705489.

Disclosures: **T. Bamfo:** None. **V.A.N. Talabattula:** None. **M. Moore:** None. **J. Sun:** None. **M. Harrington:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.19/D59

Topic: B.09. Glial Mechanisms

Support: Deutsche Forschungsgemeinschaft (DFG) grant TRR 274/1 2022
Munich Cluster for Systems Neurology (EXC 2145 SyNergy)
Gemeinnützige Hertie-Stiftung (P1150064)

Title: In vivo imaging of oligodendrocyte injury in an NMO mouse model

Authors: *S. KENET[1], M. HERWERTH[2], J. L. BENNETT[3], B. HEMMER[4,5], T. MISGELD[1,5,6];
[1]Tech. Univ. of Munich, Munich, Germany; [2]Inst. of Pharmacol. and Toxicology, Univ. of Zurich, Zurich, Switzerland; [3]Departments of Neurol. and Ophthalmology, Programs in Neurosci. and Immunol., Univ. of Colorado Sch. of Med., Aurora, CO; [4]Dept. of Neurol., Klinikum rechts der Isar, Tech. Univ. Munich, Munich, Germany; [5]Munich Cluster of Systems Neurol. (SyNergy), Munich, Germany, Munich, Germany; [6]German Ctr. for Neurodegenerative Dis. (DZNE), Munich, Germany, Munich, Germany

Abstract: Neuromyelitis optica (NMO) is an autoimmune disease predominantly affecting the spinal cord and optic nerve. The majority of NMO patients have serum antibodies (IgG) against the water channel protein aquaporin-4 (AQP4), which is in the CNS expressed on astrocytes. Despite this primary astrocytic target, demyelination is also prominent in AQP4-IgG seropositive NMO patients and is regarded as secondary to astrocyte loss. However, it remains unclear how targeting of an astrocytic antigen drives injury of other cell types, such as oligodendrocytes. Here, performing in vivo spinal cord imaging in an NMO mouse model, we explored the cellular mechanism and the time-course of glial injury during NMO lesion formation. Experimental NMO lesions were induced by local spinal application of patient-derived AQP4-IgG and human complement. Morphological assessment and calcium imaging of genetically labeled astrocytes and oligodendrocytes revealed a membrane attack complex (MAC)-mediated fast spread mechanism of NMO pathology. Within an hour of AQP4-IgG application, astrocytes displayed global calcium rise and membrane rupture, which was validated by uptake of a cell-impermeable nuclear dye and subsequent cellular fragmentation. Concurrent to the astrocytic calcium rise, oligodendrocyte processes also showed transient calcium overload, which however stabilized and reached the somata comparatively later. In contrast to the widespread, fast lytic death of

astrocytes, only some oligodendrocytes were lost at later time points. While dye exclusion experiments negated overt membrane rupture in oligodendrocytes, expression of human MAC-inhibitor protein CD59 still protected these cells from secondary damage after AQP4-IgG-mediated astrocyte injury. Glial pathology spread quickly from astrocytes to pericytes as well, but oligodendrocyte precursor cells were preserved in experimental NMO lesions. Our results imply that oligodendrocyte pathology in AQP4-IgG+ NMO is not driven by the loss of astrocytes per se, but rather evolves from MAC-dependent bystander targeting of oligodendrocytes. Our findings suggest that despite the similar starting point of MAC-mediated glial injury, the executive phase of cell damage might differ and could result in activation of distinct cell death pathways in the glial cell targets of NMO.

Disclosures: S. Kenet: None. **M. Herwerth:** None. **J.L. Bennett:** None. **B. Hemmer:** None. **T. Misgeld:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.20/D60

Topic: B.09. Glial Mechanisms

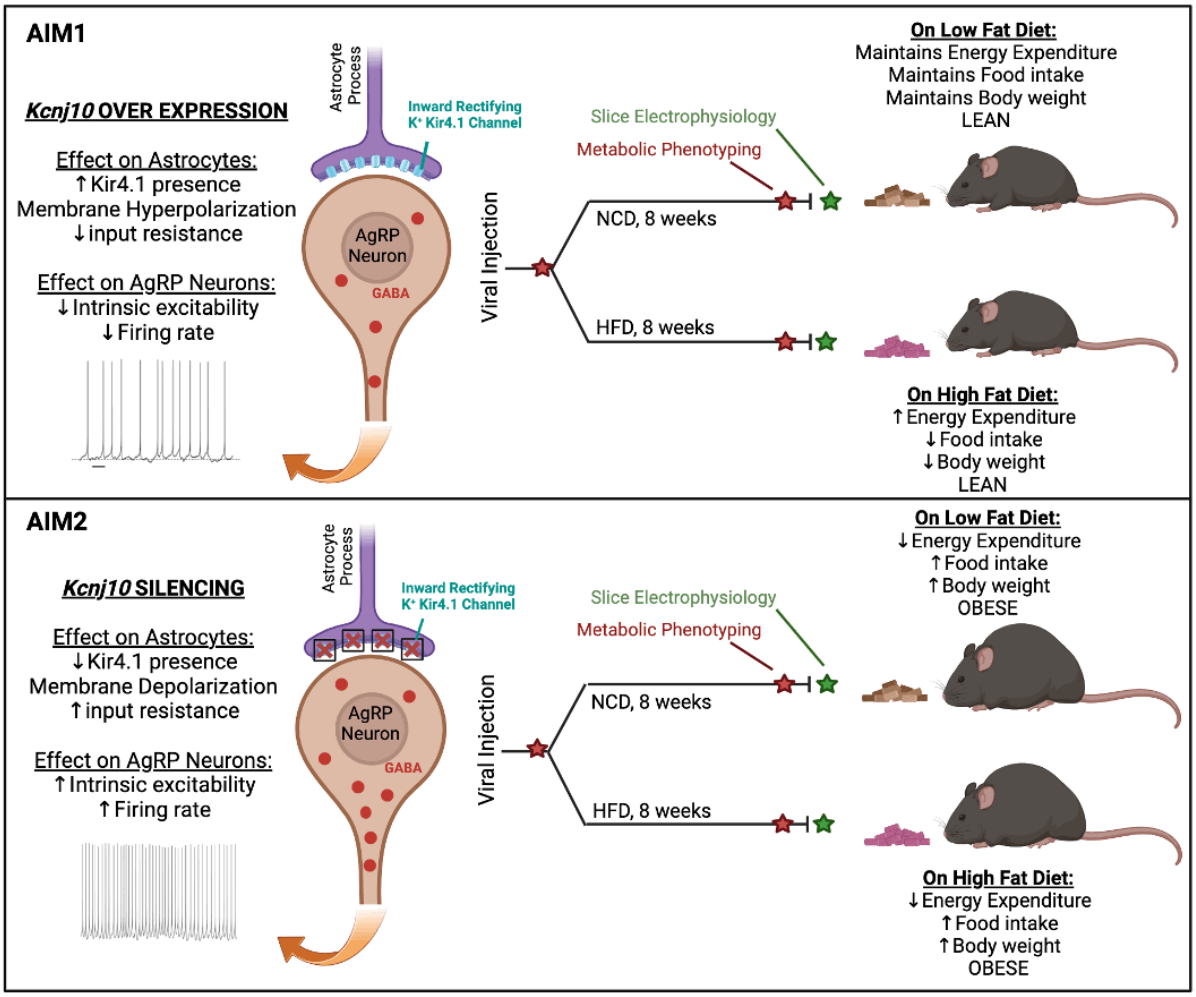
Support: NIDDK R01DK102918
NIA RF1AG059778
Jackson Laboratory startup funds to KMSO

Title: Role of hypothalamic astrocytes in the development of diet-induced obesity.

Authors: *T. B. OUELLETTE, A. KORGAN, K. M. S. O'CONNELL;
The Jackson Lab., Bar Harbor, ME

Abstract: Astrocytes are increasingly appreciated as cells which play an active role in shaping synaptic function and neuronal output. In cortical circuits, astrocytes have a significant effect on neural oscillatory patterns governing a behavioral output, but the role of astrocytes in modulation of neuronal activity in the hypothalamus and their role in shaping appetitive behavior is poorly understood. Factors like high-fat diet, which impact astrocyte expression and function likely have significant effects on neural circuitry governing food intake and energy expenditure. The goal of this project is to understand if astrocytes are necessary and sufficient for maintenance of food intake and body weight via modulation of AgRP neuronal output in the hypothalamus and whether HFD-induced changes in astrocyte K^+ handling are a causal factor in development of obesity.Astrocytes play a key role in maintenance of synaptic excitability and neuronal output by buffering neurotransmitters and ions like K^+ from the extracellular space around the synapse. The inward rectifier K^+ channel Kir4.1 (gene name *Kcnj10*) is the predominant ion channel in astrocytes. The expression of astrocytic *Kcnj10* has not been studied for its necessity in food intake, nor its context in high fat diet. **I hypothesize that changing astrocyte *Kcnj10***

expression in the arcuate nucleus of the hypothalamus is sufficient to modulate AgRP neuronal firing rate, energy expenditure and body weight of mice. In this study, we are characterizing astrocyte recruitment, morphology, expression, and function in the arcuate nucleus of the hypothalamus (ARH) of mice that are fed a high fat diet.



Disclosures: **T.B. Ouellette:** None. **A. Korgan:** None. **K.M.S. O'Connell:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.21/D61

Topic: B.09. Glial Mechanisms

Support: MOST110-2320-B-A49A-502-MY3
MOST110-2811-B-A49A-039

Title: Effects of an endogenous ligand-binding site mutant of the aryl hydrocarbon receptor on poly(I:C)-stimulated human astrocytes

Authors: *C.-C. HUNG[1,2], X.-T. LEE[1], Y.-C. HSIN[1], Y.-J. HUANG[1], Y.-L. GAN[1], P.-C. HSU[1], C.-H. LIN[3], C.-C. CHOU[4], Y.-H. LEE[1,2];
[1]Dept. and Inst. of Physiol., [2]Brain Res. Ctr., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; [3]Dept. of Nursing, Kang-Ning Univ., Taipei, Taiwan; [4]Natl. Lab. Animal Ctr., Taipei, Taiwan

Abstract: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that belongs to the bHLH/PAS family and is ubiquitously expressed in various organs, including the brain. AhR mediates gene transcription for xenobiotic metabolism, immune modulation, and cell cycle regulation. We previously reported receptor-ligand molecular docking to predict human AhR LBD consists of two ligand binding sites, one is the classical binding site (LBD-A) for TCDD and PAHs, and the other newly identified binding site (LBD-B) mainly for tryptophan-derived AhR ligands including FICZ, KYNA, I3S, etc. Tryptophan-derived AhR ligands occurs in inflammatory response due to the inflammation-induced IDO activity that catabolize tryptophan to kynurenine and KYNA. Here, we investigated the ligand selectivity of AhR LBD-B and its function in mediating proinflammatory TLR3 ligand poly(I:C)- induced AhR activity in human astrocytes. We transfected flag-tagged human AhR wild-type (WT) and an LBD-B (AhR-B) mutant into human astrocytes, and examine the A- and B-site AhR ligands-induced AhR activity as indicated by the canonical pathway target CYP1B1 gene transcription. We found that TCDD (A-site ligand), FICZ and KYNA (B-site ligand) all induced CYP1B1 mRNA expression, and both effects are enhanced by AhR-WT overexpression. Notably, AhR-B mutant enhanced TCDD-, not FICZ- or KYNA-induced AhR activity. Poly(I:C) also induced AhR activation and proinflammatory gene expressions in human astrocytes. Particularly, the Poly(I:C) effects on the IL-1β and GLT-1 expressions were selectively affected by the AhR-B mutant. Moreover, $p21^{CIP1}$, a cell cycle regulator and also a non-canonical AhR target gene, was increased by Poly(I:C) but not AhR ligands, and the Poly(I:C) effect was not affected by the AhR-B mutant in human astrocytes. Together, these data suggest that the AhR LBD-B site mediates tryptophan-derived ligands-, not TCDD-, induced gene expression. Proinflammatory stimuli-induced AhR activity in astrocytes seems to be independent of LBD-B activity, but is involved in regulation of proinflammatory responses and astrocyte-mediated brain homeostasis.

Disclosures: C. Hung: None. **X. Lee:** None. **Y. Hsin:** None. **Y. Huang:** None. **Y. Gan:** None. **P. Hsu:** None. **C. Lin:** None. **C. Chou:** None. **Y. Lee:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.22/D62

Topic: B.09. Glial Mechanisms

Support: NIH Grant AG068091
BrightFocus Foundation Grant A2019363S
Sanofi iAward
Daedalus Fund for Innovation
ADDF/AFTD Drug Discovery Award
Swid Professorship

Title: Context-dependent ROS production from mitochondrial complex III contributes to astrocyte pathology, neuroimmune signaling, and dementia-related pathogenesis

Authors: D. BARNETT[1], T. S. ZIMMER[1], C. BOOREAM[1], F. PALAGUACHI[1], S. M. MEADOWS[1], H. XIAO[2], E. T. CHOUCHANI[2], A. G. ORR[1], ***A. L. ORR**[1];
[1]BMRI, Weill Cornell Med., New York, NY; [2]Dana Farber Cancer Institute, Harvard Med. Sch., Boston, MA

Abstract: Alterations in mitochondria are implicated in aging and disease and involve increases in superoxide and other reactive oxygen species (ROS). Mitochondrial complex III (CIII-ROS) is a key driver of oxidative changes, but its exact triggers and downstream molecular, functional, and pathogenic contributions are not clear. Here we used S3QELs ("sequels"), site-selective suppressors of CIII-ROS, together with live-cell imaging of subcompartmental ROS, stoichiometric redox proteomics, transcriptomics, and complementary models of dementia-associated tauopathy and amyloid pathology to investigate the involvement of CIII-ROS in disease-related processes. We report that CIII-ROS are induced in astrocytes in a cell type-specific and context-dependent manner by select stimuli that dysregulate mitochondrial ion exchange. Increases in astrocytic CIII-ROS caused highly targeted protein oxidation and altered transcription that exacerbated disease-related gene expression, neuroimmune responses, and neuronal damage. In mice, S3QELs crossed the blood-brain barrier and were well-tolerated for up to three years of continuous oral administration. Therapeutic suppression of CIII-ROS reduced neuropathology in mouse models of dementia-related proteinopathy and extended lifespan. Rational medicinal chemistry improved solubility and blood-brain barrier penetration of next-generation S3QELs. Our findings suggest that CIII-ROS amplifies pathogenic processes in the brain and represents a new target for neurological disorders.

Disclosures: D. Barnett: None. **T.S. Zimmer:** None. **C. Booream:** None. **F. Palaguachi:** None. **S.M. Meadows:** None. **H. Xiao:** None. **E.T. Chouchani:** None. **A.G. Orr:** Other; Co-inventor on patents for treating neurological disease and other conditions with suppressors of mitochondrial reactive oxygen species production. **A.L. Orr:** Other; Co-inventor on patents for treating neurological disease and other conditions with suppressors of mitochondrial reactive oxygen species production..

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PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.23/D63

Topic: B.09. Glial Mechanisms

Support: CIHR PJT-173468

Title: Relationship between astrocyte calcium and cerebral blood flow changes during awake-sleep cycles

Authors: *F. WANG[1], J. SUN[2], G. GORDON[1];
[1]Hotchkiss Brain Institute, Dept. of Physiol. and Pharmacology, Cumming Sch. of Medicine, Univ. of Calgary, Calgary, AB, Canada; [2]CSM Optogenetics Platform, Uniceristy of Calgary, Calgary, AB, Canada

Abstract: Changes to free astrocyte calcium regulates local cerebral blood flow. Astrocyte calcium activity also changes during awake-to-sleep transitions, yet whether astrocytes help regulate the changes to cerebral blood flow that are observed when mice switch between awake, NREM and REM sleep states, remains unclear. Using in vivo two-photon fluorescence imaging in head-fixed, unanesthetized mice (10-12 weeks old), we monitored astrocyte calcium activity and diameter changes of penetrating arterioles in the retrosplenial or the barrel cortex to better understand how astrocyte calcium signals correlate with cerebral blood flow during awake-sleep cycles. We used a chronic cranial window approach with bone removal and brain nano-injection of AAV2/5 to express jGCaMP8m to astrocytes (GfaABC1D promoter, 4 weeks recovery). To determine the physiological state, we continuously measured the size of the pupil and the movement of whisker during two-photon imaging, which, when assessed in combination, are a reliable indicator of the awake state (dilated pupil with active whiskers), the NREM sleep state (medium sized pupil with stationary whiskers), or REM sleep (small pupil with active whiskers). We commenced imaging when sleep pressure was highest (7-8am). Under this condition, each mouse regularly fell asleep after approximately 1.5 hours under the microscope, followed by periods of alternating wakefulness and sleep occurrences every few minutes. We found in the quiet awake mouse, the magnitude of penetrating arteriole diameter and endfoot Ca^{2+} changes were weakly correlated. However, vessel diameter and astrocytes calcium activity concomitantly increased when each mouse woke up, revealing a strong correlation. However, we failed to see the enhanced hemodynamic changes when the mouse falls asleep previously reported. One explanation could be that our experiments did not employ a less invasive thinned skull window, which our future experiments will test. Nevertheless, our results reveal novel relationships between astrocyte calcium and vascular dynamics during awake-sleep cycles, which may be important for cerebral blood flow regulation in these unique physiological states.

Disclosures: F. Wang: None. **J. Sun:** None. **G. Gordon:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.24/D64

Topic: B.09. Glial Mechanisms

Support: Dr. John P. and Therese E. Mulcahy Endowed Professorship in Ophthalmology
Department of Veterans Affairs, grant #BX003938
The Glaucoma Foundation
Eversight
Illinois Society for the Prevention of Blindness
Richard A. Perritt M.D. Charitable Foundation

Title: Endothelin-1 overexpression elicits cellular elastinopathy in rat optic nerve head astrocytes

Authors: *C. BETANCOURT SZYMANOWSKA[1], A. K. GHOSH[1,2], T. SHIMAMURA[3,4], E. B. STUBBS, Jr.[2,5], V. R. RAO[2,5], S. KAJA[2,5,6];
[1]Loyola Univ. Chicago Neurosci. Grad. Program, Maywood, IL; [2]Res. Service, Edward Hines Jr. VA Hosp., Hines, IL; [3]Dept. of Surgery, Div. of Cardiothoracic Surgery, Col. of Med., [4]Univ. of Illinois Hosp. & Hlth. Sci. Syst. Cancer Ctr., Univ. of Illinois Chicago, Chicago, IL; [5]Ophthalmology, [6]Mol. Pharmacol. & Neurosci., Loyola Univ. Chicago, Maywood, IL

Abstract: Glaucoma is a progressive optic neuropathy manifesting with optic nerve head remodeling, damage to the optic nerve, and retinal ganglion cell loss. Optic nerve head astrocytes (ONHA) are the primary cell type in the optic nerve head. Noxious stimuli trigger reactive astrocytosis (RA), a morphological and structural remodeling associated with increased expression of glial fibrillary acidic protein (GFAP), proliferation migration, reduced stellation, changes in actin cytoskeleton and enhanced secretion of extracellular matrix proteins. RA is an early pathological process in glaucoma, underlying the characteristic optic nerve head remodeling. The molecular mechanisms underlying RA remain largely unknown. We have previously shown that RA induced by exposure of ONHA to mechanical strain resulted in phenotypes of elastinopathy associated with reduced lysyl oxidase like 1 (Loxl1) and elastin (Eln) gene and protein expression. The objective of this study was to further explore elastinopathy phenotypes in response to RA triggers. Here, we generated primary rat ONHA cultures overexpressing human V5-tagged endothelin 1 (EDN1-V5) or a FLAG-V5 (control) by lentiviral transduction. Overexpression of EDN1 was confirmed by qPCR and immunoblotting after selection with 3.5 µg/ml puromycin. Morphology was assessed by brightfield microscopy and rate of proliferation was quantified by Trypan blue cell viability assay. GFAP expression was quantified by qPCR and immunocytochemistry. Cell lysates were processed for qPCR and immunoblotting and expression of components of the elastin pathway, including fibulin-2 (Fbln2), fibulin-5 (Fbln5), lysyl oxidase like-1 (Loxl1) and elastin (Eln) were quantified. EDN1-ONHA exhibited a less-differentiated morphology and significantly increased doubling times, compared with control or non-transduced ONHA. GFAP expression, as a prototypic biomarker for reactive astrocytosis, was significantly increased. Gene expression of Fbln2, Fbln5, Loxl1 and Eln was reduced by 24% ($p<0.05$), 63% ($p<0.001$), 29% ($p<0.05$) and 63% ($p<0.01$), respectively. Changes in protein expression of Loxl1 and Eln were confirmed by immunoblotting, showing a 48% reduction in Loxl1 ($p<0.05$) and a 62% reduction in Eln ($p<0.05$). Our data suggest that EDN1 overexpression can affect elastin synthesis in ONHA through down regulation of Loxl1. The observed reduction in elastin levels is consistent with the role of Loxl1 as a cross-linking matrix enzyme required for normal elastic fiber formation and

stabilization. Loss of Eln from ONHA during RA may contribute to glaucoma progression by rendering the optic nerve head less compliant to biomechanical stress.

Disclosures: **C. Betancourt Szymanowska:** None. **A.K. Ghosh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); eyeNOS, Inc.. **T. Shimamura:** None. **E.B. Stubbs:** None. **V.R. Rao:** None. **S. Kaja:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); eyeNOS, Inc., Eyekleur, Inc.. F. Consulting Fees (e.g., advisory boards); Selagine, Inc..

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.25/D65

Topic: B.09. Glial Mechanisms

Support: MOST 111-2320-B-A49-038, from National Science and Technology Council, Taiwan.

Title: Effects of Fkbp5 Mutations on the Pro-inflammatory Signaling in Mouse Astrocytes

Authors: *W. HUANG[1], J.-Z. ZHOU[2], Y.-L. GAN[2], P.-C. HSU[2], C.-J. JENG[3,4], C.-C. CHOU[5], Y.-H. LEE[2,4];
[1]Dept. and Inst. of Physiol., [2]Physiol., [3]Anat. and Cell Biol., [4]Brain Res. Ctr., Natl. Yang Ming Chiao Tung Univ., Taipei city, Taiwan; [5]Natl. Lab. Animal Ctr., Taipei, Taiwan

Abstract: FK506 binding protein 51 (FKBP51) encoded by the *Fkbp5* gene belongs to a family of an immunophilin protein. FKBP51 is a negative regulator of glucocorticoid receptor (GR), and its genetic variations has been linked to stress-related psychiatric disorders. FKBP51 is also a multi-target cochaperone that regulates important physiological signaling pathways, including Akt and NF-κB activities, through interacting with Akt-specific protein phosphatase PHLPP1 and IKKα, respectively. Notably, recent evidence points to FKBP51 as one of the "A1" astrocyte markers, which exert pro-inflammatory and neurotoxic phenotypes. Our previous studies indicated that *Fkbp5* deficiency leads to decreased lipopolysaccharide (LPS)-induced neuroinflammation in mouse brain. Yet, the underlying mechanism of FKBP51- mediated inflammatory phenotype in astrocytes remained unclear. Here we used molecular modeling and binding site prediction between FKBP51 and IKKα. FKBP51 quadruple mutants 3AR that might disrupt the interface interaction of FKBP51-IKKα was generated to examine its effects on LPS-induced NF-κB signaling and proinflammatory response and astrogliosis in primary mouse astrocytes. We found that 3AR mutant specifically decreased the phosphorylation of p65-NF-κB but not Akt phosphorylation in LPS-stimulated astrocytes. The *Fkbp5*-3AR mutant also inhibits LPS-induced TNF-α expression, but not GR target gene expressions. Importantly, overexpression of *Fkbp5*-WT blocked LPS-induced hypertrophy, and this effect did not occur in

Fkbp5-3AR overexpressed astrocytes, indicating that the morphological regulation of FKBP51 on astrocyte reactivation is related to its binding to IKKα. In summary, our results demonstrated that FKBP51 regulates inflammatory astrogliosis via interacting with IKKα. The interaction site-specific FKBP51 mutations are useful for developing signaling target-specific FKBP51 inhibitors for treating inflammatory diseases.

Disclosures: **W. Huang:** None. **J. Zhou:** None. **Y. Gan:** None. **P. Hsu:** None. **C. Jeng:** None. **C. Chou:** None. **Y. Lee:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.26/D66

Topic: B.09. Glial Mechanisms

Support: NIH Grant R21AG060302
NIH Grant R03AG070562
NIH Grant R01AG078859

Title: Adar1 suppression causes interferon signaling and transposable element transcript accumulation in human astrocytes

Authors: *C. M. MCENTEE[1,2], A. N. CAVALIER[1,2], T. J. LAROCCA[1,2];
[1]Hlth. and Exercise Sci., [2]Ctr. for Healthy Aging, Colorado State Univ., Fort Collins, CO

Abstract: Neuroinflammation is a central mechanism of brain aging and Alzheimer's disease (AD), but the exact causes of age- and AD-related neuroinflammation are not fully understood. One potential modulator of neuroinflammation is the enzyme adenosine deaminase acting on RNA 1 (ADAR1), which regulates the accumulation of endogenous double-stranded RNA (dsRNA, a pathogen-associated molecular pattern and innate immune activator) through adenosine-to-inosine (A-to-I editing). However, the role of ADAR1 and the sources of its dsRNA targets in astrocytes, key cells involved in neuroinflammation, have not been comprehensively investigated. We used siRNA transfection to suppress ADAR1 expression (~60% reduction compared to transfection control) and function (indicated by reduced A-to-I editing) in primary human fetal astrocytes. We analyzed changes in the transcriptome using RNA-seq and found that ADAR1 knock down increased 220 differentially expressed genes (FDR < 0.1), and a majority of these genes were involved in type I interferon and pro-inflammatory signaling pathways, as indicated by gene ontology analyses. We extended on our transcriptomic findings by showing that dsRNA colocalized with melanoma differentiation-associated protein 5 (MDA5, a dsRNA sensor involved in type I interferon signaling) when ADAR1 was suppressed. Additionally, ADAR1 suppression was associated with increased ICAM-1 (a marker of pro-inflammatory astrocytes) and secreted CXCL10 and IFNβ (downstream products of type I interferon signaling), and an accumulation of transposable

element (TE) transcripts that may form dsRNA (e.g., long and short interspersed nuclear elements [LINEs and SINEs]). Finally, in secondary analyses of existing data, we found that *ADAR1* gene expression declined with aging and AD in human brain tissue ($R^2 = 0.27$; $p = 0.004$), and this correlation was associated with an age- and AD-related increase in TE transcripts. Together, our results suggest an important role for ADAR1 in astrocyte-related neuroinflammation with aging and AD through the suppression of endogenous TE-derived dsRNAs. To follow up on these observations, we are performing RNA immunoprecipitation sequencing to identify the specific TE-derived dsRNAs that accumulate after ADAR1 suppression.

Disclosures: C.M. McEntee: None. **A.N. Cavalier:** None. **T.J. LaRocca:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

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Program #/Poster #: PSTR191.27/D67

Topic: B.09. Glial Mechanisms

Support: R01 CA227149-01A1

Title: Glioblastoma hijacks astrocytic glutamate homeostasis to support tumor progression and peritumoral hyperexcitability

Authors: *M. ESCALANTE, E. FLEISCHEL, O. Y. LI, S. MENNA, H. SONTHEIMER; Neurosci., Univ. of Virginia, Charlottesville, VA

Abstract: Glioblastoma (GBM) continues to be the most aggressive primary brain tumor, with a dismal life expectancy of approximately 15 months after diagnosis. As tumor progress, patients often encounter significant complications including symptoms like headaches, vision and personality changes, memory loss, and tumor-associated epilepsy. One major factor underlying the malignant properties of GBM tumors is their ability to use the central nervous system (CNS) microenvironment to their advantage. Astrocytes, the most abundant type of glial cells in the CNS, play crucial roles in various developmental and homeostatic functions. They maintain essentially all forms of homeostasis, provide metabolic and trophic support, modulate neurovascular coupling, and regulate synaptogenesis, as well as synaptic transmission and plasticity. Although a principal role of astrocytes in glutamate homeostasis involves glutamate uptake via excitatory amino acid transporters (EAATs), they also release glutamate through system xc^- (SXC). Several studies have highlighted the involvement of SXC in epileptogenesis and excitotoxicity using different experimental models. We therefore hypothesize that gliomas may co-opt and reprogram tumor-associated astrocytes to disrupt glutamate homeostasis by upregulating SXC. Here, utilizing indirect glioma-astrocyte coculture assays, we found that GBM cells promote an increase in astrocytic SXC activity and pronounced astrocyte reactivity through secreted factors. Upon further investigation, we discovered that the increase in SXC

activity is associated with an increment of transporters at the plasma membrane and correlates with high levels of mRNA expression. Finally, using orthotopic patient-derived xenograft tumor models and in situ hybridization (ISH), we corroborate an increase of SXC mRNA transcripts in tumor-associated astrocytes. Collectively, these findings suggest that the secretome of glioma cells is sufficient to disrupt glutamate homeostasis in the tumor microenvironment, which may contribute to cortical hyperexcitability, epileptiform activity, and excitotoxicity.

Disclosures: **M. Escalante:** None. **E. Fleischel:** None. **O.Y. Li:** None. **S. Menna:** None. **H. Sontheimer:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

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Program #/Poster #: PSTR191.28/D68

Topic: B.09. Glial Mechanisms

Support: ZONMW VIDI grant 91718392
Dutch Rare Disease Foundation,Zeldzame Ziekten Fonds

Title: Aquaporin-4 and GPRC5B: Characterisation of new pathogenic variants leading to Megalencephalic Leukoencephalopathy with subcortical Cysts

Authors: *E. M. J. PASSCHIER[1,2], S. KERST[1,2], E. BROUWERS[1,2], E. M. C. HAMILTON[1], Q. BISSELING[1,2], M. BUGIANI[3], Q. WAISFISZ[4], P. KITCHEN[5], L. UNGER[5], M. BREUR[3,1], L. HOOGTERP[1], S. I. DE VRIES[6], T. E. M. ABBINK[1], M. H. P. KOLE[6,7], R. LEURS[8], H. F. VISCHER[8], M. S. BRIGNONE[9], E. AMBROSINI[9], F. FEILLET[10], A. P. BORN[11], L. EPSTEIN[12,13];
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Abstract: Water homeostasis is crucial for normal brain functioning. Brain edema due to disrupted homeostasis is a life-threatening complication of various neurological conditions. Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC) is a rare white matter disease characterized by chronic brain edema. Patients develop macrocephaly early in life and show slow cognitive decline, epilepsy, ataxia and spasticity. Patient MRI reveals an increased brain water content and subcortical cysts. At the cellular level the brain white matter shows numerous intramyelinic vacuoles. In most patients MLC is caused by pathogenic variants in either the *MLC1* or the *GLIALCAM* gene, but patients without variants in either of these genes exist. Previous studies showed that pathogenic variants in *MLC1* and *GLIALCAM* cause disturbances in astrocyte volume regulation, leading to chronic swelling of astrocytes and potentially hampering their homeostatic functions. Here, we discuss that MLC can be caused by variants in two other genes. Genetic studies in MLC patients without pathogenic variants in *MLC1* or *GLIALCAM* revealed one homozygous variant in *AQP4* in two siblings and two *de novo* heterozygous variants in *GPRC5B* in three unrelated patients. *AQP4* encodes the protein Aquaporin-4 (AQP4) which is the main water channel present in the brain. The patient variant affects an amino acid in the highly conserved NPA domain, crucial for pore formation in the cell membrane. *GPRC5B* encodes the orphan G protein coupled receptor class C group 5 member B, the function of which is still poorly understood. Similar to MLC1 and GlialCAM, AQP4 and GPRC5B are highly expressed in astrocytes, cells crucial for ion and water homeostasis. With biotinylation and calcein quenching assays, we found that the variant in *AQP4* causes a loss of AQP4 expression at the plasma membrane, ultimately altering the cellular swell and shrink kinetics. For the *GPRC5B* variants we detected increased expression levels of mutant GPRC5B in patient lymphoblasts using western blot. These patient cells also showed altered Regulatory Volume Decrease (RVD). When examining the electrophysiological properties of cells overexpressing wild-type or mutant GPRC5B, we found altered Volume Regulated Anion Channel (VRAC) activity. VRAC is an important player in normal cell volume regulation, and its disruption is a hallmark for MLC. With this work we give insight in new pathogenic variants, we discovered new disease mechanisms for MLC and open up new research pathways to study brain edema. Ultimately, our goal is to find therapeutic interventions for MLC and related forms of brain edema. With this work we highlight new targets for future research.

Disclosures: E.M.J. Passchier: None. **S. Kerst:** None. **E. Brouwers:** None. **E.M.C. Hamilton:** None. **Q. Bisseling:** None. **M. Bugiani:** None. **Q. Waisfisz:** None. **P. Kitchen:** None. **L. Unger:** None. **M. Breur:** None. **L. Hoogterp:** None. **S.I. de Vries:** None. **T.E.M. Abbink:** None. **M.H.P. Kole:** None. **R. Leurs:** None. **H.F. Vischer:** None. **M.S. Brignone:** None. **E. Ambrosini:** None. **F. Feillet:** None. **A.P. Born:** None. **L. Epstein:** None.

Poster

PSTR191. Astrocytes: Mechanisms Underlying Neurological Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.29/D69

Topic: B.09. Glial Mechanisms

Support: VIDI grant 91718392

Title: Astrocyte-specific deletion of the volume-regulated anion channel does not reproduce key aspects of Megalencephalic Leukoencephalopathy with subcortical Cysts

Authors: *S. KERST[1,2], M. BREUR[1,3], M. BUGIANI[3], R. SAH[4], H. D. MANSVELDER[2], M. S. VAN DER KNAAP[1,2], R. MIN[1,2];
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Abstract: When brain volume regulation is compromised it can lead to life-threatening brain edema. Several monogenic neurological diseases are characterized by chronic brain edema. Of these diseases megalencephalic leukoencephalopathy with subcortical cysts (MLC) is the prototype. MLC is characterized by chronic swelling of the brain white matter, extensive myelin vacuolization, motor symptoms and epileptic seizures. The most prevalent MLC-causing gene variants are in *MLC1* and *GLIALCAM*, genes which encode astrocyte proteins involved in cell volume regulation. Patient-derived lymphoblasts and isolated astrocytes with hampered MLC1 or GlialCAM expression show a defect in the swelling-induced regulatory volume decrease (RVD), a process by which cells restore their original volume. It is hypothesized that an astrocytic defect in the activation of the volume-regulated anion channel (VRAC), a key player in RVD, is the upstream event caused by the MLC-causing gene variants. Here, we investigated whether loss of VRAC function in astrocytes is sufficient to cause the MLC phenotype in mice. We generated transgenic mice lacking the essential VRAC subunit LRRC8A specifically in astrocytes, using mice which express Cre under the GFAP promoter crossed to LRRC8A$^{flox/flox}$ mice. To study whether these mice develop a motor phenotype we performed various motor tests at different ages. In 8-month-old mice we determined whether the seizure threshold upon kainate injection was reduced. Furthermore, we examined brain tissue to analyse brain water content, white matter vacuolization, astrocyte swelling, and localization of proteins involved in astrocyte volume regulation. In acute brain slices, we investigated volume regulation and accompanying changes in intracellular chloride concentration of fluorescently labelled astrocytes upon a swelling-inducing increase in extracellular potassium concentration.
Loss of VRAC function in astrocytes leads to late-onset mild ataxia and a decrease in seizure threshold. However, in contrast to what is observed in MLC mouse models and MLC patients, loss of astrocyte VRAC does not lead to an increased brain water content. Results in acute brain slices show that loss of VRAC does not alter astrocyte swelling and volume regulation. Finally, swelling-induced intracellular chloride concentration dynamics remain intact.
We reveal that the loss of functional VRAC activity in astrocytes leads to a mild late-onset disease phenotype without the MLC-characterizing chronic brain edema. This suggests that an

astrocytic VRAC defect is not the only causative factor leading to disrupted brain water and ion homeostasis in MLC.

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Poster

PSTR192. Microglia: Biology

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Topic: B.09. Glial Mechanisms

Support: AG028271
AG067061
DE014320
R25GM089571

Title: Dietary fatty acids differentially impact phagocytosis, inflammatory gene expression, and cell metabolism in microglial and neuronal cell models

Authors: *M. BUTLER[1], S. MACKEY-ALFONSO[2], N. MASSA[3], K. K. BASKIN[4], R. M. BARRIENTOS[1];
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Abstract: We have previously shown that high fat diet (HFD) consumption increases the concentration of saturated fatty acids (SFAs) in the hippocampus and elicits a neuroinflammatory response and subsequent memory impairment in aged rats. Moreover, our lab and others have shown that the omega-3 fatty acid DHA can have a neuroprotective effect and buffer against unhealthy diet consumption. However, the underlying cellular mechanisms driving these responses are poorly understood. Here, we investigate the impact of palmitic acid (PA), the abundant SFA in HFD, and the protective effects of DHA on a variety of cellular responses in microglial and neuronal cell lines. BV2 microglia or HippoE-14 neurons were treated with either vehicle or 30υM DHA for 22h prior to a 6h treatment with either vehicle or 100υM PA. Following treatment, cells were either lysed and processed for qPCR, or prepared for live cell assays to measure phagocytosis of synaptic material, and mitochondrial respiration. PA treatment increased the expression of several proinflammatory and endoplasmic reticulum (ER) stress genes in microglia and neurons, respectively, and DHA pretreatment prevented these alterations. The phagocytosis assay demonstrated that naïve BV2 cells engulfed synaptoneurosomes isolated from aged HFD-fed mice at a faster rate than synaptoneurosomes isolated from aged chow-fed mice. Interestingly, PA-treated BV2 cells exhibited slowed phagocytosis of aged-chow, but not aged-HFD, synaptoneurosomes, an effect that was mitigated by DHA pretreatment. Lastly, PA

reduced mitochondrial respiration in both microglial and neuronal cell lines, an effect that was not mitigated by DHA pretreatment. Together, these data provide compelling evidence that DHA can protect against SFA-induced perturbations in a variety of cellular processes, including inflammation, ER stress, and phagocytosis, but not changes in mitochondrial respiration. Future work will aim to disentangle the relationship between cellular metabolism and cellular stress in the context of fatty acid signaling.

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Poster

PSTR192. Microglia: Biology

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Program #/Poster #: PSTR192.02/E1

Topic: B.09. Glial Mechanisms

Support: Brain Canada Future Leaders Grant Canadian Institutes of Health Research - Tier 2 Canada Research Chair #950-232872

Title: Generation of a new model to study the impact of aging on human microglia

Authors: ***S. ARMANVILLE**, C. TOCCO, Z. HAJ MOHAMAD, J. DROUIN-OUELLET; Univ. de Montréal, Montréal, QC, Canada

Abstract: Microglia are the resident immune cells of the central nervous system (CNS) and are essential for proper brain functioning and homeostasis. With age, they adopt a dystrophic morphology which is accompanied by a disruption in their homeostatic functions. Microglial dysfunction associated with aging is believed to contribute to the progression of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease. However, how aging induces these phenotypical changes in microglia is still unknown, especially in humans given the limited accessibility of live CNS cells for molecular work. As such, we aim to develop a system in which the effect of aging on microglial function can be studied in human microglia. Modeling age-related features in microglia derived from induced pluripotent stem cells (iPSCs) is challenging because of their embryonic nature. To induce aspects of cellular aging in microglia, we used a chemical cocktail of three senescence-inducing molecules that have been shown to induce age-related features in induced neurons from iPSCs. To evaluate the impact of these chemicals on microglia, we first used an immortalized microglia-like cell line (HMC3). Our results confirms that exposure to the senescence cocktail for six consecutive days induces senescence, as characterized by a significant increase of the Beta-Galactosidase activity (n=3, p=0.003) and the senescence markers p16 and p21. Our results also show a significant increase in double-strand DNA breaks as assessed using the marker γH2AX (n=3, p=0.022). Furthermore, the senescence cocktail decreased phagocytosis (n=3, p=0.004) capabilities and increased the expression of pro-inflammatory cytokines (n=3,p=0.009), all consistent with marks of cellular

aging. Our results suggest that chemically induced senescence can induce signs of cellular aging in human microglia-like cells. In-depth characterization of iPSC-derived aged human microglia will provide more information on the potential role of altered microglia in the pathophysiology of neurodegenerative disease, such as Parkinson disease.

Disclosures: S. Armanville: None. **C. Tocco:** None. **Z. Haj Mohamad:** None. **J. Drouin-Ouellet:** None.

Poster

PSTR192. Microglia: Biology

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Topic: B.09. Glial Mechanisms

Support: NIH Grant F32MH124298
URMC SPIN Grant
R01 MH127737

Title: Frontal cortical microglia respond to dopaminergic signals from the adolescent mesofrontal circuit

Authors: *R. D. STOWELL, K. H. WANG;
Univ. of Rochester, Rochester, NY

Abstract: Dopaminergic signaling in the frontal cortex via the mesofrontal circuit regulates cognitive functions, and adolescent changes in this circuit likely contribute to the cognitive deficits associated with neurodevelopmental disorders. Previous work found that the adolescent mesofrontal dopaminergic circuit is not just vulnerable, but malleable, providing a window for intervention. Particularly, phasic activation of the adolescent dopaminergic mesofrontal circuit through wheel running or direct optogenetic activation drives dopaminergic bouton outgrowth in the frontal cortex. However, the mechanisms behind this form of adolescent plasticity remain unclear. Recent evidence suggests that microglia, the resident immune cell of the central nervous system, can respond to specific neurotransmitters and serve diverse roles in circuit refinement depending on location and timing. With a combination of transgenic, optogenetic, and viral labeling methods in mice, we investigated if microglia respond to dopaminergic signals in vivo and impact mesofrontal plasticity. Using in vivo two-photon microscopy, we tracked fluorescently labeled microglia and dopaminergic axons in the M2 frontal cortical region pre- and post- stimulation of dopaminergic ventral tegmental area (VTA) neurons. After 2-hours of wheel running, which is known to activate dopamine neurons, microglia exhibit increased arborization and parenchyma occupation as compared to their pre-run parenchyma occupation. Importantly, this effect was unique to adolescent but not adult animals. We then used the refined spatiotemporal specificity of optogenetics to assay whether this microglial response was a direct consequence of dopamine release. We found that with optogenetic stimulation of dopaminergic

axons, microglia exhibit a biphasic response characterized by an initial reduction in surveillance of the parenchyma during dopamine release, and a subsequent extension of processes into the parenchyma post-stimulation. After stimulation, microglia contact the axonal backbone at sites where new boutons will form. Microglia also make more frequent contacts with newly formed boutons and survey a greater proportion of stable boutons in stimulated animals. Pharmacological manipulation of both D1-type receptor (D1R) and D2R signaling perturbs the biphasic microglial response to VTA stimulation, attenuates microglial contacts with boutons, and blocks bouton plasticity. Our results demonstrate that microglia in the adolescent frontal cortex respond to mesofrontal dopaminergic signaling and interact with dopaminergic boutons to promote activity-dependent plasticity.

Disclosures: R.D. Stowell: None. **K.H. Wang:** None.

Poster

PSTR192. Microglia: Biology

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Topic: B.09. Glial Mechanisms

Support: NIH Grant 2R01MH113743
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Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
Charles H. Hood Foundation
Massachusetts Life Sciences Center

Title: Microglia-astrocyte crosstalk during activity-dependent synaptic remodeling

Authors: *T. FAUST[1], Y.-H. LEE[1], G. GUNNNER[1], C. O'CONNOR[1], M. BOYLE[1], A. BADIMON[2], P. AYATA[2], A. SCHAEFER[2], D. SCHAFER[1];
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Abstract: Synapses remodel in response to changes in sensory experience and neural activity. Microglia and astrocytes both contribute to activity-dependent synapse remodeling by engulfing and removing synapses from less active neurons. Yet, how these two cell types communicate to remodel synapses, while sparing others, remains an open question. Previously, we showed that microglia engulf and remove synapses in neonate mouse barrel cortex following whisker lesioning and whisker trimming-induced reductions of neural activity. This whisker lesioning-induced synapse removal was dependent on signaling between neuronal fractalkine (CX3CL1) and its cognate microglial fractalkine receptor (CX3CR1). Using this whisker lesioning paradigm, we are now exploring how astrocytes and microglia coordinate to regulate synapse remodeling. Using cell type specific translating ribosome affinity purification followed by RNAseq (TRAPseq), we are exploring transcriptional changes in microglia and astrocytes

following whisker lesioning. In the process, we have identified putative receptor-ligand signaling between these two cell types. We are also now using expansion microscopy to assess how astrocytes modify their interactions with synapses in a microglial CX3CR1 signaling-dependent manner. Together, our results provide a novel mechanism by which microglia signal to astrocytes to facilitate synapse engulfment and remodeling of cortical synapses in response to changes in neural activity.

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Poster

PSTR192. Microglia: Biology

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Program #/Poster #: PSTR192.05/E4

Topic: B.09. Glial Mechanisms

Support: R01NS119243

Title: The role of sex differences in microglial phagocytic capacity in regulating cortical astrocyte density during postnatal development

Authors: *A. S. HUGHES, U. B. EYO;
Neurosci., Univ. of Virginia, Charlottesville, VA

Abstract: Both microglia and astrocytes are essential in supporting neuronal function. However, the mechanisms that govern microglial-astrocytic communication in the maintenance of brain homeostasis are largely unknown. Moreover, though it is known that microglia exhibit sex differences in transcriptional identity and function, how these sex differences influence glial-glial interaction has been poorly interrogated, especially in development. Here, we show that wild type male mice have a higher density of cortical astrocytes than wild type females at postnatal day 15 (P15), though both males and females possess the same number of microglia, overall nuclei, neurons, OPCs, and oligodendrocytes. Interestingly, male microglia also display a reduced density of CD68+ microglia and CD68 puncta, suggesting a less phagocytic phenotype in male microglia at this developmental time point. Based on these data, we hypothesize that microglial sex differences in phagocytic capacity cause a reduced engulfment of developing astrocytes, but not other brain cell types, in males. This suggests that there is a postnatal astrocyte-specific phagocytic mechanism in place that contributes to sex differences in cortical circuit refinement by microglia. These findings could provide insight into how sex differences in glial quantity and activity make males and females differentially susceptible to neurodevelopmental disorders, such as autism spectrum disorder.

Disclosures: **A.S. Hughes:** None. **U.B. Eyo:** None.

Poster

PSTR192. Microglia: Biology

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Program #/Poster #: PSTR192.06/E5

Topic: B.09. Glial Mechanisms

Support: NIA R01 AG078964
Rainwater Foundation
NIH T32 NS082145

Title: Retrotransposon suppression in activated microglia

Authors: *M. THOMAS, F. ATRIAN, P. RAMIREZ, B. FROST;
Univ. of Texas Hlth. Sci. Center, San Antonio, San Antonio, TX

Abstract: Transposable elements are mobile genetic elements that comprise about 45% of the human genome. Transposable elements are categorized into two major classes based on their mechanism of transposition: Retrotransposons, which copy themselves and insert a new DNA copy into a new genomic location, and DNA transposons, which excise themselves from the genome and insert into a new genomic region. Retrotransposons are structurally similar to retroviruses in that they require an RNA intermediate to mobilize. Many transposable elements are embedded within highly condensed heterochromatin and are thus epigenetically silenced. Transposable element activation occurs in many human diseases including cancer and neurodegeneration, as well as in physiological aging. We and others have reported that retrotransposons are activated in Alzheimer's disease and related tauopathies as a consequence of tau-induced heterochromatin decondensation. While retrotransposon transcripts are elevated in total brain lysates from a mouse model of tau-mediated neurodegeneration, preliminary analysis of microglia from this model reveal a surprising reduction of retrotransposon transcripts. Microglia are the resident immune cells of the central nervous system and are the primary mediator of neuroinflammation. Microglia are fundamental to homeostatic neuronal function, clearance of apoptotic neurons and debris, and monitoring of the neuronal environment for potential threats. Microglial downregulation of retrotransposon expression appears to be a general feature of neuroinflammation, as preliminary analyses also suggest that retrotransposon transcript levels are reduced in response to LPS injection, which stimulates neuroinflammation. Taken together, our preliminary studies point toward a previously undocumented and surprising role of retrotransposon deactivation in activated microglia. I am currently testing the overall hypothesis that the epigenetic control of retrotransposons regulates the transition of microglia from a homeostatic to an active state.

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Poster

PSTR192. Microglia: Biology

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Program #/Poster #: PSTR192.07/E6

Topic: B.09. Glial Mechanisms

Title: Microglia modulate general anesthesia through P2Y12 receptor

Authors: *K. CAO;
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Abstract: General anesthesia (GA) is an unconscious state produced by anesthetic drugs, which act on neurons to cause overall suppression of neuronal activity in the brain. Recent studies have revealed that GA also substantially enhances the dynamics of microglia, the primary brain immune cells, with increased process motility and territory surveillance. However, whether microglia are actively involved in GA modulation remains unknown. Here, we report a previously unrecognized role for microglia engaging in multiple GA processes. We found that microglial ablation reduced the sensitivity of mice to anesthetics and substantially shortened duration of loss of righting reflex (LORR) or unconsciousness induced by multiple anesthetics, thereby promoting earlier emergence from GA. Microglial repopulation restored the regular anesthetic recovery, and chemogenetic activation of microglia prolonged the duration of LORR. In addition, anesthesia-accompanying analgesia and hypothermia were also attenuated after microglial depletion. Single-cell RNA sequencing analyses showed that anesthesia prominently affected the transcriptional levels of chemotaxis and migration-related genes in microglia. By pharmacologically targeting different microglial motility pathways, we found that blocking $P2Y_{12}$ receptor ($P2Y_{12}R$) reduced the duration of LORR of mice. Moreover, genetic ablation of $P2Y_{12}R$ in microglia also promoted quicker recovery in mice from anesthesia, verifying the importance of microglial $P2Y_{12}R$ in anesthetic regulation. Our work presents the first evidence that microglia actively participate in multiple processes of GA through $P2Y_{12}R$-mediated signaling and expands the non-immune roles of microglia in the brain.

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Poster

PSTR192. Microglia: Biology

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Topic: B.09. Glial Mechanisms

Support: P20GM109089

Title: Neuroprotective effects of exogenous hydrogen sulfide in long COVID-19

Authors: *A. A. VAKHTIN, Y. MATOS, S. NITSCHKE, K. JULIO, N. SHAFF, S. G. RYMAN;
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Abstract: Over 35% of mild COVID-19 cases produce chronic symptoms, with 88% of such post-acute sequelae involving neurocognitive issues (neuro-PASC). With mechanisms of neuro-PASC poorly understood, we proposed an indirect model for its pathogenesis via dysfunctional gut-brain interactions that persist chronically, despite SARS-CoV-2 being no longer detectable systemically. Given its common gastrointestinal disturbances, COVID-19 can induce or exacerbate gut dysbiosis, resulting in increased production and systemic proliferation of microbial byproducts. One such compound is hydrogen sulfide (H_2S) - a highly toxic gas that rapidly inhibits mitochondrial function, arrests aerobic metabolism, and induces seizures, coma, and death in relatively low doses. Despite this, extremely small, endogenous doses of H_2S are normally synthesized in the body and have beneficial biological roles, such as suppressing systemic inflammation and regulating vascular function. However, the neuroinflammatory and cognitive effects of exogenous H_2S - produced in larger volumes via gut dysbiosis that is common in COVID-19 - are unknown. Our hypothesis is that neuro-PASC symptoms are produced by persistent thalamic neuroinflammation, which is mediated by systemic H_2S that is primarily derived from the gut microbiome. We recruited 26 former mild COVID-19 patients: 12 with persistent neurocognitive symptoms ($PASC^+$) and 14 reporting full recovery ($PASC^-$). Exogenous H_2S concentrations were measured in the breath as part of a lactulose test, and were 138% higher in $PASC^+$ patients than $PASC^-$ controls. Thalamic neuroinflammation was measured using Diffusion Weighted Magnetic Resonance Spectroscopy (DW-MRS), which measures apparent diffusivity coefficient of choline (ADC_{cho}) that predominantly resides in microglia. As microglia activate, morphing from a branched to a spherical shape, ADC_{cho} increases. Greater thalamic ADC_{cho} predicted worse Working Memory (WM; $p = 0.015$), an effect driven by the $PASC^+$ ($p = 0.018$), but not $PASC^-$ patients ($p = 0.445$). Higher H_2S predicted better WM in the $PASC^+$ ($p = 0.021$), but not the $PASC^-$ group ($p = 0.659$). To elaborate this, we compared neuroinflammation across 4 groups based on $PASC^+/PASC^-$ and H_2S^+/H_2S^- factors. H_2S presence had no effect on neuroinflammation in the $PASC^-$ group. However, the $PASC^+/H_2S^-$ group had 31% more thalamic neuroinflammation than the $PASC^+/H_2S^+$, and 41% higher neuroinflammation than either $PASC^-/H_2S^-$ or $PASC^-/H_2S^+$ group. Further, H_2S presence was associated with better WM scores. These findings suggest neuroprotective effects of H_2S in PASC, which subsequently have cognitive implications in terms of WM performance.

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Poster

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Cosmos Club Foundation Scholarship Grant
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Title: Examining influences of confinement and the actin cytoskeleton in microglial complement-sensing protrusions

Authors: *S. PAULSON, J. D. ROTTY;
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Abstract: Microglia are the resident immune cells of the central nervous system. They respond to phagocytic markers to help maintain homeostasis within the brain. Phagocytic markers include members of the complement cascade, a pro-inflammatory protein cascade responsible for helping label and clear foreign bodies and debris. Complement proteins such as iC3b are also used during learning and memory to label dendritic spines for phagocytosis. Our initial studies use iC3b as an *in vitro* model substrate to understand how microglia use actin-dependent cellular protrusions to sense and respond to these phagocytic markers. Using a murine microglial cell line, BV2, and primary microglia, we utilized phagocytosis assays to measure microglial sensing of iC3b in 2D culture. As the brain is a highly confined environment, we are also interested in modeling how confinement affects complement sensing. Preliminary confinement data suggests that microglia phagocytize more efficiently and migrate more persistently during confinement. We are now poised to understand how key regulators of the actin cytoskeleton respond to simultaneous application of confinement and iC3b cues. Our initial focus will be the branched actin polymerizing Arp2/3 complex. Our overall goal is to define the molecular mechanism and *in vivo* relevance of microglial actin-based complement sensing protrusions. These studies will clarify how microglia sense and respond to complement in the brain by modeling key elements of their *in vivo* environment.

Disclosures: S. Paulson: None. **J.D. Rotty:** None.

Poster

PSTR192. Microglia: Biology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: B.09. Glial Mechanisms

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Title: Engineering an inhibitor resistant human CSF1R variant for microglia replacement

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Abstract: The replacement of microglia for the treatment of neurological diseases has been a long-term goal for many glial biologists. Researchers have tried numerous methods for microglial replacement with varying degrees of success, most of which depend on the depletion of endogenous microglia with toxic preconditioning methods such as chemotherapy or irradiation. Currently, the main method for macrophage replacement used in clinical settings is hematopoietic stem cell transplantation (HSCT) which replaces some endogenous microglia with circulation-derived macrophages but has a high mortality. Here, we sought to create a safe, specific way to replace microglia.To do this, we leveraged microglial dependency on CSF1 receptor (CSF1R) signaling and clinically available CSF1 receptor inhibitors. By engineering a CSF1R variant resistant to these pharmacological inhibitors, we created a microglia replacement strategy that does not depend on conventional chemotherapy or irradiation. With this approach, we replaced over 98% of brain macrophages with human microglia.A glycine-to-alanine substitution at position 795 of human CSF1R (G795A) confers resistance to multiple CSF1R inhibitors, including PLX3397 and PLX5622, as measured by increased survival of G795A macrophages following inhibitor treatment. Biochemical and cell-based assays show no discernable gain or loss of function. G795A- but not wildtype-CSF1R expressing macrophages efficiently engraft the brain of PLX3397-treated mice and persist after cessation of inhibitor treatment. To gauge translational potential, we CRISPR-engineered human induced pluripotent stem cell-derived microglia (iMG) to express G795A. Xenotransplantation studies demonstrate that G795A iMG exhibit nearly identical gene expression to wildtype iMG, respond to inflammatory stimuli akin to WT controls, and progressively expand in the presence of PLX3397, replacing endogenous microglia to fully occupy the brain. In sum, we found that a

G795A substitution in CSF1R confers resistance to pharmacological inhibitors, enabling widespread engraftment of human microglia and murine macrophages within the brain and providing a highly efficient strategy for robust, nontoxic replacement of microglia. We are now testing the ability of this tool to deliver gene therapy targets to the brain.

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Poster

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Topic: B.09. Glial Mechanisms

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Title: Lipofuscin-like autofluorescence within microglia and its impact on studying microglial engulfment

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Abstract: Engulfment of cellular material and proteins is a key function for microglia, a resident macrophage of the central nervous system (CNS). Among the techniques used to measure microglial engulfment, confocal light microscopy has been used the most extensively. Here, we show that autofluorescence (AF), likely due to lipofuscin and typically associated with aging, can also be detected within microglial lysosomes in the young mouse brain by light microscopy. This lipofuscin-AF signal accumulates first within microglia and increases with age, but it is not exacerbated by amyloid beta-related neurodegeneration. We further show that this lipofuscin-AF signal within microglia can confound the interpretation of antibody-labeled synaptic material

within microglia in young adult mice. Finally, we implement a robust and commercially available strategy to quench AF in mouse, marmoset, and human brain tissue.

Disclosures: J.M. Stillman: None. **F. Lopes:** None. **J. Lin:** None. **K. Hu:** None. **D.S. Reich:** None. **D.P. Schafer:** None.

Poster

PSTR192. Microglia: Biology

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Topic: B.09. Glial Mechanisms

Support: NSF GRFP DGE-1845298
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Klingenstein-Simons fellowship in neuroscience

Title: Using ER-Hoxb8 conditionally-immortalized macrophages to study microglia replacement

Authors: *K. NEMEC[1,2], F. S. PURNELL[2], Y. ORTEGA-BURGOS[3,2], N. BLANK[4,7], C. A. THAISS[4,5], M. L. BENNETT[8,6], F. C. BENNETT[2,8];
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Abstract: Microglia are the tissue-resident macrophages of the central nervous system. When depleted, the microglial niche can be reconstituted by surrogate macrophages - a process we term “microglia replacement”. Mice genetically deficient of the microglia survival receptor CSF1R lack microglia, and therefore provide a useful model for studying the dynamics of microglia replacement by transplantation. However, the use of primary macrophages as donor cells presents many experimental limitations - they are finite in number and are difficult to isolate and virally transduce. ER-Hoxb8 conditionally-immortalized myeloid progenitors provide a tempting tool for studying macrophage dynamics in a more robust manner. These cells can be expanded indefinitely in culture, are easily transduced, and readily differentiate into macrophages for downstream study. However, the degree to which Hoxb8-macrophages are comparable to microglia and bone marrow-derived macrophages remains unclear. We here demonstrate 1) the similarities and differences between these cell types in vitro and in vivo after transplantation into $CSF1R^{-/-}$ mice and 2) the advantages and limitations of using Hoxb8s to study microglia replacement, including for gain and loss of function studies.

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Poster

PSTR192. Microglia: Biology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.13/E12

Topic: B.09. Glial Mechanisms

Title: Rapid Differentiation of functional microglia from Human iPSCs by Transduction of transcription factors using Sendai Virus

Authors: *M. B. C. KILANDER[1], A. SAADIN[2], B. FRAHER[2], C. MYERS[2], L. AGBOR[2], T. TANAKA[2], A. SHAROV[2], M. KO[3], M. SEO[1];
[1]Elixirgen Scientific Inc., Baltimore, MD; [2]Elixirgen Scientific, Baltimore, MD; [3]Elixirgen Scientific, Inc., Baltimore, MD

Abstract: Microglia, the resident immunocompetent cells of the central nervous system, play vital roles in protecting neurons from pathogens and regulating neural circuit development. Recent studies demonstrate that microglia are involved in the progression of neurodegeneration, consequently resulting in disorders such as Alzheimer's disease (AD). Thus, to develop efficient therapeutic interventions, it is critical to include microglia of human origin in in vitro models of AD. However, the commercial supply of human primary microglia is limited. Therefore, establishing a reproducible protocol to differentiate microglia from human induced pluripotent stem cells (hiPSCs) is vitally important. Elixirgen Scientific has developed methods to differentiate hiPSCs into excitatory neurons, cholinergic neurons, astrocytes, and skeletal muscles by Sendai virus-based delivery of crucial transcription factors within 10 days. With this technique, namely Quick-TissueTM technology, we have developed a protocol to differentiate cells that express markers for microglia such as AIF1, SALL1, and CX3CR1 in 10 days. Quantitative RT-PCR analysis showed that AIF1 and CX3CR1 in day 10 differentiation cultures were upregulated about 100 folds and TREM2 about 30 folds relative to those in undifferentiated hiPSC culture. Furthermore, the expression levels of CD68, CD74, HEXB, ITGAM, CSF1R, and MERTK in the day 10 cultures were comparable to those in fetal microglia based on RNA-seq analysis. Immunofluorescence microscopy demonstrated highly effective differentiation by day 10, as more than 70% of cultured cells were positive for AIF1. Phagocytic abilities were thereafter confirmed by the accumulation of fluorescent beads inside these cells. We have successfully established a protocol to efficiently generate functional microglia within two weeks, which is useful for studies in drug discovery and in vitro modeling of neurodegenerative disorders such as Alzheimer's disease.

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Poster

PSTR193. Glia-Neuron Interactions in Physiology

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Topic: B.09. Glial Mechanisms

Support: NIH R01MH118441
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ASRC Seed Grant

Title: Explicit Safety Learning Engages Parvalbumin Interneurons and Neuro-Glial Interactions in the Prelimbic Cortex

Authors: *L. E. DENHOLTZ[1,2], R. KOLARIC[1,2], K. R. SURRENCE[2], I. GRUNFELD[3], E. LIKHTIK[4];
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Abstract: Stress- and anxiety-disorders such as post-traumatic stress disorder (PTSD) are characterized by fear generalization. Poor discrimination of non-threat is linked to lower medial prefrontal cortex (mPFC) volume, and white matter loss in tracts connecting the mPFC with other regions. One promising approach to mitigate excessive fear expression is safety learning, which establishes an explicit association between a cue and safety and decreases overgeneralized fear. However, the circuit-level changes exerted by safety training for this therapeutic effect are not known. We investigate whether safety training modulates neuro-glial dynamics in the mPFC, with the potential to restore mPFC communication with regions that are important for discrimination of non-threat, such as the amygdala and auditory cortex. We show that 1) male ($F(3,7)= 3.146, p<0.01$), and female ($F(3,19)=5.112, p<0.01$) mice learn to suppress fear during safety cues, 2) immunolabeling for cFos shows that parvalbumin-expressing interneurons (PV IN) of the prelimbic (PL) mPFC are more active during cued safety memory retrieval than during cued- or contextual- fear memory retrieval ($F(1.234, 4.937)= 31.00, p<0.01$), 3) Single unit recordings in the mPFC show that safety cues evoke spiking in fast-firing, putative PV INs ($p<0.05$), and 4) 3-weeks after safety learning, there is increased co-localization of satellite oligodendrocytes with non-PV cells in the mPFC ($F(2, 10) = 14.99, p<0.01$). We are currently assessing the effects of optogenetic inhibition of PV IN in the PL on safety learning and on neuroglial interactions. We hypothesize that PV INs engagement in the PL for safety learning, leads to myelin remodeling in the mPFC, thereby altering the dynamics of local mPFC activity and its long-range communication with crucial regions that partake in threat-safety discrimination.

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Poster

PSTR193. Glia-Neuron Interactions in Physiology

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Topic: B.09. Glial Mechanisms

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Title: Serotonergic neuro-glia communication

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Abstract: A brief stream of action potentials at high frequency induces the massive extrasynaptic serotonin release from the soma and axons of serotonergic Retzius leech neurons. After a few minutes, such exocytosis is followed by the activation of fictive locomotion in the isolated ganglion. The somatic release of serotonin takes hundreds of seconds and occurs onto glial cells in the absence of any synaptic connection. Here we explore the role of glia as a mediator of serotonin-dependent behavioral responses. Serotonin release and its distribution outside serotonergic neurons was studied by multiphoton excitation and fluorescence of serotonin and by the Falk-Hillarp technique. We show that the serotonin that is released from the soma becomes densely accumulated in adjacent glial processes. In ganglia devoid of serotonin after reserpine treatment, exogenous serotonin is also incorporated by glial cells. In the minutes that follow electrical stimulation, the serotonin fluorescence seen with the Falk-Hillarp technique, distributes within the whole ganglion. The time course of such serotonin redistribution correlates with the activation of the motor crawling pattern and with the elimination of incompatible behaviors. We propose that glia accumulation and transport of serotonin contribute to the long latency and duration of modulatory serotonergic responses.

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Poster

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Topic: B.09. Glial Mechanisms

Support: Lundbeckfonden 313-2019-606

Title: Investigating Satellite Glial Cell Subtypes and Heterogeneity in Dorsal Root Ganglia via Single Cell Analysis

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Abstract: Neuropathic pain, a complex and debilitating condition, remains a challenge in clinical practice due to limited understanding of its underlying mechanisms. Satellite glial cells (SGCs) in the dorsal root ganglia (DRG) play a crucial role in the modulation of neuronal function and maintenance of sensory homeostasis and have emerged as contributors to the development and maintenance of neuropathic pain. However, the classification and functional diversity of SGC subtypes in the DRG, as well as their association with different neuron subtypes, remain largely unexplored. This study aimed to investigate SGC subtypes via single-cell analysis, utilizing complementary techniques such as RNAscope and immunohistochemistry for identification of new SGC markers. We explore the distribution of SGC subtypes within the DRG and their potential associations with different neuron subtypes. To characterize SGC heterogeneity, dissociated mouse DRG cells were subjected to single-cell RNA sequencing (scRNA-seq). Unsupervised clustering of single-cell RNA-seq data identified distinct SGC subpopulations with diverse expression profiles, revealing SGC heterogeneity and revealed the existence of 5 different SGC subtypes. To validate and further characterize these subtypes, RNAscope in situ hybridization was employed to assess the spatial distribution of subtype-specific genes within the DRG. Immunohistochemical techniques were utilized to confirm the presence of different protein markers associated with identified SGC subtypes. Furthermore, an intricate SGC-neuron co-distribution pattern was mapped, with certain SGC subtypes preferentially associating with specific neuron subtypes. The findings demonstrated a heterogeneous distribution of SGC subtypes across the DRG, suggesting region-specific roles in neuronal modulation and sensory processing. The identification of novel protein markers also provided new insights into the molecular signature of SGC subtypes. In conclusion, this study provides novel insights into the complex heterogeneity and distribution of SGCs in DRG. Understanding these newly defined SGC subtypes and their specific neuron associations offers novel therapeutic opportunities for neuropathic pain, positioning SGCs as potential targets for pain management.

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Poster

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Topic: B.09. Glial Mechanisms

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Title: An in vivo genetic screen identifies immune receptor Crq as a critical regulator of glial synapse elimination in development and aging

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Abstract: During development, appropriate synaptic connections are established in a two-step process: synapse formation and selective synapse elimination. The rules that govern when and which synapses are formed and eliminated and how those rules are represented at the cellular and molecular level remain poorly defined. However, glia are critical regulators of these processes. In order to identify candidate molecules that underlie the neuron-glia interactions required for appropriate synapse development, we performed an *in vivo* genetic screen. We developed a method to rapidly read out synaptic proteins in *Drosophila* lysates by ELISA, and used this method to evaluate 1,029 lines in which genes were knocked down specifically in glia to screen for changes in synapse development. Out of 91 hits, six homologs for scavenger receptors, a class of receptors previously identified to mediate clearance of debris in immune cells, were identified. Among these, glial knockdown of Crq (CD36/SCARB2), strongly increased synaptic protein levels in the adult brain. By examining changes in synaptic proteins over time, we found that this increase in synapses was driven by an impairment in glial-mediated synapse elimination in flies lacking Crq expression. Given its role in immune cells, it was possible that Crq was simply required for glia to engage in phagocytosis. However, Crq was not required for glial clearance of neuronal debris during metamorphosis, nor for clearance of debris after neuronal injury. This demonstrates that Crq plays a more specific role in glial recognition or clearance of synapses that require elimination during development. Interestingly, it also prevented reductions in synaptic proteins observed with aging, suggesting a common mechanism underlies glial-mediated elimination of synapses during development and age-related synaptic loss. Future work will use proximity labeling to identify the neuronal ligands of Crq, and ultimately identify how those ligands are regulated to tag specific synapses for elimination. We have also generated a tool to endogenously label synapses in an inducible manner with single cell precision, which will allow us to address which subtypes of neurons undergo synapse elimination in development and aging, and address which of those events are Crq-dependent. These tools will also be used to identify the cellular and molecular pathways by which other genes identified in the screen mediate glial regulation of synapses. Ultimately, this work aims to provide insight into how neurons and glia work together to create and maintain appropriate synapses throughout the nervous system.

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Poster

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Topic: B.09. Glial Mechanisms

Support: NIH Grant NS122316
NIH Grant T32GM007308

Title: Microglia regulation of neuronal metabolism and mRNA translation

Authors: *D. ADLER[1], W. GAN[2], E. KLANN[3];
[1]New York Univ. Ctr. For Neural Sci., Brooklyn, NY; [2]Shenzen Bay Lab., Shenzen, China;
[3]New York Univ., New York, NY

Abstract: Maintaining homeostasis in the brain requires the delicate synchronization of signals and structures to ensure that rapid changes in energy demand are met, on a millisecond timescale, by adequate metabolic supply. One cell type that is known to play an important role in regulating brain tissue-homeostasis is microglia, yet little is known about how these resident-immune cells influence neuronal metabolism during physiological activity. We used a combination of AAV delivered metabolic sensors (for lactate, pyruvate, glucose, and ATP) under astrocytic (GFAP) and neuronal (hSyn1) promoters in the motor cortex of treadmill running mice and imaged these cells using two-photon microscopy to determine how neurons generate ATP during motor activity in wild type (WT) mice, and how this changes in microglia-depleted mice ($Cx3CR1^{CreERT2/+}$: $R26^{DTR/+}$). We found that upon initiation of treadmill running, astrocytes exhibited an increase in intracellular lactate, followed by an increase in extracellular lactate around astrocytes and then neurons; intracellular pyruvate increased at both L2/3 neuronal somas and L1 synapses; glucose was reduced at L1 synapses; and the NAD+/NADH ratio was increased in L2/3 and L1. Concomitantly, we found that ATP levels were modestly reduced in L1 synapses but were increased in L2/3 somas. In contrast, after microglia depletion neurons no longer exhibited increased levels of pyruvate in response to running induced activity, and intracellular ATP levels were reduced. In addition, we have developed a new method to detect the anabolic process of *de novo* protein synthesis during motor activity *in vivo* using intravenously delivered L-azidoholoalanine (AHA) for fluorescent noncanonical amino acid tagging (FUNCAT) in rotarod-trained mice. Using this method in WT mice, we detected robust labeling of cells specifically in motor cortex after one hour of rotor rod training compared to control mice injected with AHA and left in their home cage. These highly responsive motor cortical cells were predominantly CaMKII+ excitatory neurons, although S100b+ astrocytes and Iba1+ microglia also displayed increased *de novo* translation. Finally, we found that after motor training *de novo* translation no longer increased in motor cortical neurons or astrocytes in microglia depleted mice. Given the requirement of metabolic substrates for translation, these findings suggest microglia actively regulate neuronal and astrocytic metabolic fluxes in

physiological conditions and that disrupting communication between these cells results in an inability to boost protein synthesis in response to activity.

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Poster

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Topic: B.09. Glial Mechanisms

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Title: The behavioral effects in overexpression of K2p channels in glial cells and motor neurons for larval and adult Drosophila

Authors: *A. TAUL, E. ELLIOTT, R. L. COOPER;
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Abstract: The two-P-domain K^+ channels (i.e., K2P) are responsible for maintaining the resting membrane potential of cells. In the past, prior to further identification, these were referred to as leak channels. There appears to be 15 known types of K2P channels in humans and 11 known types in *Drosophila*, as well as six subfamilies (TWIK, TREK, TASK, TALK, THIK, and TRESK). TASK-3 misexpression is related to cancerous tissues and forms of epilepsy. TASK-1 is activated by halothane and isoflurane and can lead to cell hyperpolarization. Little is known about the expression of these subtypes in various animal tissues or the impact of altered expression on cellular physiology. It is well established that glia cells within the nervous system play an important role in the development and function of the nervous system, as they release gliotransmitters and cytokines. Altering glia cell activity can influence neural circuits and animal behavior. The influence of glial K2p channels on neuronal function, as well as their role in behavior, have yet to be investigated. Additionally, the *Drosophila* model allows for selective misexpression of certain neuron subsets, providing insight into individual cell types and the animal's physiology as a whole. This project examines pan-glia cells and motor neurons to examine the effects of K2p overexpression on behavior and physiology, both in larval and in adult *Drosophila*. Adult male *Drosophila* with genetic K2p overexpression (glia>ORK1) in glia show impaired climbing compared to a parental line of K2p (UAS-ORK1) ($P>0.05$, T-test, $N=10$), while overexpression in motor neurons did not show any difference from the parental line. A righting assay of single adults in vials put on a vortex for 5 seconds showed no differences among the three strains. Body wall movement (crawling) and mouth hook movement are commonly analyzed behaviors of larval *Drosophila* to assess neural dysfunction. The glia>ORK1 had more rapid mouth hook movements and fewer body wall movement than UAS-ORK1 ($P>0.05$,T-test, $N=20$). Thus, overexpression of ORK1 in glia cells alters larval behaviors.

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Poster

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Topic: B.09. Glial Mechanisms

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Title: Gpr37l1 in satellite glial cells control pain via modulation of kir channels

Authors: ***S. BANG**[1], C. JIANG[1], J. XU[1], S. CHANDRA[1], A. MCGINNIS[1], X. RUO[1], R.-R. JI[2];
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Abstract: GPR37L1 is an orphan G protein-coupled receptor (GPCR), but its role in pain regulation is largely unknown. Accumulating evidence suggests an activating role for satellite glial cells (SGCs) in the pathogenesis of pain, but the resolution mechanisms of glial cells in pain are not fully understood. Here, we report that GPR37L1 plays a role in protecting against chronic pain in mice and humans. GPR37L1 is expressed by satellite glial cells (SGCs) in the dorsal root ganglia (DRG) of mice and humans. Transgenic mice with *Gpr37l1* deficiency exhibit impaired resolution of neuropathic pain after chemotherapy and diabetic neuropathy. Knockdown of *Gpr37l1* in the DRGs of naive mice is sufficient to produce mechanical allodynia. We identified the pro-resolving lipid mediator maresin 1 (MaR1) as a possible ligand of GPR37L1. MaR1 reduced neuropathic pain in mice through GRP37L1. We measured the paclitaxel-induced reduction of inward-rectifying potassium channel (Kir) currents in ex vivo recordings from mouse SGCs. The application of MaR1 prevented paclitaxel-mediated Kir channel reduction in *Gpr37l1*$^{+/+}$ mice but not in *Gpr37l1*$^{+/-}$ mice. Furthermore, paclitaxel reduced the cell surface expression of GPR37L1 and Kir4.1 (KCNJ10) using a cell surface western blot in mouse DRGs. MaR1 application prevented paclitaxel-induced receptor internalization in mouse DRGs. We also found that MaR1/GPR37L1 signaling regulates Kir activity in both primary cultured human SGCs and cells transfected with GPR37L1 and Kir3.1 (KCNJ3) using a Ti^+ uptake assay. The results suggest that the MaR1/GPR37L1 axis in SGC plays a key role in pain resolution in both mice and humans by regulating Kir channels.

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Poster

PSTR193. Glia-Neuron Interactions in Physiology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Title: Age-dependent axo-glial interactions between midbrain dopamine neurons and oligodendrocyte lineage cells in the anterior corpus callosum

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Abstract: Oligodendrocyte progenitor cells (OPCs) receive synaptic innervation from glutamatergic and GABAergic axons and can be dynamically regulated by neural activity, resulting in activity-dependent changes in patterns of axon myelination. However, it remains unclear to what extent other types of neurons may innervate OPCs. Here, we provide evidence implicating midbrain dopamine neurons in the innervation of oligodendrocyte lineage cells in the anterior corpus callosum and nearby white matter tracts of adult and early adolescent male and female mice. Dopaminergic axon terminals were identified in the corpus callosum of DAT-Cre mice after injection of an eYFP reporter virus into the midbrain. Furthermore, fast-scan cyclic voltammetry in the adult revealed monoaminergic transients in the anterior corpus callosum, consistent with the anatomical findings. Using RNAscope, we further demonstrate that, in the adult, ~40% of *Olig2+/Pdfgra+* cells and ~20% of *Olig2+/Pdgfra-* cells in the anterior corpus callosum express *Drd1* and *Drd2* transcripts. Quantification of dopamine receptor transcripts by *Olig2+/Pdfgra+* and *Olig2+/Pdgfra-* cells during adolescence are ongoing, and preliminary analyses suggest that dopamine receptor transcript expression by these cells is strikingly greater during early adolescence compared to adulthood. Our results suggest that oligodendrocyte lineage cells may respond to dopamine released from midbrain dopamine axons, which could affect myelination. Together, this work broadens our understanding of neuron-glia interactions

with important implications for myelin plasticity across the lifespan by identifying midbrain dopamine axons as a potential regulator of corpus callosal oligodendrocyte lineage cells.

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Poster

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Topic: B.09. Glial Mechanisms

Support: CASIO SCIENCE PROMOTION FOUNDATION
JST Fusion Oriented Research for disruptive Science and Technology
KAKENHI Grant-in-Aid for Early-Career Scientists

Title: The effects of transcranial direct current stimulation on the cerebrospinal fluid-interstitial fluid exchange and brain metabolic waste clearance in mice

Authors: *Y. WANG, H. MONAI;
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Abstract: Transcranial direct current stimulation (tDCS) has received increasing attention as non-pharmacological therapeutics in recent years. In clinical research, tDCS, which involves passing a feeble direct current (1-2 mA) through the skull or scalp for 10-30 minutes, has been increasingly investigated as an adjunct to facilitate the rehabilitation of neurologic diseases. Many studies have shown that tDCS has positive therapeutic effects on Alzheimer's disease, including reducing amyloid beta (Aβ) in the brain, but the mechanisms are still not clearly understood. According to the glymphatic system, a recently proposed brain waste clearance system, the cerebrospinal fluid-interstitial fluid (CSF-ISF) exchange could facilitate the efficient clearance of metabolic waste, including Aβ. Regarding the mechanism of tDCS, Monai et al. found that adrenergic receptor blockade facilitated the recovery from motor dysfunctions after acute ischemic stroke in mice. The results suggested that the adrenergic receptor blockade facilitated the normalization of the brain extracellular ion milieu by CSF-ISF exchange. On the other hand, the cellular mechanisms of anodal tDCS have been suggested by Monai et al., in which activation of adrenergic receptors has a significant role in the mouse brain. However, it is still unclear how tDCS affects the dynamics of CSF-ISF exchange in the brain. In this study, we applied tDCS (0.1mA, 10min) to ketamine-xylazine (KX) - anaesthetized C57BL/6 mice by an anode placed on the cranial bone above the sensory cortex and a cathode inserted into the neck. To directly visualize brain fluid dynamics, we injected biotinylated dextran amine (BDA) as the

CSF tracer via cisterna magna after tDCS, then visualized BDA by Alexa 594-conjugated streptavidin (SA) using immunohistochemistry. We found that after tDCS, the influx of CSF tracer increased, suggesting that tDCS alters the CSF-ISF exchange in the clearance of brain metabolic waste. Furthermore, we found that tDCS also enhance the efflux of intra-cisterna magna injected Evans blue from the brain by in vivo lymph node imaging. These results suggested that tDCS alters the clearance of brain metabolic waste.

Disclosures: **Y. Wang:** None. **H. Monai:** None.

Poster

PSTR193. Glia-Neuron Interactions in Physiology

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Topic: B.09. Glial Mechanisms

Support: MOST 111-2320-B-006-059

Title: Post-weaning isolation in combination with nutrient imbalance induces striatal gliosis and corpus callosum myelin disruption in female mice.

Authors: *J.-T. FU, Z.-W. ZHAO, Y.-M. KUO, S.-F. TZENG;
Natl. Cheng-Kung Univ., Tainan, Taiwan

Abstract: Chronic stress, which is linked to obesity, has been recognized as a risk factor for several diseases such as diabetes, cardiovascular disease, and depression. Gliosis, which involves the activation of the CNS glial cells including microglia and astrocytes, plays a vital role in regulating the neuronal dysfunction caused by chronic stress. In male C57BL/6 mice, exposure to early-life social stress through post-weaning isolation (PWI) resulted in the observation of demyelination and gliosis in the corpus callosum. In a subsequent continuous study, we investigated the effects of nutrient imbalance by feeding male and female mice a high-fat diet (HFD) in accompany with subjecting them to PWI for a duration of 12 weeks. The findings of the study revealed that the combination of PWI and HFD feeding significantly caused myelin reduction and loss of oligodendrocytes in the corpus callosum of female mice. Furthermore, the presence of astrocytic aquaporin-4 (AQP4) and the integrity of the vascular system, assessed by examining the vascular marker CD31 in the caudate putamen (CPu), were disrupted in female mice exposed to PWI and HFD. Interestingly, these effects were found to be modulated by dopamine D1 receptor (D1R)-associated signaling, as intracortical administration of the D1R agonist was able to restore the decline in AQP4 and CD31 levels induced by PWI. These findings highlight that early-life social stress coupled with nutrient imbalance can impair AQP4-mediated water flux in the CPu and lead to myelin impairment in the corpus callosum of obese female mice, with the potential for restoration through the involvement of the D1R-associated signaling pathway. (The study was supported by MOST 111-2320-B-006-059).

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Poster

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Program #/Poster #: PSTR193.11/E23

Topic: B.09. Glial Mechanisms

Title: Myelin lipid synthesis modulates neural activity and is essential for motor learning

Authors: *Y. AOYAMA[1], D. KATO[1], K. NISHIDA[2], Y. TAKAHASHI[3], T. SAKAMOTO[3], I. TAKEDA[1,4], T. TATEMATSU[1], S. GO[1], Y. SAITO[1], S. KUNISHIMA[1], J. CHENG[1], H. LINGNAN[1], Y. TACHIBANA[2], S. SUGIO[1], R. KONDO[1], *A. YUKI[5], F. ETO[3,6], S. SATO[3], A. MOORHOUSE[7], K. KADOMATSU[1], I. YAO[3,6], M. SETOU[3], H. WAKE[8,1];
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Abstract: Lipids are one of the major components of myelin and contribute to the insulation of action potentials of nearby axons. These myelinated axons form white matter and act as cables to propagate information to distinct brain regions (Nave, 2010). Human MRI studies show that learning and training, such as playing the piano and juggling, lead to structural changes in white matter (Sampaio-Baptista and Johansen-Berg, 2017; Scholz et al., 2009). In mice, MRI studies have also found similar structural changes associated with increased expression of myelin-related proteins (Sampaio-Baptista et al., 2013). Impaired regulation of myelin related protein caused myelin dis-regulation resulted in motor learning deficits (Kato et al., 2020). Accumulated evidence suggested that motor learning requires activity-dependent myelination and regulation of temporal pattern of the neural activity which is essential for effective learning process. However, it is unclear whether lipid synthesis changes during motor learning and if so, whether this change contribute to neural populational activity regulation that required for motor learning. Here, we conducted in vivo two-photon imaging to quantify lipid synthesis changes during a lever-pull task in the primary motor cortex (M1). Three motor learning phases were examined: early, middle, and late. Increased movement-related calcium activity amplitudes correlated positively with both early and late lever-related neural activity. Next, to identify if myelin specific lipids are altered in response to changes in the neural circuitry associated with motor learning. We performed MALDI-IMS and LC-MS/MS of the M1 for mice in each phase of motor learning process, and quantified levels of different sphingomyelin (SM), galactosylceramide (GalCer) and sulfatide. We showed that the SM are associated with the increase in task-related neural activity during early stage of learning, while the increase in GalCer is associated with synchrony of neural activity during the late stage. As GalCer is synthesized from SM via the galactosyltransferase (CGT) enzyme, we further evaluate its role in triggering or maintaining motor learning neural synchrony by oligodendrocyte (OLs) specific CGT inhibition using adeno associate virus induced short-hairpin RNA (shRNA). Finally, inhibition of GalCer synthesis via OLs specific CGT shRNAi resulted in motor learning impairment. These results suggest that the

myelin lipid synthesis is regulated in a neural activity-dependent during motor learning. This study will be the key to understanding the mechanisms in neurodegenerative diseases related to altered lipid synthesis.

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Poster

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Title: Cross-modal plasticity after early visual deprivation depends on microglial elimination of inhibitory synapses

Authors: *A. HASHIMOTO[1], N. KAWAMURA[2], E. TARUSAWA[2], I. TAKEDA[1], Y. AOYAMA[1], N. OHNO[3,4], M. MATSUMOTO[4,5], M. INOUE[1], M. KAGAMIUCHI[1], D. KATO[1], Y. HASEGAWA[1], J. NABEKURA[4,6], A. SCHAEFER[7,8], A. J. MOORHOUSE[9], T. YAGI[2], H. WAKE[1,4,10,6,11];
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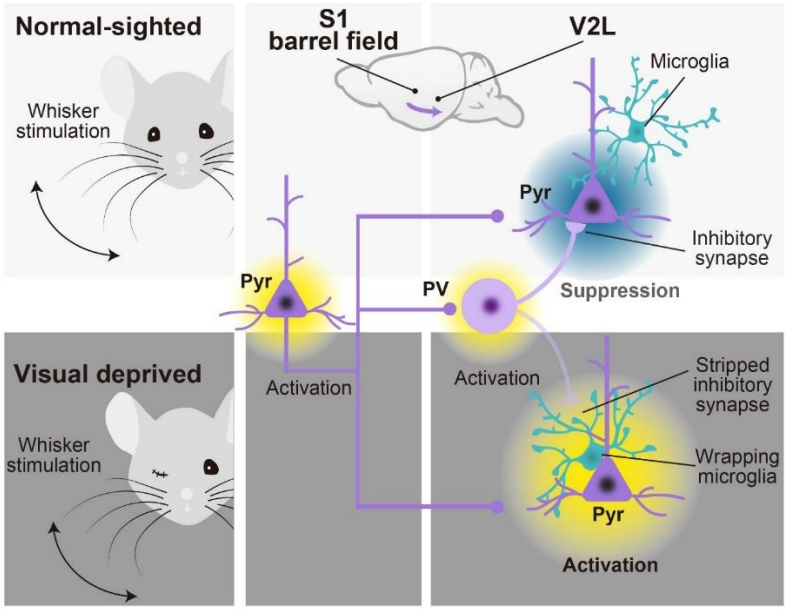
Abstract: Sensory inputs are essential to detect the external environment, but a part of them is disturbed in the blind and deaf. Traditionally, the concept of cross-modal plasticity has been raised, which an impaired sensory input is compensated by the other sensory systems and even promote the remained sensory abilities. Previous study showed whisker-dependent activation of visual cortex in the eye enucleated mice. These days we reported visual deprivation induces the microglial stripping of inhibitory synapses in extrastriate visual cortex (V2L), resulting in the acquisition of the responsiveness to the tactile sensation of neurons in V2L and promotion of the tactile discrimination ability. Actually, the depletion of microglia with Pexidartinib around monocular deprivation period reduced the neuronal activation with whisker stimulation. Furthermore, the depletion of microglia more than 20 days after monocular deprivation also results in a loss of responsiveness in V2L. This finding suggests that microglia might be involved in not only introducing but also maintaining cross-modal plasticity and that microglia might dynamically and continuously regulate inhibitory synapses in response to neuronal activity. Therefore, we are now trying to reveal how microglia modulate the excitatory and inhibitory synapses, visualizing the neurotransmission with the glutamate/GABA-sensitive fluorescent reporters (iGluSnFR/iGABASnFR). This might allow us to analyze what attract microglia to neuronal soma in response to visual deprivation and how it modulates synapses in terms of their functional as well as structural aspects. Combining the technic of two-photon holographic optogenetics, it would be possible to assess the effect of microglial synaptic modulation on the neuronal connectivity. This study will be an important clue to understand the physiological function of multi-sensory cortex and the microglial experience-depended dynamic synaptic plasticity.



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Poster

PSTR193. Glia-Neuron Interactions in Physiology

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Topic: B.09. Glial Mechanisms

Support: AFAR Grant MCKNIGHT21003

Title: Reward-based foraging behavior alters microglia-hyaluronan interactions in the ventral tegmental area during healthy brain aging

Authors: *D. T. GRAY[1], A. GUTIERREZ[1], C. CHARWAY[1], L. DE BIASE[2];
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Abstract: Genome-wide association studies in healthy older humans indicate that the gene-expression profile of microglia mediates the relationship between pathophysiology and cognitive decline in the healthy aged brain. This suggests that microglial physiology impacts a brain's ability to adapt to vulnerabilities in function that arise across the lifespan. Brain aging is accompanied by region-specific changes in microglial density and inflammatory profile, and one region in which microglial aging phenotypes emerge relatively early in the aging progression is in the ventral tegmental area (VTA). Recently, hippocampus microglia have been shown to respond to activity-dependent cytokines released by neurons by secreting matrix metalloproteinases and other enzymes that degrade extracellular matrix (ECM) structures to facilitate experience-dependent structural plasticity. Whether behavior-induced neuronal activity results in a similar remodeling of microglia-ECM interactions in the VTA, and whether these dynamics change across the lifespan in the absence of disease is not known. To this end, we have developed a reward-based foraging task in mice that is sensitive to age-associated changes in reward-driven aspects of behavior. Immunohistochemical and imaging-based analyses of the ECM scaffold hyaluronan indicates that behavioral engagement results in a reduction of hyaluronan density in both adult and late-middle-aged mice. In the middle-aged mice, this reduction coincides with a significant decrease in hyaluronan fragment size that was not observed in young animals. Assessments of microglial tissue coverage from the same brain sections suggest that behavioral engagement results in an increase in microglial territory in young mice that is significantly attenuated in middle-aged mice. Conversely, behavioral engagement results in an increase in microglia density in middle-aged mice that is not observed in young mice. Together, these results indicate that VTA microglia in older animals do not respond to behavior in the way that young VTA microglia do, and one possible contributor to this difference is the impact of reduced hyaluronan fragment size as there is evidence that

smaller hyaluronan fragments induce proinflammatory phenotypes in immune cells throughout the body. Future experiments will use direct manipulations of hyaluronan abundance and size alongside manipulations of microglial properties to test causality in these relationships.

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Poster

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U.S. Department of Veterans Affairs N2864-C
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Alzheimer's Association Award ABA-22-965518

Title: Characterization of endogenous extracellular matrix in 3D primary cortical cell culture

Authors: *A. DREXLER[1,2], S. BROWN[1,2], D. A. BORTON[1,2,3,4];
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Abstract: Reproducible and representative *in vitro* models of the brain may provide substantial insight into the etiology of neurological and neurodegenerative disease. However, methods to model the mammal brain, its complex activity and cellular diversity, *in vitro* are inherently challenged as such models must mimic *in vivo* intercellular interactions within the brain across three-dimensional volumes and long periods of time. Cell-to-cell and cell-to-extracellular matrix (ECM) interactions within an *in vitro* neuro-microenvironment are essential for maintaining homeostasis and promoting cellular development. We have developed a 3D primary cortical cell culture model (microtissue) that contains endogenous ECM. Using immunohistochemical analysis, characterized cell-type diversity of the microtissue and cell-type dynamics over a long-term culture of 60 days. In this study, we focused primarily on characterizing the ECM, namely laminin, collagen IV, proteoglycans, and glycoproteins, as they provide structural and homeostatic support in cortical tissue. We evaluated the presence of these components and how they change over the life of the microtissue. In addition, we tracked the presence and morphological changes in neurons, astrocytes, and microglia. The evolving presence of these cell types in our microtissues over the course of the 2-month culture speaks to the stability of our model and enables the evaluation of a heterogeneous cell population during microtissue development.

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Poster

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Topic: B.09. Glial Mechanisms

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Owens Family Foundation

Title: P2ry12 deficiency results in sex-specific locomotory hyperactivity in adult female mice

Authors: *O. J. UWERU[1], K. A. OKOJIE[1], A. TRIVEDI[1], J. BENDEROTH[1], L. S. THOMAS[2], G. DAVIDSON[1], K. COX[1], U. B. EYO[1];
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Abstract: P2RY12 receptor is evolutionarily conserved, highly, and selectively expressed by microglia in the homeostatic brain. Recent studies have shown that this receptor provides directionality for microglia process movement and mediates crosstalk between microglia and neurons in the basal state. Thus, P2RY12-mediated microglia-neuron crosstalk may undergird normal brain function. However, there is paucity of literature on the physiological significance of P2RY12-mediated microglial functions at the behavioral level, especially across sex. Accordingly, we investigated the behavioral consequences of a genetic deletion of this receptor to ascertain its physiological importance. First, using flow cytometry to profile P2RY12 expression from development to adulthood, we find a temporal increase and sex-age interaction with adult females showcasing higher expression of the receptor than males in wildtype mice. Given the scarcity of literature on microglia in deep brain structures like the striatum, we wondered how a P2RY12 deficiency might impact their morphology in the hippocampus-CA1 and dorsal striatum (DS). Our 3D Sholl analysis shows significant sex-genotype interactions in both regions with dramatic de-ramification of P2RY12-deficient microglia in females but not males when compared to their littermate P2RY12-sufficient counterparts. Also, using the Golgi technique to assess dendritic spine density, we find significant sex-genotype interaction in both regions with P2RY12-deficient females showcasing a reduction in spine density in the DS when compared to their P2RY12-sufficent counterparts. Because of the critical role of the DS in the control of movement, we wondered how these P2RY12-deficient mice might perform in the open field locomotory test. With the goal being to examine their basal locomotory behavior, the open field test was conducted over the course of two days with day one serving a habituation purpose, which presumably should allow the animals to exhibit their naturalistic locomotion on day two. Despite a lack of gross motor balance and coordination abnormality in the accelerated rotarod test, P2RY12-deficient females, but not males, show hyper-locomotory behavior when compared with their littermates P2RY12-sufficient counterparts. Further, we find that the P2RY12-deficient females showcase impaired habituation and increased velocity. Consequently, we hypothesize that microglial perturbation induced by a P2RY12 deficiency triggers dysfunctional inhibitory systems in a sexually dimorphic manner. Collectively, our data suggest a sex-specific role of microglia P2RY12 in the regulation of basal locomotory behavior.

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Poster

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Topic: B.09. Glial Mechanisms

Support: Programa de fortalecimiento de institutos, centros y laboratorios de investigación 2023

Title: Acute stress differentially affects the expression of c-Fos in neurons and glia in stress-sensitive brain regions

Authors: *A. N. AGUILAR DELGADILLO, F. CRUZ MENDOZA, N. L. HERRERA LOZA, S. LUQUIN DE ANDA, Y. RUVALCABA-DELGADILLO, F. JÁUREGUI-HUERTA; Univ. De Guadalajara, guadalajara, Mexico

Abstract: Stress involves a challenge to homeostasis that triggers a set of reactions allowing the organism to respond in the most adaptative way posible. Acute stress is capable of activating multiple stress-sensitive brain regions that can activate circuits and impact organism adaptation. c-Fos, a product of the c-fos gene, is a protein frecuently used as a marker of activation in the study of the effects of external factors on the central nervous system. However, most of the changes in c-Fos expression have been attributed to neurons, ignoring the dynamics of activation of other brain cell lineages. Despite this, mounting evidence supports the notion tha glial cells also express c-fos. Therefore, we studied the proportional differences of neurons and glia in the expression of c-Fos in stress-sensitive brain regions. Four male Wistar rats were exposed to varied acute stress model and sacrificed two hours after the initiation of the stimulus. The obtained tissue was processed by fluorescent immunohistochemistry on 35-micrometer sections for c-Fos with GFAP, Olig-2, Ng2, Iba-1 and Neun in the medial prefrontal cortex, claustro, lateral septum, paraventricular nucleus of the hypothalamus, amygdala and hippocampus. We demonstrated a differential expression of c-Fos in neurons and glia, with variable proportionality in the different stress-sensitive brain regions. This evidence reinforces the idea of considering c-Fos as a marker of activation not exclusive to neurons, contributes to the understanding of activation dynamics, and enhances the comprehensive perspective in brain activity studies.

Disclosures: **A.N. Aguilar Delgadillo:** None. **F. Cruz Mendoza:** None. **N.L. Herrera Loza:** None. **S. Luquin de Anda:** None. **Y. Ruvalcaba-Delgadillo:** None. **F. Jáuregui-Huerta:** None.

Poster

PSTR193. Glia-Neuron Interactions in Physiology

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR193.17/E29

Topic: B.09. Glial Mechanisms

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NSERC (RGPIN-2015-05571)
Plum Foundation (2017-19, 2020-22)

Title: Effects of sleep deprivation on somatic appositions of GABA transporters and inhibitory transmission in orexin and MCH neurons in the lateral hypothalamus

Authors: *T. GOLOVIN[1], M. HIRASAWA[3], K. SEMBA[2];
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Abstract: Orexin (ORX) and melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus (LH) promote wakefulness and sleep, respectively. Their complementary roles in sleep regulation may involve astrocytic modulation of synaptic transmission to these neurons. We previously showed that sleep deprivation (SD) for 6 h bidirectionally altered the apposition of primarily astrocytic glutamate transporter-1 with ORX and MCH neurons, which differentially modulated excitatory transmission to these functionally discrete neurons (Briggs et al., 2018, J Neurosci). ORX and MCH neurons also receive GABAergic inputs including those from sleep-promoting neurons to ORX neurons. Thus, we investigated whether GABA transporters (GATs) modulate GABAergic transmission to ORX and MCH neurons and whether this is altered by SD, by examining GAT appositions and inhibitory transmission to these neurons.
Adult male rats were sleep deprived or left undisturbed (Rest) for 6 h at ZT0-6, followed by double-label immunohistochemistry, confocal imaging, and automated apposition analysis, or patch-clamp recordings. We first confirmed that neither GAT1 nor GAT3 colocalized with VGAT, indicating that both are astrocytic in the LH. After SD, the density of GAT1/GAT3 appositions (count per 10 µm perimeter length) on ORX and MCH neurons did not change. However, in ORX but not MCH neurons, SD reduced the mean apposition length with GAT1, but not GAT3, by 18.7%. Overall, there was a 14.9% decrease in the percentage of ORX cell perimeter that was in apposition with GAT1. To investigate the functional consequence of this reduction in GAT1-ORX apposition, we recorded from ORX neurons of both D- and H-subtypes. Surprisingly, SD affected neither evoked nor spontaneous inhibitory transmission in either ORX neuron subtype. However, co-application of GAT1- and GAT3-specific inhibitors, but not each alone, reduced evoked IPSC amplitude.
Overall, SD selectively reduced GAT1 apposition with ORX neurons, while it had no effects on GAT3 or MCH neurons. Interestingly, this anatomical plasticity of GAT1 was not associated with any changes in basal inhibitory transmission to ORX neurons. This is likely due to a compensatory action of GAT3 when GAT1 apposition is reduced. It is possible that the previously shown presynaptic inhibition of glutamatergic transmission is sufficient to regulate ORX neuron excitability for sleep homeostasis.

Disclosures: **T. Golovin:** None. **M. Hirasawa:** None. **K. Semba:** None.

Poster

PSTR193. Glia-Neuron Interactions in Physiology

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NIPS EM Facility

Title: Sleep history-dependent astrocytic structural remodeling at synapses to orexin neurons in the lateral hypothalamus

Authors: *K. SEMBA[1], T. GOLOVIN[2], C. BRIGGS[1], J. BURNS[1], S. HATADA[3], S. DEURVEILHER[1], Y. KUBOTA[3,4];
[1]Med. Neurosci., [2]Physiol. & Biophys., Dalhousie Univ., Halifax, NS, Canada; [3]Natl. Inst. Physiol. Sci. (NIPS), Okazaki, Japan; [4]RIKEN CBS, Wako, Japan

Abstract: Sleep-wake cycles are regulated by alternate activation of sleep- and wake-promoting neurons. We previously showed that astrocytes dynamically regulate excitatory transmission to wake-promoting orexin (ORX; also known as hypocretin) neurons and sleep-promoting melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus by modulating glutamate transporter-1 distribution and glutamate uptake in a cell type-specific manner and according to sleep history (Briggs et al., 2018, J Neurosci). One possible mechanism for this synaptic plasticity is structural remodeling of perisynaptic astrocyte processes, such as protrusion and withdrawal at the synapse.
To investigate this possibility, we conducted serial 3D reconstruction of ORX neurons with correlative light-electron microscopy using hypothalamic sections from adult male rats that were either sleep deprived (sleep deprivation, SD) or left undisturbed (Rest) for 6 h from ZT1-7 (n=2 per group). A total of 11 ORX neurons (n=5 SD; n=6 Rest), each including the soma typically with proximal dendrite(s), were serially reconstructed, and astrocytic processes at synapses were quantitatively analyzed.
We found that overall synaptic density (count/area) did not change between Rest and SD conditions, but that synaptic densities were 3-fold higher on dendrites than on somata of ORX neurons. The vast majority (94%) of the synapses on ORX neurons were associated with astrocytic processes at Rest. After SD, the number of synapses contacted by astrocytes decreased substantially (to 82%). This decrease in the number of synapses contacted by astrocytes was

accompanied by a reduction in astrocytic coverage (% of cleft edge with astrocytic process) per synapse with SD. Astrocytic coverage per synapse, however, was not correlated with the distance between astrocytic processes and the synaptic cleft, which only decreased at somatic synapses after SD.
These results indicate that astrocytic processes at synapses to wake-promoting ORX neurons show dynamic structural remodeling, with some variation for somatic and dendritic synapses, following 6 h of SD, a procedure that also induced presynaptic inhibition of glutamatergic transmission to ORX neurons (Briggs et al., 2018). We propose that structural remodeling of perisynaptic astrocytes represents a cellular mechanism for sleep homeostasis.

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Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

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Topic: C.01. Brain Wellness and Aging

Support: NSF BOI/IOS 1556968 to TST
NSF BOI/IOS 2034864 to TST
NJ Governor's Council for Medical Research and Treatment of Autism CAUT22AFP008 to JD
RU-GSN Dean's Dissertation Fellowship to JB

Title: Secreted semaphorin signaling regulates aging-associated dendritic modifications and cognitive function

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[1]Biol. Sci., Rutgers Univ., Newark, NJ; [2]Col. of Arts and Sci., Heritage Univ., Toppenish, WA

Abstract: There is accumulating evidence of a significant loss of neuronal dendritic complexity that occurs with age, which may account for the synaptic loss and cognitive decline seen in aged mammals, including humans. However, the molecular mechanisms underlying these changes are unclear. Some of the molecular players involved in dendritic morphogenesis are the class 3 secreted semaphorins. In development, semaphorin 3A (Sema3A) signals through its receptors neuropilin (Nrp)1/plexin (Plxn)A4 to induce dendritic elaboration in layer V cortical neurons, while Sema3F signals with Nrp2/PlxnA3 to induce spine pruning on the apical dendrite of the same neurons. Interestingly, Sema3A and 3F are also expressed in adult and aged rodent and human cortices, but their role in dendritic remodeling in aging is unknown. To address this, we established an *in vitro* model of aging mouse primary cortical neurons and examined the effects of Sema3A and 3F in dendritic remodeling in aged versus young neurons. First, we confirmed

that significantly more long-term (15 & 30 days *in vitro*, DIV) cultured neurons express the aging-associated senescence marker β-gal compared to 5DIV neurons. Next, we demonstrated that treating with either Sema3A or Sema3F induces significantly more dendritic branching starting at 5DIV and 15DIV, but not by 30DIV. This suggests changes in the cellular response to Sema3A and 3F with time in culture, and possibly with aging. In addition, 30DIV neurons treated with equal amounts of both Sema3A and Sema3F simultaneously had a significant increase in dendritic branching compared to single treatments, indicating a synergistic effect of the two ligands at the aged timepoint. This pathway may be a therapeutic target for loss of dendritic elaboration seen with age. Furthermore, we see a significant decrease in PlxnA4 receptor expression in wildtype (WT) cortical lysates from aged 24-month-old mice compared to 1-month-old mice, suggesting a role for semaphorin-plexin signaling in the loss of dendritic elaboration seen with age. Finally, aged WT and adult *Plxna4*$^{-/-}$ mice were challenged with a reversal learning task to assess memory and cognitive flexibility. We found that both adult *Plxna4*$^{-/-}$ and aged WT mice perform significantly worse than adult WT mice, suggesting PlxnA4 is required in aged and developing animals for cognitive function. Collectively, our study provides novel insights into the involvement of semaphorin-plexin signaling in the loss of dendritic complexity seen with aging, which may lead to cognitive decline. This work may serve as a platform for future experiments to investigate the role of semaphorin signaling in neurodegenerative diseases.

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Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.02/E32

Topic: C.01. Brain Wellness and Aging

Support: Israel science foundation 828/21

Title: Neuronal SMC3 regulates weight, body composition, and hormonal balance in parallel with sex-dependent effects on anxiety behavior

Authors: *N. SALEEV, D. GETSELTER, E. ELLIOTT;
Medecine faculty, Bar-Ilan Univ., Safed, Israel

Abstract: SMC3 is a major component of cohesin complex that regulates higher-order chromatin organization and gene expression. Human genetic studies reveal that de novo mutations in *SMC3* gene, found in patients with Cornelia de Lange syndrome (CdLs). This syndrome characterized by intellectual disabilities, and behavioral patterns as self-injury. Nonetheless, little is known about the exact role of SMC3 in neuronal maintenance and gene expression especially in adulthood. This study aimed to determine the role of SMC3 in adulthood

brain, using *in-vivo* models of adulthood excitatory neuron SMC3 knockout in male and female mice. Neuron-specific SMC3 knockout mice displayed dysregulated anxiety-like behavior and self-injury in males and females compared to wild-type littermates. Of interest, female knockouts displayed less anxiety while males displayed more anxiety, although both displayed self-injury, a known phenotype in the human condition. In parallel, significant metabolic changes were displayed in both male and female mice, including overweight phenotype, loss of muscle mass, differences at respiratory exchange, heat production and hormonal changes after knockout of SMC3 gene in excitatory neuron cells of adult brain. RNA-seq in the hypothalamus reveals changes in several peptides that moderate proper hormonal balance. This is interesting, considering reports of adultness obesity in a subset of individuals with CdLs.
This knowledge provides a novel basis for potential treatment or improvement of quality of life for diagnosed people with CdLs or people who diagnosed with side effects of instable SMC3 function.

Disclosures: **N. Saleev:** None. **D. Getselter:** None. **E. Elliott:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

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Topic: C.01. Brain Wellness and Aging

Support: JSPS KAKENHI Grant 23H02633
JST Grant JPMJSP2130

Title: Reelin regulates lipid composition of the neuronal plasma membrane

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Abstract: The plasma membrane of neurons is composed of a variety of lipid molecules that have distinct biophysical characteristics and functions. For example, sphingomyelin (SM) constitutes a small fraction called lipid rafts that affect the localization and function of various proteins, including GPI-anchored proteins. Therefore, elucidation of the mechanisms that regulate the lipid composition of the neuronal plasma membrane is important for understanding the mechanisms that control neuronal morphology and information transmission. Reelin is a large secreted protein expressed mainly in the central nervous system. Reelin is important for neuronal migration, synaptic plasticity, spine formation, and so on. We have previously found that the lipid composition of the embryonic brain of Reelin-deficient mice is different from that of the wild type. In addition, previous studies using non-neuronal cells have shown that the activation of tyrosine kinase Fyn (also activated by Reelin in neurons) increases the amount of

SM in the plasma membrane. It is possible that Reelin affects the SM amount of the neuronal plasma membrane. Here we aim to clarify the effect of Reelin on the lipid composition of the neuronal plasma membrane. Primary cultured neurons treated with Reelin were more strongly stained by Equinatoxin2-GFP, an SM-binding probe, than neurons without Reelin, indicating that Reelin increases the amount of SM in the plasma membrane. Reelin also increased the amount of GPI-anchored protein expressed on the plasma membrane. Non-targeted analysis of lipid components in extracted postsynaptic density regions revealed that the composition of phosphatidylcholine, ceramide, and diacylglycerol, which are lipid species involved in SM synthesis and degradation, was altered in Reelin-deficient mice. These results suggest that Reelin increases the amount of SM in the plasma membrane and affects neuronal function. We are currently exploring the detailed molecular mechanisms by which Reelin increases SM on the neuronal plasma membrane. In addition, we have comprehensively analyzed the distribution of lipid molecules in the mice brains using mass imaging techniques and found that certain lipid molecules have a characteristic distribution in the brain of wild-type mice. We are currently analyzing whether the distribution of these lipid molecules is altered in Reelin-deficient mice.

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Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

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Program #/Poster #: PSTR194.04/E34

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant R01AG066027
NIH Grant U19MH114830

Title: Cell-type specific molecular signatures of aging revealed in a brain-wide transcriptomic cell type atlas

Authors: *K. JIN, Z. YAO, C. T. J. VAN VELTHOVEN, E. S. KAPLAN, J. GOLDY, K. RONELLENFITCH, M. REDDING, M. DESIERTO, A. RUIZ, B. LEVI, N. DEE, L. ESPOSITO, K. A. SMITH, B. TASIC, H. ZHENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Biological aging can be defined as a gradual loss of homeostasis across various aspects of molecular and cellular function. Aging is a complex and dynamic process which influences distinct cell types in a myriad of ways. The cellular architecture of the mammalian brain is heterogenous and diverse, making it challenging to identify precise areas and cell types of the brain that are more susceptible to age than others. Here, we present a high-resolution single-cell RNA sequencing dataset containing over 1.2 million high-quality single-cell transcriptomic profiles of brain cells from young adult (2 months old) and aged (18 months old)

mice across both sexes, including areas spanning the forebrain, midbrain, and hindbrain. We find age-associated gene expression signatures across nearly all 130+ neuronal and non-neuronal cell subclasses we identified. We detect the greatest numbers of gene expression changes in non-neuronal cell types, suggesting that different cell types in the brain vary in their susceptibility to aging. We identify specific, age-enriched clusters within specific glial, vascular, and immune cell types from both cortical and subcortical regions of the brain. We also identify genes with expression changes across multiple cell subclasses, pointing to certain mechanisms of aging that may occur across wide regions or broad cell types of the brain. Finally, we discover the greatest gene expression changes in cell types localized to the third ventricle of the hypothalamus, including tanycytes and *Tbx3*+ neurons found in the arcuate nucleus that are part of the neuronal circuits regulating food intake and feeding behavior. These findings suggest that the area surrounding the third ventricle in the hypothalamus may be a hub for aging in the mouse brain. In summary, we reveal a dynamic landscape of cell-type-specific transcriptomic changes associated with normal aging in the brain that will serve as a foundation for the investigation of functional changes in the aging process and the interaction of aging and diseases.

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Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

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Program #/Poster #: PSTR194.05

Topic: C.01. Brain Wellness and Aging

Support: NIH R00AG061231

Title: A new variant of AQP4 water channel attenuates neuromyelitis optica and edema pathology

Authors: H. LALAMI[1], P. SURESH[1], Z. SHI[1], M. LEVY[2], B. GREENBERG[3], J. D. DOUGHERTY[4], ***D. SAPKOTA**[5];
[1]Biol. Sci., Univ. of Texas at Dallas, Richardson, TX; [2]Neurol., Harvard Med. Sch., Boston, MA; [3]UT Southwestern, Dallas, TX; [4]Genet. and Psychiatry, Washington Univ. Sch. of Med., Saint Louis, MO; [5]Biol. Sciences, Neurosci., Univ. of Texas, Dallas, Richardson, TX

Abstract: A new variant of AQP4 water channel attenuates neuromyelitis optica and edema pathology
Aquaporin 4 (AQP4) is a water channel expressed in astrocytes, with roles in the maintenance of water balance, clearance of waste products, and pathogenesis of various brain disorders. Recently, we identified a novel variant of AQP4, termed AQP4X, which has an extended C-

terminus resulting from stop codon readthrough of the *Aqp4* transcript and localizes exclusively at the blood-brain barrier. Using the $Aqp4^{No\_X}$ mouse line that specifically lacks AQP4X, we demonstrated that this variant facilitates the clearance of amyloid beta from the brain. Here, we generated the $Aqp4^{All\_X}$ mouse model that overexpresses AQP4X and used it to unravel additional roles of this protein variant. Remarkably, the $Aqp4^{All\_X}$ mouse exhibits diminished reactivity to sera from patients with neuromyelitis optica, a demyelinating disorder caused by autoantibodies targeting AQP4. Biochemical investigations reveal that the $Aqp4^{All\_X}$ mouse has a significant reduction in the formation of supramolecular complexes that AQP4 is known to form in astrocyte membranes. Furthermore, the $Aqp4^{All\_X}$ mouse displays attenuated edema pathology in comparison to wildtype and $Aqp4^{No\_X}$ mice. Our findings highlight AQP4X as a potential therapeutic target for managing neuromyelitis optica, as well as conditions like stroke and brain injuries that can lead to brain edema.

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Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

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Topic: C.01. Brain Wellness and Aging

Support: AHA Fellowship 903523
NINDS Grant R03NS095063
NINDS Grant R01NS102337

Title: Methylprednisolone rescues inherent barrier defects of Friedreich's Ataxia blood-brain barrier model

Authors: *F. SMITH, D. KOSMAN;
Biochem., State Univ. of New York, Buffalo, Buffalo, NY

Abstract: Methylprednisolone rescues inherent barrier defects of Friedreich's Ataxia blood-brain barrier model Frances M. Smith and Daniel J. KosmanDisclosures: FMS; none, DJK; none. Friedreich's Ataxia (FRDA) is the most inherited ataxia, affecting ~1:50,000 US citizens. FRDA is caused by GAA expansion repeats in the first intron of the Frataxin (FXN) gene, causing transcriptional repression and expression deficit of this essential mitochondrial iron chaperone protein. Patients first experience a burst of neurodegeneration to the Cerebellar Dentate Nuclei and the Dorsal Root Ganglion, with *progressive* brain iron accumulation in the former. That the brain iron accumulation is progressive and mirrors the decline in patient quality of life before premature death, we question if the blood-brain barrier is responsible for aberrant iron influx to the patient brain, leading to neuroinflammation. Brain iron accumulation and degradation of the blood-brain barrier is indeed seen in other neurodegenerative disorders including Alzheimer's

and Parkinson's diseases. Individual cells of the blood-brain barrier rely on the cytoskeleton to anchor tight junction proteins for proper sealing of adjacent cells and the formation of a paracellularly *impermeable* barrier. In the early 2000s, cytoskeletal defects were identified in FRDA patient fibroblasts, but unfortunately the extent of the knowledge of how this affects cell pathology ended there. We have previously identified that Frataxin-deficient (shRNA) human brain microvascular endothelial cells (hBMVEC), an *in vitro* blood-brain barrier model, do indeed display inherent barrier defects, including: 1) loss of whole-cell F-actin, 2) loss of F-actin at the cell membrane, 3) tight junction protein deficit, and 4) increased paracellular barrier permeability. Methylprednisolone (MPO) is a corticosteroid that stabilizes tight junctions, and treatment of our shFXN hBMVEC shows improvement of barrier physiology including: 1) increased tight junction production at both the transcriptional and translational levels, and 2) decreased paracellular permeability. We are particularly interested in MPO as a small clinical trial dosing FRDA patients with MPO reported clinical improvement in the timed 1-minute walk. Therefore, we propose that the FRDA BBB has inherent barrier defects, but treatment with MPO stabilizes BBB physiology, and is potentially neuroprotective.

Disclosures: F. smith: None. **D. Kosman:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.07/E36

Topic: C.01. Brain Wellness and Aging

Support: NIDA/NIH IRP

Title: Differential steady state expression of KDEL receptor isoforms and their influence on protein homeostasis

Authors: *L. K. GREER, E. S. WIRES, K. A. TRYCHTA, C. T. RICHIE, B. K. HARVEY; NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD

Abstract: The endoplasmic reticulum (ER) is the site of many cellular processes crucial to maintaining homeostasis, including protein synthesis, lipid metabolism, and intracellular calcium storage. Resident proteins localized to the ER lumen carry out these processes and are retained in the ER through interactions with the Golgi-localized, transmembrane protein, KDEL receptor (KDELR). Our lab has previously demonstrated that when ER calcium stores are depleted, ER luminal proteins are secreted out of the ER, overwhelming the KDEL receptors, and are released into the extracellular space in a process called exodosis. ER calcium depletion occurs in neurological diseases such as ischemic stroke, Parkinson's disease, and substance abuse disorders, therefore, understanding the underlying factors that modulate exodosis may provide insight into new therapeutic strategies. Our lab has previously reported that overexpression of KDELR isoforms, KDELR1 and KDELR2, attenuates exodosis, though the isoforms are

differentially expressed between cell line and tissue types. In addition, KDELR2, but not KDELR1 transcript expression is increased in response to activation of the unfolded protein response. To better understand the biology of KDEL receptors and their subsequent role in modulating exodosis, we further investigated the differences between KDELR isoforms. We identified isoform-specific steady-state differences and narrowed down the transmembrane domain responsible for these differences. Modification or swapping of the transmembrane domain altered ability of KDELRs to attenuate exodosis. The KDEL receptors' contribution to proteostasis in the context of ER calcium dysregulation is relatively unexplored in neurological diseases and we report new insights into isoform specific features of KDEL receptors and their role in modulating proteostasis.

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Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

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Program #/Poster #: PSTR194.08/E37

Topic: C.01. Brain Wellness and Aging

Support: JSPS KAKENHI Grant Number JP21K05296

Title: Humanin, a bioactive peptide,suppressescellular senescence in neurons

Authors: A. KOZUKA, ***T. NIIKURA**;
Sophia Univ., Tokyo, Japan

Abstract: Humanin (HN), a 24-residue polypeptide, suppresses amyloid beta-induced neuronal death in vitro and ameliorates memory deficit of Alzheimer's disease (AD) mouse models. We recently found that HN directly enhances exocytosis in neuronal cells and increases acetylcholine level in hippocampus in normal young mice, suggesting that HN functions as a modulator of neurotransmitter release under the normal physiological condition. HN is a secretive peptide and the HN level in circulation decreases age-dependently. It is thus assumed that the change in HN level is implicated in aging-associated cognitive decline. Cellular senescence contributes to tissue aging as well as progress of age-associated diseases such as AD. In this study, we assessed the effect of S14G-HN, a highly potent HN derivative, on cellular senescence in neurons. In primary mouse cortical neurons, long-term in vitro culture increased the number of β-galactosidase (SA-β-gal) positive cells, a marker of senescence. S14G-HN attenuated the number of positive cells. S14G-HN also reduced the number of SA-β-gal positive cells in primary chromaffin cells, which are widely used as neuroendocrine cells. To test the effect of HN on neurotransmitter release, we used rat pheochromocytoma PC12 cells as a neuronal cell model. Glutamate treatment caused cell senescence in differentiated PC12 cells. S14G-HN dose-dependently suppressed the increase in the number of SA-β-gal positive cells. In glutamate-

induced senescent cells, acetylcholine-evoked dopamine release was significantly attenuated. Fifteen minutes pretreatment of S14G-HN enhanced acetylcholine-induced dopamine release in neuronal differentiated PC12 cells but not in glutamate-induced senescent cells. These results suggest the potential role of HN in suppressing aging-related cellular senescence.

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Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

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Topic: C.01. Brain Wellness and Aging

Support: The National Institute on Aging (1RF1AG068292)
The Glenn Foundation and the American Federation for Aging Research (AFAR BIG21042).

Title: The role of G-quadruplex helicase DDX5 in astrocytic senescence

Authors: *V. M J[1], E. WHEELER[2], N. TANDON[2], D. MONCHAUD[3], A. S. TSVETKOV[1,4,5]; [1]Neurol., Univ. of Texas Hlth. Sci. Center, Houston, Houston, TX; [2]Neurosurg., Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [3]ICMUB CNRS, Inst. de Chimie Moléculaire de l'Université de Bourgogne, Dijon, France; [4]The Univ. of Texas Grad. Sch. of Biomed. Sci., Houston, TX; [5]UTHealth Consortium on Aging, The Univ. of Texas McGovern Med. Sch., Houston, TX

Abstract: Cellular senescence is characterized by DNA damage, chromatin remodelling, dysfunctional autophagy, and metabolic reprogramming. In brain cells, senescence plays a crucial role in aging and age-related brain disorders. During aging, neurons and astrocytes are subjected to different stresses (reactive oxygen species, DNA damage, protein aggregation, and dysregulated gene expression) that contribute to senescence. However, the mechanistic link between stress and senescence remains to be understood, as well as the differences in pathways between healthy aging and pathological conditions. Guanine-rich DNA and RNA can fold into non-canonical four-stranded structures called G-quadruplexes (G4s, G4-DNA, G4-RNA). G4-DNA plays important roles in transcription and replication, among other mechanisms, while G4-RNA regulates various RNA functions including translation. Stabilized G4-DNA induces genomic instability, whereas stabilized G4-RNA disrupts RNA-dependent processes, leading to cellular senescence. We found that brain samples from aged mice contain more G4s than those of young mice. Mice treated with a small molecule G4 stabilizer develop cognitive impairment and accelerated brain aging. Senescent astrocytes contain more G4-RNA compared to non-senescent astrocytes. DDX5 is an ATP-dependent G4 helicase that unfolds G4-DNA and G4-RNA inside the cells. Here, we demonstrate that DDX5 is downregulated in astrocytes in a mouse model of tauopathy—aged Tau P301S mice—compared to aged control mice, and also in cultured senescent human astrocytes. We hypothesize that reverting G4 stabilization by increasing DDX5

will prevent/reduce senescence phenotypes in human astrocytes. Our results demonstrate an important G4-dependent molecular mechanism of aging and senescence in the brain. These studies will create a strong foundation to decode the vital functions of G4-DNA, G4-RNA, and G4 helicase DDX5 in regulating cellular senescence and brain aging.

Disclosures: V. M j: None. **E. Wheeler:** None. **N. Tandon:** None. **D. Monchaud:** None. **A.S. Tsvetkov:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.10/E39

Topic: C.01. Brain Wellness and Aging

Support: NS105638
AG073994

Title: Gaba signaling triggered by tmc-1/tmc delays neuronal aging by inhibiting the pkc pathway in c. elegans

Authors: *J. WU, L. WANG, D. KO, D. YAN;
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Abstract: Aging causes functional decline and degeneration of neurons and is a major risk factor of neurodegenerative diseases. To investigate the molecular mechanisms underlying neuronal aging, we developed a new pipeline for neuronal proteomic profiling in young and aged animals. While the overall translational machinery is down-regulated, certain proteins increase expressions upon aging. Among these aging-up-regulated proteins, the conserved channel protein TMC-1/Tmc has an anti-aging function in all neurons tested, and the neuroprotective function of TMC-1 occurs by regulating GABA signaling. Moreover, our results show that metabotropic GABA receptors and G protein GOA-1/Goα are required for the anti-neuronal aging functions of TMC-1 and GABA, and the activation of GABA receptors prevents neuronal aging by inhibiting the PLCβ-PKC pathway. Last, we show that the TMC-1-GABA-PKC signaling axis suppresses neuronal functional decline caused by a pathogenic form of human Tau protein. Together, our findings reveal the neuroprotective function of the TMC-1-GABA-PKC signaling axis in aging and disease conditions.

Disclosures: J. Wu: None. **L. Wang:** None. **D. Ko:** None. **D. Yan:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.11/E40

Topic: C.01. Brain Wellness and Aging

Support: NIG-JOINT
Hoansha
The Japan foundation for Aging and Health
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TMU strategic research fund for social engagement

Title: The depletion of axonal mitochondria impairs autophagy via eIF2 signaling

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Abstract: Mitochondria play critical roles in cell survival, such as energy production and signaling. In neurons, mitochondria are actively transported to the axon to meet local energy demands, while mitochondrial transport declines in aging. The number of functional mitochondria decreases in synapses in the brains of patients suffering from neurodegenerative diseases, and the genetic depletion of axonal mitochondria causes neurodegeneration. In several neurodegenerative diseases, the accumulation of abnormal proteins and declines in protein degradation pathways, such as autophagy, are thought to contribute to neurodegeneration. However, the molecular links between the mislocalization of mitochondria and protein degradation pathways are not understood.Here we report that disruption of eIF2 signaling mediates proteostasis collapse and neuronal dysfunction in the brains with the depletion of axonal mitochondria. Neuronal knockdown of milton, an adaptor protein for axonal transport of mitochondria, causes mitochondria depletion from the axon and causes age-dependent neurodegeneration in *Drosophila*. We found that ubiquitinated proteins are accumulated, and autophagy was impaired, in the brains with neuronal knockdown of milton. Autophagic impairment precedes neuronal dysfunctions and neurodegeneration, suggesting their causative roles in deleterious phenotypes caused by axonal depletion of mitochondria. Proteome analysis revealed that milton knockdown significantly affects expression of proteins associated with translational initiation factor eIF2, which regulates switching between global translation and integrated stress response (ISR). We also found that milton knockdown increased eIF2β and decreased eIF2α, accompanied by different patterns in a sucrose gradient ribosome fractionation and lower phosphorylation of eIF2α suggestiong that ISR was dysregulated.We also found that eIF2β overexpression caused decreased eIF2α, impairment of autophagy, and neuronal dysfunctions, as observed with milton knockdown flies. Interestingly, lowering eIF2β expression rescued autophagy impairment and neuronal dysfunction by milton knockdown. These results suggest that loss of axonal mitochondria caused autophagy impairment via eIF2 dysfunction.Our results suggest that mitochondrial mislocalization and eIF2 signaling trigger proteostasis collapse and induce neuronal dysfunctions. These findings enhance our understanding of the molecular mechanisms underlying the onset and progression of age-related neurodegenerative diseases.

Disclosures: **K. Shinno:** None. **E. Suzuki:** None. **Y. Miura:** None. **K. Ando:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.12/E41

Topic: C.01. Brain Wellness and Aging

Title: In vitro model of TMEM106B cytoplasmic aggregation and its effects on nuclear pore complex and nucleocytoplasmic transport

Authors: ***M. DU**[1], L. RUAN[2], L. JIN[2], S. O. VIDENSKY[2], J. D. ROTHSTEIN[2,1];
[1]The Solomon H. Snyder Dept. of Neurosci., [2]Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Pathological protein aggregation is a hallmark of multiple neurodegenerative diseases. Recently, three independent groups characterized a previously unidentified amyloid fibril composed of the C-terminal fragment of transmembrane protein 106B (TMEM106B) in postmortem brain tissue. Intriguingly, TMEM106B amyloids were detected in patients with a range of neurodegenerative diseases as well as neurologically normal patients in an age-dependent manner. TMEM106B regulates intracellular lysosomal function and is reportedly a potent genetic modifier of many neurodegenerative diseases and biological aging. The recent discovery of cytoplasmic TMEM106B aggregates suggests a potential gain-of-function that may affect cellular function more broadly. However, whether TMEM106B aggregation is a benign by-product of aging or an active contributor to neurodegeneration remains unknown. Here, we utilized overexpression (OE) to artificially induce aggregation of the C-terminal fragment of TMEM106B (AA120-274, TMEM-C) in cell models. We showed that this method can rapidly introduce cytoplasmic TMEM106B inclusions that are insoluble, thioflavin-S positive and whose morphology partially resembles what has been observed in postmortem human tissue staining. Interestingly, the induced TMEM106B aggregates are perinuclear and associated with pathologic invaginated nuclear envelop, implying their potential impact on nuclear structure and function. Previously, our lab discovered that pathological aggregates such as tau, mutant huntingtin, and C9orf72 dipeptide repeat proteins could disrupt the nuclear integrity of neurons, as evidenced by reduced number of nuclear pore complex (NPC), sequestration and cytoplasmic mislocalization of specific nucleoporins (NUPs), and compromised nucleocytoplasmic transport (NCT). Using the in vitro aggregation model, we found that, as compared to TMEM106B full-length (TMEM-FL) or mCherry OE control, TMEM-C caused a significant reduction in the level of several NUPs ($p<0.05$), including Nup62 and POM121 previously implicated in Alzheimer's disease and amyotrophic lateral sclerosis. In addition, TMEM-C significantly reduced the nucleus-to-cytoplasm ratio of the endogenous nuclear protein such as TDP-43 ($p<0.05$), suggesting a defect in NCT and/or faithful maintenance of the nucleus-cytoplasm compartmentalization. Ongoing studies are underway to examine if a similar relationship between TMEM106B and the NPC exists in human tissue and neurons. Taken together, our study suggests TMEM106B aggregates

can affect the structural and functional integrity of the nucleus and may have a broader impact on brain aging.

Disclosures: **M. Du:** None. **L. Ruan:** None. **L. Jin:** None. **S.O. Videnskiy:** None. **J.D. Rothstein:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.13/F1

Topic: C.01. Brain Wellness and Aging

Support: NSF Graduate Research Fellowship
NIH R01 NS041435

Title: Mhc class ii induction in oligodendroglia varies with age

Authors: ***R. B. CATENACCI**[1], M. D. SMITH[2], A. RHODES[2], P. A. CALABRESI[2,1], S. PHILLIPS[2];
[1]Solomon H. Snyder Dept. of Neurosci., [2]Dept. of Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: Oligodendrocytes (OLs) and their progenitor cells (OPCs) generate myelin sheaths that enable saltatory conduction along the axon. However, more recent data show that OLs and OPCs play key roles beyond myelination, such as participation in inflammation. Studies of multiple sclerosis (MS) have revealed a subtype of cells known as immune OLs (iOLs) and OPCs (iOPCs), characterized by the expression of major histocompatibility complex (MHC) molecules normally restricted to antigen presenting cells of the immune system. Therefore, it has been suggested that iOPCs/iOLs sit at the nexus of inflammation and demyelination in MS and could be a viable therapeutic target. However, much of the work examining the function of iOPCs/iOLs has been executed *in vitro,* using neonatal or epigenetically reprogrammed cells that lack features of aging, which plays a major role in MS progression. We hypothesized that iOPC/iOL induction may be dependent on age. To test this hypothesis, we isolated O4+ cells from neonatal (p7-9) and young adult (9-12 weeks) mice and treated them with IFNγ to induce iOPCs/iOLs. MHC class II induction as detected by qPCR (fold induction neonatal: 712.6±474.1 adult: 177.5±190.3 p=0.02 2-way ANOVA, n=3) and immunohistochemistry (%MHC class II+ neonatal: 21±3.6 adult: 0.4±0.7, 2-way ANOVA, n=3) was higher in neonatal compared to young adult O4+ cells. MHC class I induction was not significantly different between the two groups (fold induction neonatal: 142.8±83.6 adult: 79.8±1 p=0.17 2-way ANOVA, n=3), suggesting a specific deficit in MHC class II signaling, rather than generalized response to IFNγ. In addition, treating neonatal OPCs with a c-Myc inhibitor previously shown to induce an aged phenotype suppressed MHC class II induction after IFNγ treatment. To determine if oligodendroglia show differences in MHC class II with age *in vivo,* we took advantage of CD74-TdTomato mice previously generated by our lab, in which MHC class II-expressing cells are

labeled with TdTomato (TdT). Comparison of brain and spinal cord from neonatal, young adult, or aged (52 weeks) naïve CD74-TdT mice by immunohistochemistry showed that Olig2+TdT+ cells could be detected only in the spinal cord of aged mice (1.7±1.6% TdT+, n=4). In contrast, Olig2+TdT+ cells were not significantly altered with aging in MHC class I reporter mice, once again illustrating the specificity of the effect of age on MHC class II (young adult: 2.7±1.3%TDT+ aged:1.3±0.3%TdT+, p=0.08, unpaired T-test, n=3). These results contribute to the growing understanding of roles for oligodendroglia outside of myelination and how changes in oligodendroglia across the lifespan can contribute to aging and disease.

Disclosures: R.B. Catenacci: None. **M.D. Smith:** None. **A. Rhodes:** None. **P.A. Calabresi:** None. **S. Phillips:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.14/F2

Topic: C.01. Brain Wellness and Aging

Support: Junior Investigator Research Grant (#957578) by the Texas Alzheimer's Research and Care Consortium
R21 (1R21AG075750) by the National Institute on Aging
Career Development Award (#856061) by the American Heart Association

Title: G-quadruplex stabilization alters cycling expression of the core circadian genes in cultured mouse-derived cerebral endothelial cells

Authors: *S. MONGA[1], B. NOH[1], S. M. RAHMAN[1], Y.-J. LAI[2], J. MORUNO MANCHON[1]; [1]Neurol., UTHealth Sci. Ctr. at Houston, Houston, TX; [2]Solomont Sch. of Nursing, Zuckerberg Col. of Hlth. Sci., Univ. of Massachusetts Lowell, Lowell, MA

Abstract: In mammals, every cell has the core-clock genes that regulate the rhythmic expression of genes, involved in many biological functions, such as transcription regulation, metabolism, DNA repair, and cell cycle. However, the circadian clock function declines with aging, becomes a major risk factor for cerebrovascular function, which is importantly maintained by endothelial cells. Proper function of cerebral endothelial cells (CECs) is critical for the protection of the cerebrovasculature and to maintain the integrity of the blood brain barrier (BBB). Indeed, the permeability of the BBB is dynamically regulated by circadian rhythms. Guanine quadruplex (G4), is a non-canonical secondary structure of DNA and RNA that regulates gene expression and translation. Previously, we observed that 20-months old (m/o) mouse-derived CECs showed enhanced G4s, compared with 4-m/o CECs, suggests G4 formation is associated with aging. Thus, we hypothesized that stabilization of G4s negatively affects circadian clock in an age-dependent manner in cultured CECs. Cultured primary CECs derived from young (4-m/o),

middle aged (9-m/o), and aged (20-m/o) mice were isolated. CECs were synchronized with dexamethasone and treated with a G4-stabilizing agent, pyridostatin (PDS). RNA was isolated from CECs at different time points (24 - 48 h), and the relative expression of core circadian genes (*i.e., Clock, Cry1, Per1, and Arnt1)* was analyzed. We found that the expression of the core circadian genes was significantly altered by PDS in 20-m/o group, but not in young CECs. From RNA-seq analysis shows that among multiple helicases, proteins that unwind G4s, *Ddx39* was significantly downregulated in aged mouse-derived CECs than young CECs. Our data suggest that young CECs are more resilient to G4 stabilization, and that *Ddx39* downregulation contributes to the loss of the ability to resolve G4s with aging in cultured CECs. This study proposes a novel molecular mechanism that may explain the decline of circadian clock in brain vasculature with aging, and it may help to develop therapeutic strategies to prevent/mitigate cerebrovascular problems associated with aging.

Disclosures: S. Monga: None. **B. Noh:** None. **S.M. Rahman:** None. **Y. Lai:** None. **J. Moruno Manchon:** None.

Poster

PSTR194. Molecular and Cellular Changes with Age and Environment

Location: WCC Halls A-C

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Program #/Poster #: PSTR194.15/F3

Topic: C.01. Brain Wellness and Aging

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KIST 2E32211

Title: Investigating the age-dependent features of ER and mitochondria contacts in cortical neurons

Authors: *H. JUNG[1], S. SUGA[2], S. KIM[1,3], K. LEE[4], Y. HIRABAYASHI[2], S.-K. KWON[1,5]; [1]Korea Inst. of Sci. and Technol., Seongbuk-gu, Seoul, Korea, Republic of; [2]Dept. of Chem. and Biotechnology, Sch. of Engin., The Univ. of Tokyo, Tokyo, Japan; [3]Dept. of Systems Biotech., Konkuk Univ., Seoul, Korea, Republic of; [4]Advance Analysis Ctr., Korean Inst. of Sci. and Technol., Seoul, Korea, Republic of; [5]Div. of Bio-Medical Sci. & Technol., Univ. of Sci. and Technol. (UST), Seoul, Korea, Republic of

Abstract: Ca^{2+} ions play important roles for neuronal function, including basal synaptic transmission, synaptic plasticity, and cellular viability. In addition, dysregulation of Ca^{2+} has been known as one of the hallmarks of neuronal aging. The endoplasmic reticulum (ER) and

mitochondria are able to regulate Ca^{2+} level in neuronal dendrites via their contacts. Ca^{2+} can be transferred from the ER to the mitochondria; therefore, tightness of these interactions significantly affect cytosolic Ca^{2+}levels. However, due to the technical limitations, conclusions are controversial and lack consistency. In order to gain a comprehensive understanding of brain aging, it is crucial to elucidate the fundamental features of Ca^{2+} homeostasis mediated by the ER and mitochondria. Here, we applied senescence-associated β-galactosidase as cellular aging markers to define aged neurons. Additionally, we employed Ca^{2+} indicators that specifically target the mitochondria, ER, and cytosol to visualize their Ca^{2+} dynamics. We revealed that aged neurons had reduced mitochondrial Ca^{2+} uptake ability while ER Ca^{2+} dynamics remained unchanged. Furthermore, the amount of cytosolic Ca^{2+} was increased in aged neurons. Based on these findings, further investigations are needed including; (1) comparative analysis of structural changes in neuronal ER-mitochondria contacts between aged and young brains using 3D-electron microscopy, and (2) examination of the causal relationship between the alteration of age-dependent Ca^{2+} dynamics and ER-mitochondria contacts by employing molecular tools for ER-mitochondria tethering. This study will provide reliable information regarding changes in organelle-mediated neuronal Ca^{2+} homeostasis during aging and suggest potential targets for mitigating the effect of brain aging.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.01/F4

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

Support: NIH Grant R01AG061785-A1

Title: Alzheimer's Disease Clock Gene Expression Alterations in Parvalbumin Interneurons

Authors: *M.-M. COOPER[1], E. D. ROBERSON[3], K. L. GAMBLE[2], R. COWELL[4]; [2]Psychiatry, [1]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Neurol., Univ. of Alabama, Birmingham, Birmingham, AL; [4]Southern Res., Birmingham, AL

Abstract: Alzheimer’s disease (AD) features subclinical epileptiform activity predominantly during the inactive circadian phase. Parvalbumin (PV) interneurons are the most abundant interneuron type in the hippocampus and cortex, and therefore could likely contribute to the imbalance in excitation and inhibition. Because epileptiform activity in AD follows a circadian rhythm of expression, we hypothesized that clock gene dysfunction in PV interneurons of the hippocampus and cortex contributes to AD-related hyperexcitability. To begin testing this hypothesis, we asked if there are alterations in the transcription of core clock genes in PV interneurons in the hippocampus and cortex of the hAPPJ20 mouse model of AD. Mice were

entrained using controlled lighting and brains were collected after 2 days of constant darkness across 6 circadian timepoints. RNAscope was then used to measure the gene expression of the chosen clock genes within the PV cells in our respective regions of interest. Our results aim to confirm the existence of clock gene alterations within PV cells in order to elucidate the connection between circadian dysfunction and epileptiform activity in AD. Future studies include the analysis of additional clock genes and regions of interest.

Disclosures: **M. Cooper:** None. **E.D. Roberson:** 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.; R01AG061785-A1. F. Consulting Fees (e.g., advisory boards); AGTC, Lilly, Genetech. Other; Editorial board of Journal of Neuroscience. **K.L. Gamble:** 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 funded by R01AG061785-A1. **R. Cowell:** 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.; 5R01 AG061785-02.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.02/F5

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

Support: NIHR01NIGMS146257
The Wooten Foundation.

Title: Planar and Spatial Analysis of 3xTg-AD Mouse Brain Revealed RhoA-LIMK signaling Axis Dysregulation

Authors: *S. NIK AKHTAR[1], Q. LU[2];
[2]Brody Sch. Med. East Carolina Univ., [1]East Carolina Univ., Greenville, NC

Abstract: Background: Alzheimer’s Disease (AD) is associated with amyloid plaque deposition, neurofibrillary tangle formation, and synaptic loss. A key class of proteins that modulate synapse formation is the Rho family of small GTPases. RhoA, one of the most studied Rho GTPase proteins, is widely reported to be dysregulated in AD. However, its targeting to modify disease outcomes demonstrated mixed results. To address this issue, we hypothesize that the activity states of RhoA and its downstream effector LIMK are different in the cortex and subregions of the hippocampus along their respective spatial and planar dimensions. **Objectives:** We intended to elucidate the plane and spatial dependent changes in RhoA/LIMK activity in wild-type (WT) and 3xTg-AD mouse model hippocampus and cortex using phosphor-specific

antibodies. **Methods:** We used antibody pRhoA that recognizes an inactive state of RhoA (S188 phosphorylation) and antibody pLIMK against the active state of LIMK (T508 phosphorylation) to investigate RhoA signaling in WT and 3xTg-AD mouse models. For spatial and planar RhoA-LIMK axis studies, we prepared serial sections from the rostral to caudal coronal planes of the entire cortex and hippocampus followed by immunofluorescence labeling with pRhoA and pLIMK antibodies. **Results:** pRhoA showed an increasing trend in expression in the cortex but a decreasing trend in the dentate gyrus of the 3xTg-AD mouse hippocampus. On the contrary, pLIMK expression showed a trend to decrease in the cortex but increase in the dentate gyrus of 3xTg-AD mouse hippocampus. pRhoA showed a nuclear to cytoplasmic redistribution in the cortex in a plane-dependent manner. To address this, rostral to caudal analysis of the mouse brain was performed demonstrating that both pRhoA and pLIMK elicited a plane dependent expression pattern. pRhoA and pLIMK corroborated each other along the spatial and planar axis, i. e. a higher expression in pRhoA expression corresponded to a lower expression of pLIMK. On the other hand, only pRhoA demonstrated dynamic redistribution between the nucleus and cytoplasm whereas pLIMK did not show such redistribution. Two-way ANOVA analysis showed that the same region of the brain among different planes had significant differences in the expression of pRhoA and pLIMK. **Conclusion:** Phosphorylation-dependent RhoA-LIMK signaling axis is dysregulated in 3xTg-AD mouse brains with such spatial dysregulation further being manifested along the rostral-caudal plane dimensions.

Disclosures: S. Nik Akhtar: None. **Q. Lu:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.03/F6

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

Support: Department of Defense grant (AZ1701452) (G.E.S)

Title: Common pathogenic signaling mechanisms of Alzheimer's disease and traumatic brain injury

Authors: *E. K. WEBBER[1], D. F. STEINBRENNER[2], N. M. BARRINGTON[2], J. MCDAID[2], G. E. STUTZMANN[3];
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Abstract: Traumatic brain injury (TBI) is a significant risk factor for development of Alzheimer’s disease (AD). These two conditions share several comorbidities, such as deficits in memory function and behavioral regulation. We hypothesize that these shared qualities stem from alterations to Ca^{2+} homeostasis, resulting in similar maladaptive changes to neuronal

physiology. The long-term effects of repeat impact TBI (rTBI), single TBI (sTBI), and their contribution to network and single-cell deficits remain unknown. Our research objective is to identify connections between AD, rTBI, and sTBI at the single-cell and network levels, and to characterize the combined pathology via immunohistochemistry and behavior. To accomplish this, we subjected male and female 3xTg-AD mice and age-matched non-transgenic controls to rTBI and sTBI consisting of either one or three closed-head controlled-cortical impacts or sham treatment protocols. 30-days post procedure, brain slices were harvested for electrophysiological recordings of hippocampal synaptic transmission and plasticity properties at the Schaffer collateral CA1-synapse, 2-photon calcium imaging and whole-cell electrophysiology of cortical neurons, or immunostaining of histopathological markers. Prior to brain dissection and analysis, mice completed both Barnes maze (BM) and open field (OF) behavioral tests to assess long-term spatial memory, mobility, and anxiety-like behaviors. Current data indicate that rTBI-induced synaptic deficits contribute further to alteration of long-term potentiation, the cellular correlate of learning and memory, and sTBI groups show altered BM behavior. Histopathological findings indicate a reduction in phospho-tau when sTBI AD mice are treated with Dantrolene, a negative allosteric modulator of the ryanodine receptor, a key contributor to intracellular Ca^{2+} dyshomeostasis. To date, our findings support common upstream pathogenic signaling mechanisms in TBI and AD, such as Ca^{2+} dyshomeostasis, which may play a role in increased vulnerability to developing dementia after brain injury.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.04/F7

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

Support: AHA CDA 856826

Title: Upregulation of long-chain acyl-CoA synthetase 3 alleviates neuropsychiatric symptoms in Alzheimer's disease

Authors: L. XU, Y. ZHANG, P. ZOU, R. H.-C. LEE, ***C. WU**;
LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: The comorbidity of depression and anxiety are the most prevalent neuropsychiatric symptoms in Alzheimer’s disease (AD). Despite w-3/w-6 fatty acids ratio is highly related to neuropsychiatric disorders, the etiology of the comorbidity among *lipid dyshomeostasis* and depression remains unknown in AD. As such, biosynthesis of omega-3 fatty acids (ω3-FA) through long-chain acyl-CoA synthetases (ACSL) is one of the essential elements for cell function and survival. This is due to ω3-FA’s imperative role in modulating plasma membrane

configuration, energy production, mitochondrial function, and inflammation. Thus, ω3-FA deficiency dictated by downregulation of ACSL is one of the main etiologies of neurodegenerative diseases. We thus study whether ACSL reduction is the essential element causing depression in AD. The depression- and anxiety-like behavioral responses were investigated in young (1-3 months) and aged (9-12 months) 3xTg-AD mice using forced swim test (FST), tail suspension test (TST), elevated plus maze (EPM), and sucrose preference test (SPT). We also studied the potential trophic factors involved in and related genes dysregulation in the hippocampus and cortex via immunofluorescence (IF) staining and RNA sequencing. We discovered that depression- and anxiety-like behavior can be detected in aged 3xTg-AD mice, which is accompanied by lower ACSL3 levels as compared to young 3xTg-AD mice. Interestingly, upregulation of ACSL3 via AAV alleviated depression- and anxiety-like behavior in aged 3xTg-AD mice. Finally, results from immunofluorescence (IF) staining and RNA sequencing suggest that low ACSL3-induced depression-like behavior in aged 3xTg-AD mice is mediated by brain-derived neurotrophic factor (BDNF) signaling. The present study indicates that upregulation of ACSL3 is a novel target to treat depression and anxiety in AD.

Disclosures: **L. Xu:** None. **Y. Zhang:** None. **P. Zou:** None. **R.H. Lee:** None. **C. Wu:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.05/F8

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

Support: Kaneb Grant

Title: The Potential Role of Ephrins and Calbindin in Anterior Cingulate of Alzheimer's Disease

Authors: D. XIAO[1], K. WANG[4], ***N. HIDDEN**[2], E. EVANGELISTA[2], H. SABOLEK[3]; [1]Biol. and Neurosci., [3]Psychology, [2]Regis Col., Weston, MA; [4]West Virginia Univ., Morgantown, WV

Abstract: Ephrins and calcium binding proteins have been implicated in the pathogenesis of Alzheimer’s Disease (AD) in the hippocampus of rodent model of AD and postmortem human brains. However, it is unclear whether there are changes of ephrins and calbindin proteins in the anterior cingulate cortex (ACC) and which predispose individuals to disease. Previous research has demonstrated that ephrins, a large family of tyrosine kinases with A and B subtypes (EphA and EphB), play an important role as wiring molecules to form brain circuitries in the developing brain and adult neuroplasticity. Specifically, EphA4 and EphB2 are implicated in mediating synapse morphology, and neuroprotective against Amyloid-beta (Aβ) -induced neurotoxicity, respectively. Additionally, calcium binding proteins have been shown to play an important role in calcium homeostasis for memory formation and cell survival. We used postmortem brains of AD and CN (cognitive control, n=5 for each) and immunohistochemistry to study the two

ephrins and calbindin. Firstly, we found no changes in ACC tissue area and total number of cells in ACC in AD compared to CN individuals. However, we found that the nucleus of cells tends to be rounder, reduced in area, perimeter and optical density in AD compared to CN. Secondly, we found no changes in EphA4, or EphB2, or calbindin levels in the ACC in AD compared to CB individuals. However, we found that the size of calbindin cells is smaller in the upper layers of anterior cingulate area 24 with deformed morphology, suggesting disrupted calcium metabolism and calbindin at ACC plays an important role in AD pathogenesis. Further studies of these biomarkers and their contribution to synaptic morphology and dysfunction could aid in the development of potential therapeutic strategies to treat the cognitive decline in AD.

Disclosures: D. Xiao: None. **K. Wang:** None. **N. Hidden:** None. **E. Evangelista:** None. **H. Sabolek:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.06/G1

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

Support: NIH/NIA RF1AG081203

Title: Neuronal ABCA7 deficiency aggravates neuritic dystrophy and mitochondrial dysfunction in 5xFAD mice

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Abstract: Alzheimer's disease (AD) is the most common form of dementia and the sixth leading cause of death in the United States. ß-amyloid (Aß) plaque and tau fibrillary tangles are two hallmarks in AD patients' brains. The AD pathogenesis is associated with synaptic loss, inflammation, endoplasmic reticulum stress, mitochondrial dysfunction, etc. Despite extensive research on AD pathogenesis, no effective treatment to cure AD or prevent its progression due to unclear mechanism. *ABCA7* gene encodes an ATP-binding cassette sub-family A member 7 (ABCA7) and participants in lipid metabolism. Several loss-of-function variants in the *ABCA7* gene are genetically reported to be associated the late-onset AD. However, few studies have identified the loss-of-function of the *ABCA7* gene in neurons contributing to AD pathogenesis. By crossing *Abca7*-floxed 5xFAD mice with *Abca7*-floxed Camk2aCre mice, we generated the neuron-specific knock-out mice (n*Abca7*$^{-/-}$) with 5XFAD background, to investigate how the loss of neuronal ABCA7 affects AD pathogenesis. Behavioral tests showed a slightly worse learning and anxiety phenotype in 5xFAD;n*Abca7*$^{-/-}$ mice (10-12-month-old) than those in 5xFAD mice. Immunochemistry studies found more Aß deposits and dystrophic neurites in the cortex of 5xFAD;n*Abca7*$^{-/-}$ mouse model. Using bulk RNA-seq analysis on the cortex, we found impaired

mitochondria and synaptic dysfunction in the knock-out mice. These results suggest that neuronal loss of ABCA7 is associated with exacerbated AD pathogenesis and that restoring ABCA7 function may be a potential target for treating AD.

Disclosures: **N. Wang:** A. Employment/Salary (full or part-time):; Mayo Clinic. **S. Starling:** None. **D. Haskell:** None. **K. Kawatani:** A. Employment/Salary (full or part-time):; Mayo Clinic. **X. Ma:** None. **T. Aikawa:** None. **T. Parsons:** A. Employment/Salary (full or part-time):; Mayo Clinic. **R. Perkerson:** A. Employment/Salary (full or part-time):; Mayo Clinic. **Y. Pan:** None. **Y. Ren:** A. Employment/Salary (full or part-time):; Mayo Clinic. **T. Kanekiyo:** A. Employment/Salary (full or part-time):; Mayo Clinic.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

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

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Research Grants Council of Hong Kong (the Collaborative Research Fund [C6027-19GF] and the Theme-Based Research Scheme [T13-605/18W])
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Title: Epha4 signaling is involved in hippocampal synaptic dysfunction in alzheimer’s disease

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Abstract: Alzheimer’s disease (AD), the most common form of dementia, is characterized by irreversible memory loss. Emerging evidence along with our previous findings suggest that dysregulated erythropoietin-producing hepatocellular A4 (EphA4) signaling is involved in the abnormal hippocampal synaptic function in AD. Thus, it is important to study the precise roles of

EphA4 in hippocampal synaptic plasticity impairment in AD. Here, we showed that blockade of EphA4 activity using a specific peptide EphA4 inhibitor, KYL, rescued the impairment in hippocampal synaptic basic transmission and plasticity in the APP/PS1 transgenic AD mouse model. Moreover, KYL administration restored the loss of excitatory synapses in the hippocampus of APP/PS1 mice, potentially through decreasing the microglial and astrocytic phagocytosis of synaptic proteins. To illustrate how EphA4 inhibition rescues the hippocampal synaptic dysfunction in AD, we conducted single-nuclei RNA sequencing analysis in the hippocampus of KYL-treated APP/PS1 mice. Results revealed that the restoration of hippocampal functions may be associated with genes/pathways involved in excitatory synapse formation and maintenance. Together, our findings show that modulation of EphA4 signaling restores hippocampal excitatory synaptic functions in AD.

Disclosures: **X. Yang:** None. **Y. Wang:** None. **J. Lin:** None. **W. Fu:** None. **A. Fu:** None. **N.Y. Ip:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

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

Support: 2021 SGR 00357
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021 FISDU 00182

Title: Novel therapeutic strategy based on m6A epigenetic mark for Alzheimer’s disease

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Abstract: RNA m6A modifications are one of the most common in the human brain, and are key players in the posttranslational regulation of messenger RNA (mRNA) splicing, translation, and degradation. The fat mass and obesity-associated protein (FTO) is an RNA demethylase that is an important regulator of central nervous system development, neuronal signalling and disease. Interestingly, it has been shown that genes associated with m6A control may play a role in conferring risk of dementia, and thus FTO gene could be associated with neurological disorders

such as Alzheimer's disease (AD). As we observed a decreased levels of m6A in AD human patients, we hypothesized that the pharmacological inhibition of FTO, would increase levels of m6A and recover the cognitive impairment in an AD context. Here we used a mouse model of AD linked to the aging process, the senescence-accelerated mouse prone 8 (SAMP8). We performed a proof-of-concept for AD treatment, using a well-established FTOi, the FB23, which cannot cross BBB, and our hit candidate FTOi, a new chemical scaffold, high potency micromolar (μM), exhibiting a high PAMPA-BBB permeability, and good PK/PD. Interestingly, we found a better cognitive performance in SAMP8 treated group with our candidate at 3 mg/Kg in comparison with FB23 groups that did not reach rescued cognition. Those results were correlated with enhancement in synaptic plasticity evaluated through the density of spines and length of dendritic branches by using Golgi Staining. Therefore, from this *in vivo* study using two different pharmacological and *in vitro* models to elucidate the role of m6A enzymes as new epigenetic targets for a beneficial CNS effect. Thus, our findings deepen the description of the role of m6A epigenetic mark-related enzymes as a potential target for neurodegenerative diseases, being FTO inhibition a novel therapeutic strategy for AD.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

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Program #/Poster #: PSTR195.09/G4

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

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Title: Characterization of synaptic plasticity and memory deficits in female and male mice of a non-transgenic $A\beta_{1-42}$ amyloidosis model

Authors: R. JIMENEZ-HERRERA, A. CONTRERAS, S. DJEBARI, J. MULERO-FRANCO, G. IBORRA-LÁZARO, D. JEREMIC, *J. NAVARRO-LOPEZ, **L. JIMENEZ-DIAZ**; Neurophysiol. & Behavior Lab., Univ. of Castilla-La Mancha, Ciudad Real, Spain

Abstract: Hippocampal dysfunction induced by amyloid-β (Aβ) accumulation is one of the neuropathological hallmarks of Alzheimer's disease (AD), characterized by excitatory/inhibitory imbalance, impairments in synaptic plasticity and oscillatory activity, and profound memory deficits. Although AD is more prevalent in women than men, the potential sex difference remains largely unexplored, with contradictory findings from amyloidosis transgenic mice models. Thus, to address the lack of data on early amyloidosis stages in females, our study aimed to systematically characterize the effect of intracerebroventricular (*icv.*) injection of oligomeric

A$\beta_{1\text{-}42}$ (oA$\beta_{1\text{-}42}$) on hippocampal-dependent memory and synaptic plasticity in the CA1-CA3 hippocampal synapse, in both male and female mice. For that purpose, we assessed long term potentiation (LTP) through ex vivo electrophysiological recordings and both i) spatial (working, short- and long-term) and ii) exploratory habituation memory, using Barnes maze and open field habituation tasks, respectively. Our results showed that oA$\beta_{1\text{-}42}$ impaired both memory types in a long-lasting manner (up to 17 days post-injection), regardless the sex. Furthermore, LTP was inhibited at a postsynaptic level, both in males and females, and instead, long-term depression (LTD) was induced for the same prolonged period, which could contribute to the memory deficits. In conclusion, our results provide further evidence of the shifting of LTP/LTD threshold due to a single *icv*. oA$\beta_{1\text{-}42}$ injection, which might underly cognitive deficits in early stages of AD. The reported long-lasting cognitive and functional alterations in males and females validate this model for the study of early amyloidosis in both sexes, thus offering a robust alternative to the inconsistence of amyloidosis transgenic mice models.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.10/G5

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

Title: A new model mice with cholinergic dysfunction and amyloid pathogenesis for Alzheimer's disease

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Abstract: The absence of a model mice with cholinergic dysfunction and amyloid pathogenesis might delay drug discovery improving clinical symptom in Alzheimer’s disease (AD). The neural functioning peptide, hippocampal cholinergic neurostimulating peptide (HCNP), induces acetylcholine synthesis via increasing the amount of choline acetyltransferase in the medial septal nucleus. We previously reported that HCNP precursor protein conditional knockout (HCNP-pp cKO) mice might be an adequate genetic model for cholinergic functional impairment in septo-hippocampal interactions. Here, we tried to generate a new model mouse with cholinergic dysfunction and amyloid pathogenesis. The cKO mice of HCNP-pp was crossed with amyloid precursor protein (APP) with three pathogenic mutation knock-in (*App*$^{NL\text{-}G\text{-}F}$ KI) mice. The model mice were evaluated by the behavioral test, and physiological phenotype by theta power of local field potential and field excitatory postsynaptic potential (fEPSP) during long-

term potentiation (LTP) in hippocampus. The involvement of some molecules associated with the cholinergic and glutamatergic terminals in hippocampal function, or amyloid pathogenesis was evaluated. The impairment of memory, novel object recognition test, became evident from earlier period, 9-months old, in HCNP-pp cKO×*App* KI mice compared with *App* KI mice, 12-months old. The reduction of fEPSP during LTP was confirmed in the hippocampus of HCNP-pp cKO×*App* KI mice compared with *App* KI mice. The theta oscillation, indicative of cholinergic function, in the hippocampus of HCNP-pp cKO×*App* KI mice was also reduced compared with *App* KI mice. Morphological assessment showed no significant difference associated with amyloid pathogenesis between the two groups. Evaluation of HCNP-pp cKO×*App* KI mice hippocampus by western blotting revealed reduced levels of choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT) than *App* KI mice. The level of NR2A constituting NMDA type glutamate receptor of HCNP-pp cKO×*App* KI mice was reduced compared with control mice. The dysfunction of cholinergic interaction in septo-hippocampus network via HCNP-pp cKO may phenotypically accelerate the impairment of cognitive function in amyloid pathogenic mice, *App* KI. This new model mice could be utilized as a pathological animal model with cholinergic dysfunction and amyloid pathogenesis for Alzheimer's disease.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.11/G7

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

Title: Impaired Mitochondrial Ultrastructure in Peripheral Neurons under Cholinergic Receptor Muscarinic 1 (CHRM1) Loss: Implications for Alzheimer's Disease and Sensory Neurodegeneration

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Abstract: Cholinergic Receptor Muscarinic 1 (CHRM1) is a G protein-coupled receptor expressed in both the central and peripheral nervous systems (CNS and PNS). The degeneration of cholinergic neurons and cholinergic hypofunction are pathologies associated with Alzheimer's disease (AD). Our recent studies have demonstrated a severe loss (≥50% decrease compared to non-demented individuals) of CHRM1 protein levels in the postmortem temporal cortices, which is associated with poor survival in AD patients. Moreover, investigations utilizing an enriched cortical synaptosomal mitochondrial fraction from wild-type and Chrm1 knockout ($Chrm1^{-/-}$) mice have revealed that Chrm1 loss leads to altered supramolecular assembly of oxidative phosphorylation-associated protein complexes and changes in the ultrastructure of cortical

mitochondria, correlating with functional deficits in respiration. These findings directly link Chrm1 loss to an impaired mitochondrial phenotype in the CNS, emphasizing its relevance to AD pathogenesis. While the impact of CHRM1 loss in the CNS and its association with AD pathogenesis have been the focus of previous research, the significance of CHRM1 loss in peripheral neurons in AD cannot be overlooked. Reports of declining peripheral nerve conduction in AD patients prompted this study to characterize mitochondrial deficits in mouse dorsal root ganglion (DRG) neurons under Chrm1 loss conditions. Overexpression of C-terminal green fluorescent protein (GFP)-tagged Chrm1 and red fluorescence protein (MitoRFP) tagged with a mitochondrial localization signal peptide in cultured primary DRG neurons resulted in the localization of both proteins to the mitochondria, revealing the mitochondrial localization of Chrm1. Confocal time-lapse fluorescence imaging demonstrated their comigration in the neurites, suggesting potential Chrm1 localization in mitochondria. Additionally, transmission electron microscopy analysis revealed a spectrum of mitochondrial structural abnormalities, including disruption of cristae, in adult mouse DRG neurons following Chrm1 loss, thus suggesting a direct link between Chrm1 loss and mitochondrial degeneration in peripheral neurons. The observed Chrm1-GFP colocalization with mitochondria aligns with the localization of a truncated form of the homologous Chrm2 protein, which has recently been demonstrated to localize in the mitochondria. Overall, our study point to hitherto unknown localization of Chrm1 in neuronal mitochondria and implies that Chrm1 hypofunction in peripheral neurons may underlie mitochondrial malfunction, leading to sensory neurodegeneration.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

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

Support: Colorado State University
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Title: Selective cholinergic activation prevents memory loss and thein vivogrowth of amyloid plaques in Alzheimer’s disease

Authors: E. BLACK[1], R. LEE[1], M. DOOLITTLE[1], A. STEGER[2], *S. KIM[1];
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Abstract: Alzheimer’s disease (AD) is the most common form of dementia with no known cause and cure1. Studies suggest that one of the main causes of AD is disruptions in synaptic activity of GABAergic inhibitory interneurons by beta-amyloid peptide (Aβ). This in turn decreases

inhibitory activity to increase excitation in pyramidal excitatory neurons in the hippocampus, resulting in network hyperexcitability. Hyperexcitability in the hippocampal network also promotes Aβ secretion and accumulation, leading to the formation of amyloid plaques, a central pathology of AD. This suggests that the Aβ-induced reduction of hippocampal inhibition is a crucial trigger for the development of AD. Therefore, enhancing hippocampal interneuron activity is thought to be neuroprotective against AD. We thus hypothesize that Aβ-induced hippocampal hyperexcitation promotes the in vivo rapid growth of amyloid plaques, which can be reversed by increasing hippocampal inhibition. To activate hippocampal inhibition, we injected drugs to stimulate α7- and α4β2-nicotinic acetylcholine receptors (nAChRs) into 5-month-old amyloid pathology model (5XFAD) mice. hippocampal sections from these mice were stained with Thioflavin S to visualize amyloid plaques. We found that in vivo co-stimulation of α7- and α4β2-nAChRs significantly reduced the total area and average size of amyloid plaques in the 5XFAD hippocampus when compared to the control hippocampus. This suggests that co-activation of these two receptors significantly reduces the growth of amyloid plaques in 5XFAD mice by preventing hyperexcitation in hippocampal pyramidal cells.

Disclosures: **E. Black:** None. **R. Lee:** None. **M. Doolittle:** None. **A. Steger:** None. **S. Kim:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

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

Support: Colorado State University
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Title: Co-activation of selective nicotinic acetylcholine receptors improves hippocampal brain rhythms and memory in the mouse of Alzheimer’s disease

Authors: *R. LEE[1], M. DOOLITTLE[1], E. BLACK[1], A. STEGER[2], S. KIM[1];
[1]Colorado State Univ., Fort Collins, CO; [2]Rocky Mountain High Sch., Fort Collins, CO

Abstract: It has been suggested that reduced activity in GABAergic inhibitory interneurons disrupts neural oscillations in the hippocampus, which leads to memory loss in Alzheimer's disease (AD).A prominent AD pathology in the human brain is the loss of cholinergic neurons and nicotinic acetylcholine receptors (nAChR). Aβ is known to interact with these receptors and impair their function. nAChRs are expressed more in GABAergic inhibitory interneurons, thus cholinergic deficiency is a prime suspect for Aβ-induced impairment of inhibitory dysfunction in

the hippocampus and cognitive decline in AD.Our previous findings, using cultured mouse hippocampal neurons show Aβ selectively interacts with α7- and α4β2-nAChRs, but not α3β4-nAChRs, and decreases activity in inhibitory interneurons, but induces hyperexcitation in excitatory neurons. We thus hypothesize that Aβ reduces hippocampal GABAergic activity by selectively inhibiting α7- and α4β2-nAChRs, resulting in hippocampal oscillatory disruption and memory loss in AD.To test our hypothesis, the AD mouse model, 5XFAD transgenic mice, and wild type (WT) littermates were treated intraperitoneally with α7- and α4β2-nAChR agonists. Saline was given to control mice.Fear conditioning was performed to see if agonists improved memory. We found that 5XFAD mice showed clear deficit in contextual memory which was successfully reversed by co-stimulation of α7- and α4β2-nAChR agonists.Stereotaxic surgery was then performed to measure local field potentials of theta and gamma oscillations, key components for learning and memory. During memory consolidation, theta, slow and fast gamma activities were significantly reduced in 5XFAD mice. Co-activation of α7- and α4β2-nAChR agonists was sufficient to restore normal rhythmic activities in 5XFAD mice.Ca2+ imaging with nicotine uncaging further reveals that cultured hippocampal neurons expressed α7- and α4β2-nAChRs in parvalbumin-positive (PV+) and somatostatin-positive (SST+) cells, respectively. These two major types of GABAergic inhibitory interneurons play key roles in hippocampal network activity and learning and memory. Thus, co-activation of the two receptors is important for restoring hippocampal activity and memory in 5XFAD mice.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

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

Support: NIH grant R01AA027768
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Title: Cognitive Deficits in the 5xFAD Mouse Model of Alzheimer's Disease: Insights from Corticostriatal Hyperactivity and Reduced Cholinergic Function

Authors: *Y. HUANG[1,2], X. XIE[2], W. WANG[2,3], R. CHEN[2,3], K. DE SOUZA[2], X. WANG[2], C.-H. FU[4], Q. TONG[5], J. CHIN[4], J. WANG[2,3,1];
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Abstract: Alzheimer's disease is characterized by the deposition of Abeta and cognitive inflexibility. However, the relationship between Abeta deposition and cognitive deficits remains unclear. In this study, we conducted behavioral, histological, confocal imaging, and physiological investigations to examine the potential mechanisms by which Abeta deposition affects cognitive function. Our behavioral studies revealed deficits in reversal flexibility during instrumental learning tasks and spatial reversal learning in a Barnes maze in 5xFAD mice, an Alzheimer's disease mouse model. Histological analyses indicated an increased recruitment of striatal neurons in reversal learning, suggesting a failure to suppress previously learned behavior. Furthermore, physiological studies demonstrated hyperactivity in glutamatergic cortical neurons and GABAergic striatal direct-pathway medium spiny neurons (dMSNs) in 5xFAD mice, while cholinergic neurons in the striatum and basal forebrain exhibited reduced activity compared to wild-type mice. Additionally, both cholinergic neurons receive GABAergic inputs from dMSNs. Lastly, ex vivo confocal imaging using genetically encoded sensors indicated lower acetylcholine release in 5xFAD mice. Taken together, these findings suggest that the hyperactive corticostriatal pathway may lead to reduced cholinergic activity and impaired cognitive function. Our research provides new insights into the neurobiological changes underlying the cognitive deficits observed in Alzheimer's disease.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

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

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The Don and Nancy Mafrige Professor in Neurodegenerative Disease Endowment (B.K.)
Mitchell Center for Neurodegenerative Diseases

Title: Functional assessments of Phospholipase D1 using wild-type and 3xTg-AD mice: A spatial and temporal mechanism for the progression of neurodegeneration.

Authors: *S. SREENIVASA MURTHY[1], C. NATARAJAN[2], K. GARZA[3], J. CURRIE[1], K. ARAYA[1], M. MALLIPUDI[1], H. ONUORAH[1], K. SHETH[1], E. WONG[1], S. BOSWORTH[1], J. DORNAK[1], B. KRISHNAN[4];
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Abstract: Background: Alzheimer's disease (AD) is the memory-related neurodegenerative disorder, which contributes to 70% of the cases globally. Synaptic dysfunction is a well-known early event that causes progressive cognitive decline in AD. The latest AD therapeutics on the forefront only offer a moderate symptomatic relief with significant off-target effects. Therefore, understanding the mechanism for AD pathogenesis and developing novel therapeutic targets are urgently needed. Our lab has recently reported an anomalistic increase in phospholipase D isoform 1 (PLD1), that breakdown phospholipids in AD postmortem brain samples, compared to control subjects. Moreover, the effect of elevated PLD1 driven by amyloid-ß and tau deposits has been well-established in wild type mice and in 6-month-old 3xTg-AD model mice. In the present study, we assess the novel role of PLD1 in modulating cellular mechanisms involved in synaptic dysfunction in AD. **Methods:** Here, we studied the spatial and temporal expression of PLD1 in 3xTg-AD model mice, treated with a small molecule PLD1 inhibitor (VU0155069), in an age-dependent manner. Furthermore, the brain-region specific mechanisms of PLD1 were evaluated by utilizing adeno-associated viral 2 (AAV2) vectors via intracerebroventricular route in 6, 12, and 24-month-old wild-type and 3xTg-AD model mice. Following VU0155069/AAV2 administration, the mice cohorts were subjected to behavioral studies specific to learning and memory, such as the NOR (novel object recognition), Y-maze, and elevated plus maze. Synaptic dysfunctions were studied using high frequency stimulation long-term potentiation, HFS-LTP as well as low frequency stimulation long-term depression, LFS-LTD. Finally, the synaptic strength in frozen synaptosomal P2 fractions was determined by utilizing previously standardized novel *in vitro* assay called the Fluorescence-Assisted Single Synaptosome-Long Term Potentiation (FASS-LTP). **Result:** In WT aged mice we noted differential effects of PLD1 over expression and attenuation. Additionally, we corroborate our results with diseased aging seen in 3xTg-AD using pharmacological and molecular approaches with AAV2 vectors. **Conclusion:** Our research provides a novel insight into how PLD1 contributes to progressive functional deficits associated with synaptic dysfunction by impinging on critical cellular signaling events compromised in early and late stages of Alzheimer's disease.

Disclosures: S. Sreenivasa murthy: None. **C. Natarajan:** None. **K. Garza:** None. **J. Currie:** None. **K. Araya:** None. **M. Mallipudi:** None. **H. Onuorah:** None. **K. Sheth:** None. **E. Wong:** None. **S. Bosworth:** None. **J. Dornak:** None. **B. Krishnan:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.16/H2

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

Support: GRF 17102120
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JRS N_HKU 735/21
HMRF 09200966

Title: In vivo imaging of neurovascular unit of the hippocampus during early disease progression in 5XFAD mice

Authors: *W. XIE, C. S. LAI;
THE UNIVERSITY OF HONG KONG, HONG KONG, China

Abstract: *In vivo* imaging of neurovascular unit of the hippocampus during early disease progression in 5XFAD mice

Wenyuan XIE[1], Cora Sau Wan LAI[1,2]1School of Biomedical Sciences.[2]State Key Laboratory of Brain and Cognitive Sciences.The University of Hong Kong, Hong Kong

Abstract:
Alzheimer's disease is a common neurodegenerative disorder characterized by the progressive loss of memory and cognitive functions. Based on previous findings, the neurovascular unit, which protects neurons and provides them with energy, plays an important role in the pathophysiology of Alzheimer's disease. Neurovascular dysregulation, amyloid accumulation and neuronal synaptic impairment may be intricately linked early in AD progression. In mammals, the hippocampus is involved in the process of learning and memory, and it has been reported to exhibits amyloid-dependent structural and functional degradation in the early stage of AD. Therefore, hippocampus is an extremely important region for understanding the mechanisms of memory encoding and recall impairment in Alzheimer's disease. The role of neurovascular unit damage in the hippocampus in the early pathogenesis of AD has been attracting more attention recently but still needs more study. Here we used *in vivo* longitudinal imaging method to examine amyloid plaque, blood vessel, and dendritic spine dynamic changes in the hippocampus of 5XFAD mice from early to late stages of Alzheimer's disease development. This study could be useful for investigate the functional and structural changes and underlying physiological relationships of neurovascular unit at cellular and subcellular resolutions, that would be important for the evaluation of treatment and therapeutic efficacy in the mouse model of Alzheimer's disease.

Disclosures: W. Xie: None. **C.S. Lai:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.17/H3

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

Support: Jeanne Kempner Fellowship (C.N.)
AARG-17-533363
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NIA R01 – AG063945
The Don and Nancy Mafrige Professor in Neurodegenerative Disease

Endowment
Mitchell Center for Neurodegenerative Diseases

Title: Pld1 signaling in neurodegenerative states - looking at brain regions and cellular components

Authors: *K. GARZA, C. NATARAJAN, S. GOPALKRISHNA SHETTY SREENIVASA MURTHY, S. M. BUDHWANI, K. SHETH, E. WONG, B. KRISHNAN;
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Abstract: Background: Phospholipase D (PLD)belongs to a superfamily of lipolytic enzymes that are conserved from bacteria to humans. PLD isoforms have been observed to play roles in all the cell compartments. Their main function involves the breakdown of phospholipids, primarily phosphatidylcholine (PC) which is a major component of the neuronal membranes. Due to its conserved status, this enzyme is also involved with several signaling pathways, including synaptic neurotransmission important for the expression of memory. As a result, we are currently using conventional methods, particularly biochemistry approaches such as Western blots and co-immunoprecipitation to understand the signaling mechanisms that are altered. Moreover, the PLD1 expression patterns, both in human postmortem samples as well as animal models of neurodegeneration, particularly, the 3xTg-AD model of late-onset AD (LOAD), demonstrate that there are differences in the expression pattern between brain regions. As a result, we are studying different brain regions and looking at the expression of known signaling partners for PLD1 in healthy states that contribute to dendritic spine integrity, namely, the mechanical target of rapamycin (mTOR), protein kinase alpha (PKCalpha) and cofilin. We will also be focusing on the temporal profile of these expression patterns in different cellular compartments to get a complete picture of the signaling state. **Methods:** Synaptosome isolation and other biochemical approaches including Western blotting, and co-immunoprecipitation were used to study the role of PLD in altered signaling mechanisms as well as PLD1 expression patterns in different regions of the brain such as the hippocampus, frontal cortex, cerebellum, midbrain, and parietal cortex. **Conclusions:** We will be presenting data that demonstrates the differential expression patterns depending on the temporal as well as the spatial profile in the transgenic mouse models providing an insight into how PLD1 affects neurodegeneration and how small molecule inhibition of PLD1 in the transgenic mouse model provide resilience to the progression of ADRD.

Disclosures: K. Garza: None. **C. Natarajan:** None. **S. Gopalkrishna Shetty Sreenivasa Murthy:** None. **S.M. Budhwani:** None. **K. Sheth:** None. **E. Wong:** None. **B. Krishnan:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.18/H4

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

Title: Investigating region- and cell-specific changes of major cholesterol regulators in an unorthodox Alzheimer's disease model using volume imaging

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Abstract: Title: Investigating Region- And Cell-Specific Changes Of Major Cholesterol Regulators In An Unorthodox Alzheimer's Disease Model Using Volume Imaging
Haylee Mesa, Lawry Soto, Hadley Edwards, Jonathan Meade, Joel Edouard, Qi Zhang
Alzheimer's Disease (AD) is a neurodegenerative disease, and the most prevalent yet currently untreatable form of dementia. Mounting evidence suggests profound changes in lipid metabolism in the AD brain. Particularly, cholesterol (Chol) has been linked to AD pathogenesis by clinical, genetic, and biochemical data from numerous studies. Our recent work further connected Chol to AD by showing a new functional link between presynaptic membrane Chol and amyloid precursor protein (APP), which involves its Chol-interactive motif. Therefore, we propose that APP regulates Chol trafficking and homeostasis at neuronal axons. Accordingly, we hypothesize that the deletion or mutation of APP will cause Chol dysregulation in brain cells, which can be tested using APP-null mice. Intriguingly, brain Chol experiences a progressive decrease during aging, the primary risk factor for AD. Here, we assess our hypothesis in the brains of aging APP-null mice by measuring three major Chol regulators (i.e., SREBP-2, the master regulator for Chol metabolism; INSIG-1, an ER membrane protein patterned with SREBP-2 for its trafficking and proteolysis; and SCAP, a regulator of SREBP-2). Our investigation requires a suitable strategy as AD is neurodegeneration in a region-specific and network-selective manner. To do so, we use age-matched wild-type and APP-null mice at 18 to 30 months old. We begin with tissues from different brain regions (i.e., hippocampus, midbrain, cortex, and cerebellum) based on their susceptibility to AD. In brain slices, we validate all antibodies we use and their ability to survive tissue clearing procedure. Using iDisco, a tissue-clearing method for large tissue samples like whole mouse brains, we clear the aging brains of wild-type and APP-null mice and conduct volume imaging using a light-sheet microscope, Blaze UltraMicroscope. We use Imaris software to stitch the image tiles and reconstruct them in 3D space. Based on immunofluorescence signals, we analyze the amount of those three Chol regulators in astrocytes and neurons in different neuronal networks and brain regions that are affected by AD differently. By so doing, we create a comprehensive landscape of Chol regulation in the aging brain and possibly in AD. By elucidating these molecular changes, our research aims to shed light on the underlying mechanism of neurodegeneration in AD and potentially identify novel therapeutic targets for this devastating disease.

Disclosures: **H. Mesa:** A. Employment/Salary (full or part-time):; FAU Stiles-Nicholson Brain Institute, Dr. Qi Zhang. **Q. Zhang:** A. Employment/Salary (full or part-time):; FAU Stiles-Nicholson Brain Institute.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.19/H5

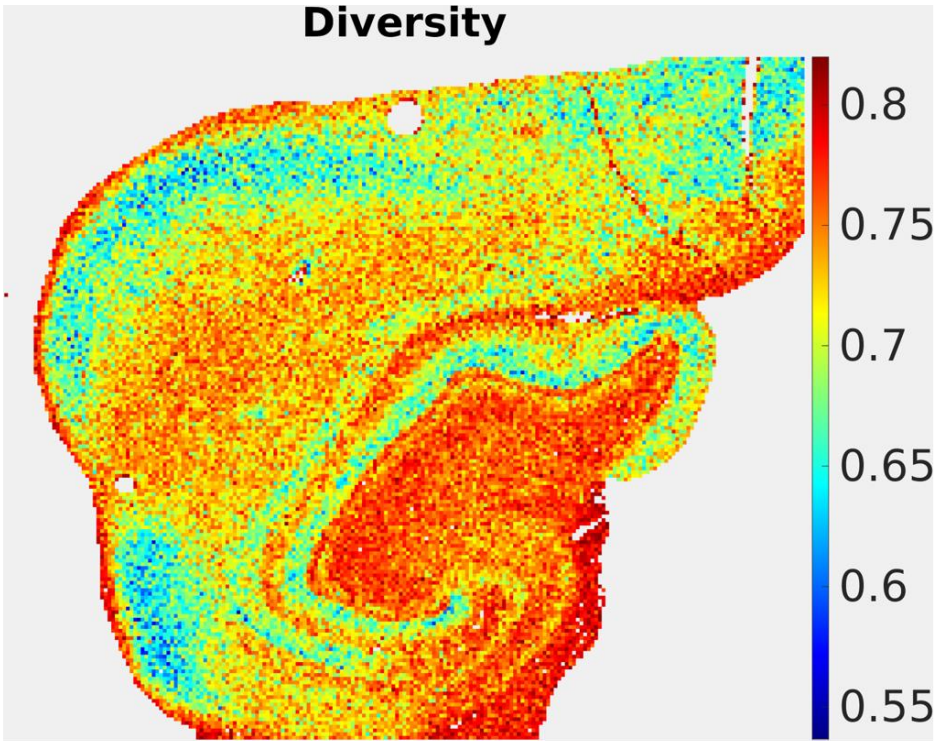
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Wellcome Trust Grant 218293/Z/19/Z
ERC Advanced Grant 885069
Wellcome Trust Grant 221295/Z/20/Z

Title: Synaptome architecture of the human hippocampus is progressively and spatially altered in Alzheimer's disease

Authors: *Z. QIU[1], O. E. CURRAN[2], B. NOTMAN[1], C. SMITH[1], S. GRANT[1];
[1]Ctr. for Clin. Brain Sci., The Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Cell. Pathology, Univ. Hosp. of Wales, Cardiff, United Kingdom

Abstract: Excitatory synapse loss is thought to underlie the cognitive impairments in Alzheimer's disease (AD). The development of molecular imaging methods that measure the protein composition and morphological parameters of billions of individual synapses in mouse brain has revealed very high synapse diversity, described by the term 'synaptome' (Neuron 2018, 99, 781-799; Science 2020, 369, 270-275,). Here we report the adaptation of synaptome mapping developed in mice for the study of the human hippocampal formation (HPF), a key structure for learning and an early and consistent locus of AD pathology.
Excitatory synapses were fluorescently labelled using antibodies to PSD95, an abundant postsynaptic scaffold protein, then imaged on a high-throughput spinning disk confocal microscope. Images from billions of individual synaptic puncta were analysed using advanced deep-learning methods, providing a data-driven catalogue of excitatory synapse subtypes. Analysis of 16 subregions of the HPF in 17 control subjects revealed 7 excitatory synapse subtypes, each with a unique spatial distribution. Next, we examined the HPF of 11 early-stage (Braak II) and 10 late-stage (Braak VI) AD cases. Subtypes were differentially affected compared with the control, with some being resilient and others vulnerable at different stages of disease progression. The loss of vulnerable subtypes correlated with changes in Aβ deposition. Our synaptome map of excitatory synapses in the human HPF provides a multiscale reference resource for this key brain region and a baseline for neuropathological studies. Identification of vulnerable and resilient subtypes of excitatory synapses may suggest new diagnostic and therapeutic approaches in AD and other synaptopathies



Unsupervised synapse diversity maps in the human HPF. Pixel size: 20 x 20 um

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.20/H6

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

Title: Positive modulation of NMDA receptors in the hippocampus and neocortex rescues Hebbian synaptic plasticity and cognitive deficit via BDNF/TRKb signaling in APP/PS1 mice

Authors: *S. CHUNG[1], J.-H. JEONG[2], J.-W. AHN[2], Y.-H. KIM[2];
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Abstract: Positive modulation of NMDA receptors in the hippocampus and neocortex rescues Hebbian synaptic plasticity and cognitive deficit via BDNF/TRKβ signaling in APP/PS1 miceAuthors *S. CHUNG[1,2], J-H. Jeong[1], J-W. AHN[1], *Y-H. KIM[1]; [1]BnH Research Co. LTD., Goyang-si, Gyeonggi-do, 10594, Republic of Korea; [2]Brain Korea 21 Plus Project for

Medical Science, Department of Physiology, Yonsei University College of Medicine, Seoul, 03722, Republic of Korea**Disclosures S. CHUNG, ,J-H. Jeong, J-W. AHN and Y-H. KIM** declare potential conflicts of interest arising from a filed or issued patent on BNH101 as co-inventors, not patentees. **Abstract**One of the most significant characteristics of AD is anterograde amnesia followed by retrograde amnesia. In addition, the cerebral cortex has been known to be the significant long-term repository for many aspects of declarative memory. Therefore, it is postulated that synaptic plasticity of the cerebral cortex, long-term memory is stored, must be considered as well as the hippocampus to deal with memory loss in a moderate to severe stage of AD. We, therefore, developed BNH101, a positive NMDA modulator targeted at a GluN2B-binding site. BnH101, enhanced synaptic efficacy at thalamocortical (TC) input of the layer 4 barrel cortex and LTP induction at the Schaffer collateral input to CA1 hippocampus in dose-dependent manners with the max dose of 10 mg/kg in 15month APP/PS1 mice. Meanwhile, as a positive control, Aducanumab has shown increased hippocampal LTP induction without affecting the TC synaptic efficacy. Still, the effect of BnH101 on the TC potency was synergically potentiated by co-applying Aducanumab in 15month APP/PS1 mice. In addition, the t of BnH101 was nearly entirely prevented by applying R0-25-6981 (5 μM), a GluN2B-selective antagonist to the layer 4 barrel cortex. In terms of a molecular mechanism for the BnH101 effect, BnH101 significantly increased expression of GluN2b and BDNF protein expression in the barrel cortex and hippocampus in 15-month APP/PS1 mice. This effect of BnH101 on BDNF expression was significantly hindered by the blockade of GluN2B-containing NMDA receptor with R0-25-6981. Finally, BnH101-treated APP/PS1 mice exhibited significantly increased avoidance responses compared with vehicle-treated ones. These results suggest Positive modulation of NMDA receptors in the hippocampus and neocortex may rescue Hebbian synaptic plasticity and cognitive deficit via BDNF/TRKβ signaling in the rodent Alzheimer disease model.

Disclosures: S. Chung: A. Employment/Salary (full or part-time):; BnH research. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BnH research. **J. Jeong:** A. Employment/Salary (full or part-time):; BnH research. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BnH research. **J. Ahn:** A. Employment/Salary (full or part-time):; BnH research. **Y. KIm:** A. Employment/Salary (full or part-time):; BnH research. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BnH research.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.21/H7

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

Support: UK DRI Ltd (award XCPD2019–01)

Title: Cortical synaptome architecture is disrupted in Alzheimer's disease patients

Authors: *J. GRIFFITHS[1,2], Z. QIU[2], B. NOTMAN[2], T. WONG[2], P. M. MATTHEWS[1], C. SMITH[2], J. JACKSON[1], S. G. N. GRANT[2];
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Abstract: Introduction: Synapse pathology is a leading determinant of the onset and progression of Alzheimer's disease (AD). Synapses in the mammalian brain are highly diverse in protein composition, protein turnover rate, nanoarchitecture and morphology. This diversity is encapsulated by the terms 'synaptome', which describes the set of all synapse types and subtypes, and 'synaptome architecture', which describes the spatiotemporal distribution of the synaptome in the brain. We have developed systematic methods for examining the molecular features of billions of individual synapses in mouse and human brain tissue. Here, we leverage these methods to uncover how the synaptome and synaptome architecture change in the cortex of patients with AD. Aim: To systematically characterise the synaptome and synaptome architecture in the cortex of AD patients and identify vulnerable and resilient synapses throughout disease progression. Methods: We developed a panel of antibodies to survey presynaptic (SV2A, SYP, SYN1, VGAT) and postsynaptic (PSD95, GLUN1, GPHN) proteins of excitatory and inhibitory synapses and studied cortical regions representing areas affected by early-, mid-, and late-stage AD pathology: the primary visual (control n=29; AD n=35), frontal (control n=12; AD n=31), and temporal (control n=20; AD n=29) cortex, respectively. By combining immunofluorescent labelling of these synaptic proteins, single-synapse resolution spinning disk microscopy, and advanced computer vision and machine learning algorithms, we implemented a comprehensive synaptome mapping pipeline for human post-mortem tissue. Results: Our study revealed significant and differential changes in synaptome architecture across each cortical region, uncovering not just a loss of specific synaptic proteins in AD but a difference in the overall composition of those that remain. Classification of excitatory synapse subtypes within these regions exposed certain subtype vulnerability and resilience. Conclusion: We report the first human cortical synaptome map of AD, providing direct insight into the impact of AD progression on synapse diversity. This map will inform the fundamental mechanisms underlying cognitive decline and, through recognition of vulnerable and resilient subtypes, help identify synapse-specific biomarkers and therapeutic strategies.

Disclosures: J. Griffiths: None. **Z. Qiu:** None. **B. Notman:** None. **T. Wong:** None. **P.M. Matthews:** None. **C. Smith:** None. **J. Jackson:** None. **S.G.N. Grant:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.22/H8

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

Support: NIH grant R01NS105615
NIH grant R01MH124827
NIH grant R21AG072178

Title: Hyperfunction of Post-synaptic Density Protein 95 Promotes Seizure Susceptibility in Early-Stage Aβ Pathology

Authors: *Y. YOOK, K. LEE, S. LIZARAZO, N.-P. TSAI;
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Abstract: Amyloid-beta(Aβ) is a cleaved fragment of amyloid precursor proteins, an integral membrane protein. Excessive Aβ accumulation can form toxic aggregates that cause synaptic damage, neuroinflammation, and Ca^{2+} dyshomeostasis. Traditionally, Aβ has been thought to contribute to lower neuronal activity and neurodegeneration in Alzheimer's disease (AD) because of its toxicity. In contrast, recent studies have demonstrated that Aβ can induce hyperexcitability and epileptiform activity during the earlier stage of accumulation, even before AD onset. Interestingly, AD patients with seizures showed a faster cognitive decline than those without epileptic history, implying that seizures may aggravate the progression of the disease. However, the mechanism by which Aβ induces seizures is still elusive. Postsynaptic Density 95 (PSD-95) is a major scaffolding protein at the postsynaptic density (PSD), clustering ion channels, neurotransmitter receptors, cell-adhesion proteins, and other scaffolding proteins. PSD-95 enhances excitatory synapse maturation and synaptic transmission by stabilizing protein complexes at PSD. In this study, we reveal that PSD-95 is a key mediator in Aβ-induced seizure vulnerability. First of all, our western blot analysis showed that PSD-95 is elevated, resulting from reduced interaction with ubiquitin E3 ligase murine double minute 2 (Mdm2) and decreased ubiquitination. Second, we revealed that Aβ increases PSD-95-dependent elevation of excitatory synapses and the surface expression of the α-amino-3-hydroxy-5- methyl-4-isoxazole propionic acid (AMPA) receptor. Lastly, inhibition of PSD-95 significantly reduces seizure vulnerability in 8-week-old APP/PS1 mice. Taken together, our results demonstrate the mechanism underlying elevated seizure susceptibility during early-stage Aβ pathology and introduce PSD-95 as a potential therapeutic target for treating seizures in AD.

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Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.23/H9

Topic:

Support: Alzheimer Nederland
Brain Foundation Netherlands

Title: Amyloid-β driven synaptic depression is triggered via disrupted trafficking of GluA3 containing AMPA-receptors

Authors: *N. R. REINDERS[1], S. SPEK[3], R. KLAASSEN[4], K. KOYMAN[5], H. D. MACGILLAVRY[2], A. B. SMIT[6], H. W. KESSELS[7];
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Abstract: Background: The synaptic deficits associated with soluble oligomeric amyloid-β (Aβ) are a highly probable cause for cognitive deficits in Alzheimer's disease. Aβ weakens synapses by removing AMPA-type glutamate receptors (AMPARs). We previously found that neurons are fully resistant to Aβ-mediated synaptic depression when they lack GluA3-subunit containing AMPARs, implying that GluA3-containing AMPARs are selectively targeted by Aβ. This study further elucidates the critical role of GluA3 in Aβ-mediated synaptic impairments. **Method:** We studied Aβ-mediated effects on synapses and GluA3 trafficking with electrophysiology and fluorescence microscopy using different Aβ model systems. By altering a PDZ-binding motif in GluA3, we manipulated the ability of GluA3 traffic in and out of hippocampal synapses. **Results:** Synapses of CA1 pyramidal neurons become vulnerable to Aβ when they express AMPAR subunit GluA3. We found that Aβ-oligomers reduce the levels of GluA3 immobilized at spines, indicating they deplete GluA3-containing AMPARs from synapses. These Aβ-driven effects critically depended on the PDZ-binding motif of GluA3. When GluA3 was expressed with a single amino acid mutation in its PDZ-binding motif that prevents GRIP binding, it did not end up at dendrites and spines and Aβ failed to trigger synaptic depression. GluA3 with a different point mutation in the PDZ-motif, which leaves GRIP-binding intact but prevents its endocytosis, was present at spines in normal amounts but left synapses fully resistant to the effects of Aβ. Aβ-oligomers not only caused GluA3-containing AMPARs to be removed from synapses but also targeted them towards endo-lysosomal compartments for degradation. Correspondingly, a selective reduction of GluA3 levels in hippocampus was observed in synaptosome fractions from APP/PS1-transgenic mice at an early age. **Conclusion:** We here show that GluA3 renders synapses vulnerable to the effects of Aβ. Together, our findings outline a model where the endocytosis and lysosomal degradation of GluA3-containing AMPARs is a critical early step in the cascade of events through which Aβ accumulation causes a loss of synapses.

Disclosures: **N.R. Reinders:** None. **S. Spek:** None. **R. Klaassen:** None. **K. Koyman:** None. **H.D. Macgillavry:** None. **A.B. Smit:** None. **H.W. Kessels:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.24/H10

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

Title: Aberrant Expression of GABA-Related Genes in the Hippocampus of 3xTg-AD Model Mice from the Early to End Stages of Alzheimer's Disease

Authors: *H. MORI[1,2], Y. YOSHINO[1], J.-I. IGA[1], S. OCHI[1], Y. FUNAHASHI[1], K. YAMAZAKI[1], H. KUMON[1], Y. OZAKI[1], S.-I. UENO[1];
[1]Ehime Univ., toon city, ehime prefecture, Japan; [2]Dept. of Neuropsychiatry, Shokokai Imabari Hosp., Imabari, Japan

Abstract: Background: We explored the gene expression levels in the brain of 3xTg-AD model mice to elucidate the molecular pathological changes from the early to end stages of Alzheimer's disease (AD). **Objective:** We re-analyzed our previously published microarray data obtained from the hippocampus of 3xTg-AD model mice at 12 and 52 weeks of age. **Methods:** Functional annotation and network analyses of the up- and downregulated differentially expressed genes (DEGs) in mice aged 12 to 52 weeks were performed. Validation tests for gamma-aminobutyric acid (GABA)-related genes were also performed by quantitative polymerase chain reaction (qPCR). **Results:** In total, 644 DEGs were upregulated and 624 DEGs were downregulated in the hippocampus of both the 12- and 52-week-old 3xTg-AD mice. In the functional analysis of the upregulated DEGs, 330 gene ontology biological process terms, including immune response, were found, and they interacted with each other in the network analysis. In the functional analysis of the downregulated DEGs, 90 biological process terms, including several terms related to membrane potential and synapse function, were found, and they also interacted with each other in the network analysis. In the qPCR validation test, significant downregulation was seen for Gabrg3 at the ages of 12 ($p = 0.02$) and 36 ($p = 0.005$) weeks, Gabbr1 at the age of 52 weeks ($p = 0.001$), and Gabrr2 at the age of 36 weeks ($p = 0.02$). **Conclusion:** Changes in immune response and GABAergic neurotransmission may occur in the brain of 3xTg mice from the early to end stages of AD.

Disclosures: H. mori: None. **Y. Yoshino:** None. **J. Iga:** None. **S. Ochi:** None. **Y. Funahashi:** None. **K. Yamazaki:** None. **H. Kumon:** None. **Y. Ozaki:** None. **S. Ueno:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.25/I1

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

Support: Intramural Research Program of the NIH; Z01 ES090089

Title: Hippocamposeptal GABA release during aging in 5XFAD mice

Authors: *J. C. DAMBORSKY, J. L. YAKEL;
Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC

Abstract: Hippocamposeptal (HS) projection neurons are GABAergic neurons in the hippocampus that send projections to the medial septum/diagonal band of Broca (MS/DBB) region of the basal forebrain. These HS neurons are part of a reciprocal septo-hippocampo-septal circuit that connects the hippocampus and MS/DBB, and is critical for mediating spatial and episodic memory. Previous studies have shown that HS neurons may be sensitive to the deleterious effects of pathological exposure to amyloid-β (Aβ), as would occur in Alzheimer's disease (AD). However, it is not known how Aβ affects GABA release from HS terminals in the MS/DBB in early stages of amyloid pathology, or how this may change over time. In order to address these questions, we used the 5XFAD mouse model of Alzheimer's disease (AD), which exhibits rapid accumulation of pathological levels of Aβ, and memory deficits that can be detected by around 4 months of age. Most HS neurons co-express somatostatin (SST), so to target these neurons in 5XFAD mice, we crossed SST-Cre mice to 5XFAD hemizygous mice and performed stereotaxic injections of Cre-dependent AAV containing mCherry/channelrhodopisin-2 (ChR2) bilaterally into the hippocampus. To examine how HS synaptic transmission is affected by age and pathological exposure to Aβ, we used optogenetics to selectively stimulate HS terminals while performing whole-cell patch clamp recordings from MS/DBB neurons in acute slices taken from mice 2-3 weeks following injection at 4 different ages: 4, 6, 9, and 12 months. We performed paired-pulse optogenetic stimulation protocols and analyzed the amplitude of inhibitory postsynaptic currents (IPSCs), as well as the paired-pulse ratio (PPR) at 3 different inter-pulse intervals; 50, 100, and 200 ms. We found that there were no differences in IPSC amplitude or PPR between 5XFAD mice and littermate controls in either males or females at any of the ages recorded. This suggests that there is not a large loss of GABA release, or change in release probability, from HS terminals in the MS/DBB, even at an age at which large deficits in cognitive abilities can be observed. Future studies will determine if there are more subtle changes in the regulation of HS GABA release that may contribute to memory impairment, of if this pathway remains largely unchanged during the early and mid-stages of AD-like pathology. Understanding how HS release is affected by amyloid pathology will provide a key piece to the puzzle of determining how activity within the larger septo-hippocampo-septal circuit is related to memory impairment in AD.

Disclosures: **J.C. Damborsky:** None. **J.L. Yakel:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.26/I2

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

Support: 1R01AG06583601

Title: Role of extracellular matrix in Alzheimer's Disease-associated memory loss

Authors: *L. CHAUNSALI, B. TEWARI, C. PRIM, H. SONTHEIMER;
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Abstract: Alzheimer's disease (AD) is characterized by a progressive loss of memory and cognition. AD pathology includes the deposition of beta-amyloid plaques and hyper-phosphorylated tau tangles. Accumulating evidence suggests that alterations in the brain extracellular matrix (ECM) are also a significant core pathological feature of AD. In several parts of the brain, including the cerebral cortex and hippocampus, ECM condenses to form highly organized structures, known as perineuronal nets (PNNs). PNNs surround not only the majority of parvalbumin-expressing GABAergic inhibitory neurons but also the excitatory pyramidal neurons in the hippocampal CA2 area. PNNs are known to stabilize synapses and regulate neuronal plasticity, and are thereby implicated in stabilizing the memory trace. Recent studies have shown alterations in the PNNs in various neurological disorders including AD. We therefore, hypothesized that memory deficits and cognitive decline associated with AD may be associated with alterations in ECM and PNNs. To study this question we immunostained PNNs in 5XFAD, a widely used transgenic mouse model of AD where we observed significant degradation of PNNs specifically in the CA2 region of the hippocampus, and are implicated in social cognition memory. Loos of PNNs were visible as early as 6 months and correlated well with impaired social cognition memory in behavioral test. To elucidate the mechanism of PNN disruption, we assessed the ECM remodeling machinery and observed upregulated gene expression of several enzymes known to regulate PNNs. These findings suggest that increased expression of proteolytic enzymes, most likely secondary to amyloid pathology, degrade PNNs in the hippocampus CA2 area and contribute to deficits in social memory dysfunction. Preventing PNN disruption by inhibition of ECM remodeling agents may therefore constitute a novel intervention strategy to delay AD-associated memory loss.

Disclosures: L. Chaunsali: None. **B. Tewari:** None. **C. Prim:** None. **H. Sontheimer:** None.

Poster

PSTR195. Alzheimer's Disease: Mechanisms of Synaptic Dysfunction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.27/I3

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

Support: ERC #951294

Title: Investigating Extracellular Space in Alzheimer's Disease Using Shadow Imaging and single particle tracking in APP PS1 Mouse Model

Authors: *G. PORRAS[1], J. ESTAUN-PANZANO[2], I. CALARESU[3], Y. DEMBITSKAYA[3], S. NANDI[4], Q. GRESIL[4], L. COGNET[4], V. U. NÄGERL[3], L. GROC[3], E. BEZARD[5];
[1]Motac, Cheshire, United Kingdom; [2]Institu des Maladies Neurodegeneratives, Bordeaux,

France; [3]Inst. Interdisciplinaire en Neurosciences, Bordeaux, France; [4]Inst. d'Optique, Talence, France; [5]Inst. of Neurodegenerative Dis., Bordeaux, France

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterised by the accumulation of amyloid-beta (Aβ) plaques targeted by active and passive immunisation approaches. The accessibility to the antigen, i.e. the penetrability of the anatomopathological landmarks, is a major limitation factor. Aβ plaques are generated outside neurons in the extracellular space (ECS). This nanometric maze is one of the least known structures of our brain, primarily due to only recently overcome technological limitations. Understanding ECS diffusion dynamics in disease, within the plaques and in the brain as a whole reveal crucial for better-understanding AD pathogenesis, implications, and, even more importantly, for the rational design of therapeutics. We applied our ground-breaking techniques to study the brain ECS properties around and within Aβ plaques in the APP/PS1 mouse transgenic model. First, we performed shadow imaging experiments (SUSHI). This microscopy technique uses the freely diffusing calcein dye in the ECS to get negative images of unlabeled brain cells in their anatomical context. SUSHI unravels, in 3D, a dense amyloid core surrounded by packed cellular bodies, suggestive of a structure difficult to penetrate. Actual ECS characteristics within Aβ plaques were further studied using the single-particle tracking of certain fluorescent nanomaterials such as Infra-red Single Wall Carbon Nanotubes (IR-SWCNTs) or Quantum Dots (QDs). These approaches, complementary to SUSHI, have become powerful tools to quantify ECS properties at the nanoscale. Our preliminary findings reveal that, at a bulk level, there is a significant increase in the extracellular diffusion rates in some of the most affected regions, such as hippocampal CA1 and CA3 regions or cortical layer VI. We then characterised the hindered diffusion and altered dynamics within the plaques themselves. Using the auto-fluorescent properties of these plaques and diffusing quantum dots in acute slices, we characterised the extracellular diffusion alterations near, around, and inside Aβ plaques. The dense and fibrillar structure of Aβ plaques and the corona of cells surrounding it presents a physical barrier that greatly restricts, but not forbids, the entry of larger particles, including quantum dots, into the core of the plaques. These results present the nanometric organisation of brain tissue and ECS around and within Aβ plaques providing unprecedented insights into their difficult penetration by large molecules. partially the poor bioavailability of certain therapeutic agents inside the plaques themselves and offer new leads for future therapeutic developments.

Disclosures: G. Porras: None. **J. Estaun-Panzano:** None. **I. Calaresu:** None. **Y. Dembitskaya:** None. **S. Nandi:** None. **Q. Gresil:** None. **L. Cognet:** None. **V.U. Nägerl:** None. **L. Groc:** None. **E. Bezard:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.01/I4

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

Support: Louisiana Biomedical Research Network/NIH

Title: Identification of small molecule therapeutic drugs for Alzheimer's and Parkinson's disease using cell line and Drosophila models

Authors: D. ROBERTS[1], H. BROKENBERRY[1], M. CONNELL[1], ***S. D'MELLO**[2];
[1]Louisiana State Univ. Shreveport, Shreveport, LA; [2]Louisiana State Univ. Shreveport, Dallas, TX

Abstract: We previously described that chemical compounds with a 3'-indolone core were neuroprotective in a cell culture model of developmentally-regulated neuronal death. In the current study we extended our analysis to a cell line model in which HT22 cells, a mouse hippocampal neuroblastoma cell line, was treated with glutamate to induce oxidative stress. In this model of Alzheimer's disease two commercially available 3'-indolone compounds, SU6656, a Src inhibitor, and PKR inhibitor negative control, were both fully protective against glutamate-induced toxicity. Another 1,4-benzoxazine compound, NGN-006, was also fully protective against glutamate-induced death of HT22 cells. We extended our studies to SH-SY5Y cells treated with either MPP+ or rotenone, two widely used cell line models of Parkinson's disease (PD). SU6656 was only partially protective against rotenone-induced toxicity whereas PKR inhibitor negative control was not protective. Both SU6656 and PKR inhibitor negative control were partially protective against MPP+ toxicity. We are currently identifying signaling pathways regulated by these compounds in HT22 and SH-SY5Y cells. We are also testing NGN-006 in *Drosophila* models of neurodegenerative diseases. Results of these ongoing studies will be presented.

Disclosures: D. Roberts: None. **H. Brokenberry:** None. **M. Connell:** None. **S. D'Mello:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.02/I5

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

Support: NIH [R01AG057555]
Hunter RISE grant[#2R25GM060665-21]
City University of New York (Neuroscience Collaborative program, The Graduate Center
The Graduate Center

Title: Sex-specific effects of the HDAC inhibitor RG2833 in mitigating spatial memory performance deficits in an Alzheimer's disease transgenic rat model.

Authors: *K. NDUKWE[1,2], L. XIE[3], P. SERRANO[4], P. ROCKWELL[2], M. FIGUEIREDO-PEREIRA[2];
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Abstract: Alzheimer's disease (AD) is one of the most common causes of dementia. In the United States, AD affects about 5.8 million Americans and is projected to reach 13.8 million individuals by 2050. Epigenetic alterations such as histone-modifications play a role in memory function, and disruption in the epigenetic processes is linked to the pathogenesis of neurodegenerative and neuropsychiatric diseases. Currently, there are no effective therapies for AD, and therapies targeting epigenetic mechanisms such as pharmacologic inhibitors of histone deacetylases (HDACs), are effective in improving cognitive performance in short-term studies using animal models of AD. The long-term functional consequences of such therapeutic intervention have so far not been investigated. In our study, we describe the long-term therapeutic effects of oral administration of a brain-penetrant HDAC1/3 inhibitor RG2833 in a Fisher transgenic 344-AD (TgF344-AD) rats, a model of AD.We show that there is no obvious toxicity on the TgF344-AD rats up to 6 months of treatment (starting at 5 months of age administering the drug in chow at 30mg/kg of body weight). The drug treatment prevented spatial memory deficits and significantly improved hippocampal-dependent spatial memory performance in TgF344-AD rats compared to wild type littermates at 11 months of age. We also report a female sex-specific drug effect, which has not previously been reported. Even though RG2833 treatment failed to ameliorate amyloid beta or tau paired helical filament accumulation in female rats, we observed a decrease in gliosis manifested by fewer amoeboid and ramified microglia in the hippocampal CA1 region compared to untreated TgF344-AD littermate controls. Furthermore, RNAseq analysis of hippocampal tissue from TgF344-AD rats showed that drug treatment upregulated the expression of immediate early genes (Arc, Egr1 and c-Fos) and other genes involved in synaptic plasticity and memory consolidation in females but not in males. These data indicate that histone modifying therapies can improve cognitive behavior by improving the expression of neuroprotective genes and by modulating the activation of immune cells and possibly dampening the inflammatory response. Based on our data, we propose that RG2833 could be an effective therapeutic that could benefit female patients with AD.

Disclosures: K. Ndukwe: None. **L. Xie:** None. **P. Serrano:** None. **P. Rockwell:** None. **M. Figueiredo-Pereira:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.03/I6

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

Support: Ministry of Science and Technology, Taiwan/MOST111-2326-B-182-001-MY3
Chang Gung Memorial Hospital, Taiwan (CMRPD1M0291)

Title: Alda-1, an ALDH2 activator, exerts anti-neuroinflammatory effect in Alzheimer's disease.

Authors: *C.-C. CHIU;
Chang Gung Univ., Taoyuan, Taiwan

Abstract: Alzheimer’s disease (AD), characterized by selective neurodegeneration in brain regions involved in emotional and cognitive function, is the most prevalent cause of dementia among older people. Microglial-mediated neuroinflammation, the inflammatory response of CNS, is involved in the etiopathogenesis of AD. Proinflammatory microglia accelerates ROS generation, leading to neurodegeneration. TOMM40 genetic variants are believed to increase the risk of AD in Taiwanese population. TOMM40 genetic variants, rs157581 (c.339T > C, p.Phe113Leu, F113L) and rs11556505 (c.393C > T, p.Phe131Leu, F131L), were associated with an increased risk of AD. We further utilized cell models to examine the role of TOMM40 variation in mitochondrial dysfunction that causes microglial activation and neuroinflammation. Activation of mitochondrial aldehyde dehydrogenase 2 (ALDH2) is expected to alleviate oxidative stress and ER stress. In this study, we examined t anti-neuroinflammatory benefit of Alda-1, an activator of ALDH2, on BV2 microglial cells. BV2 microglial cell transfected mutant (F113L) or (F131L) TOMM40 caused mitochondrial dysfunction and oxidative stress-induced activation of microglia and NLRP3 inflammasome. The level of TNF-α, IL-1β, and IL-6 pro-inflammatory cytokines was increased in the BV2 microglial cells expressing mutant (F113L) or (F131L) TOMM40. The culture medium from BV2 microglial cells transfected mutant (F113L) or (F131L) TOMM40 led to neurotoxicity of hippocampal neurons by inducing the activation of microglia and NLRP3 inflammasome and the release of pro-inflammatory cytokines. Our results showed that pretreatment of Alda-1 attenuated mutant TOMM40-induced mitochondrial ROS generation in BV2 microglial cells. Alda-1 could be used for preventing and treating AD by targeting microglial mitochondria.

Disclosures: C. Chiu: None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.04/I7

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

Support: NIH Grant AG068992

Title: Developing platforms with AD GWAS iPSC-derived Microglia for High-Throughput Drug Screening

Authors: A. MURCHISON[1], B. SCHMID[2], M. W. NICHOLSON[1], A. MAROOF[1], P. ZHOU[1], S. COVARRUBIAS[1], M. S. MACAULEY[3], B. HOLST[2], ***W. POON**[1,4];
[1]NeuCyte, Mountain View, CA; [2]Bioneer A/S, Hoersholm, Denmark; [3]Univ. of Alberta, Edmonton, AB, Canada; [4]Inst. of Memory Impairments and Neurolog. Disorders, Univ. of California, Irvine, Irvine, CA

Abstract: Recent AD Genome-wide Association Studies (GWAS) have uncovered single nucleotide polymorphisms (SNPs) within genes predominantly expressed in microglia (*TREM2, TYROBP, CD33, CR1, PILRA/B, PLCG2, INPP5D, BIN1, MS4* gene family) or upregulated in microglia in association with pathology (*APOE, TREM2*) suggesting that microglia and innate immunity play a critical role in disease etiology. Many of the AD GWAS genes lack murine homologs making modeling of those genes in animal models difficult. One such gene is CD33, in which the murine homolog is functionally distinct from the human isoform. The emergence of methods that enable the generation of human iPSC-derived brain cells has led to the development of iPSC models that better recapitulate disease phenotypes. Recently, it was shown that a co-culture model could be established to generate both diffuse and compact Aβ plaques in a 2D system. Such platforms can lead to improved drug screening efforts to identify new therapeutic targets. One identified AD GWAS risk SNP is closely associated with the CD33 gene, confers protection, but is in linkage disequilibrium with rs12459419. The rs12459419 SNP is located at the 5′ intron-exon junction of CD33 exon 2 and promotes exon skipping during CD33 pre-mRNA splicing in microglia. Exon 2 skipping removes the immunoglobulin domain from full-length CD33 (CD33M) leading to a truncated isoform (CD33-D2). Here, microglia were generated from exon-2 deleted iPSCs ($CD33^{\Delta 2/\Delta 2}$) derived from patient iPSCs ($CD33^{C/C}$) in order to investigate the impact of exon-2 skipping on microglia function. These microglia were utilized in different platforms that enable interrogation of both cell autonomous and non-cell autonomous CD33 protective allele function providing novel phenotypic assays for drug discovery.

Disclosures: **A. Murchison:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **B. Schmid:** A. Employment/Salary (full or part-time):; Bioneer. **M.W. Nicholson:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **A. Maroof:** A. Employment/Salary (full or part-time):; Neucyte. **P. Zhou:** A. Employment/Salary (full or part-time):; NeuCyte. **S. Covarrubias:** A. Employment/Salary (full or part-time):; NeuCyte. **M.S. Macauley:** None. **B. Holst:** A. Employment/Salary (full or part-time):; Bioneer. **W. Poon:** A. Employment/Salary (full or part-time):; NeuCyte, Inc.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.05/I8

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

Support: CIHR grant (MOP-84480)

Title: Attenuation of Aβ seed-induced tau aggregation by native PLGA nanoparticles and its relevance to Alzheimer's disease pathology

Authors: *S. KAR[1], P. S. PAUL[1], T. PATEL[2], J.-Y. CHO[3], A. YARAHMADY[2], A. KHALILI[3], V. SEMENCHENKO[3], H. WILLE[2], M. KULKA[3], S.-A. MOK[2];
[1]Med. (Neurology), [2]Biochem., [3]Nanotechnology Res. Ctr., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Evidence suggests that increased levels/accretion of beta-amyloid (Aβ) peptide, together with enhanced phosphorylation/aggregation of tau protein, underlie the degeneration of neurons and development of Alzheimer's disease (AD), the most common cause of dementia affecting the elderly. While Aβ-induced tau hyperphosphorylation is essential to the loss of neurons, misfolded tau seeds facilitate propagation of disease pathology, highlighting a synergistic/cooperative interaction between Aβ and tau in AD pathology. Thus, many studies have pursued a variety of small molecules, including nanoparticles conjugated with various drugs to interfere with Aβ and/or tau aggregation and toxicity as a strategy for AD treatment. We reported earlier that a family of FDA-approved biodegradable PLGA nanoparticles without conjugation to any drug/agent can inhibit Aβ aggregation and attenuate disease phenotype in cellular and animal models of AD. In this study, we evaluated the effects of native PLGA on Aβ seed-induced aggregation of tau protein using a variety of biophysical, structural and spectroscopic approaches. Our results show that $A\beta_{1\text{-}42}$ seeds enhanced aggregation of tau protein in the presence and absence of heparin and the effect was dose-dependently attenuated by native PLGA nanoparticles. Interestingly, PLGA inhibited aggregation of both 4R and 3R tau isoforms involved in the formation of neurofibrillary tangles in AD brains. Furthermore, Aβ seed-induced tau aggregation observed in the presence of arachidonic acid was also suppressed by native PLGA. Collectively, these results suggest that native PLGA nanoparticles can inhibit the Aβ seed-induced aggregation of different tau protein isoforms highlighting their therapeutic potential in the treatment of AD pathology.

Disclosures: S. Kar: None. **P.S. Paul:** None. **T. Patel:** None. **J. Cho:** None. **A. Yarahmady:** None. **A. Khalili:** None. **V. Semenchenko:** None. **H. Wille:** None. **M. Kulka:** None. **S. Mok:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.06/J1

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

Support: NIH Grant U54AG065187
NIH Grant R21CA274620
NIH Grant P50CA217691

Mary Kay Ash Foundation Grant for Cancer Research
NIH Grant P30CA138292

Title: Targeting p38/MK2 protein-protein interaction for therapeutic discovery in Alzheimer's disease

Authors: M. HU, Y. DU, H. FU, *A. IVANOV;
Pharmacol. and Chem. Biol., Emory Univ., Atlanta, GA

Abstract: P38 kinases are essential components of nervous system signal transduction. Four known p38 isoforms (p38α, p38β, p38γ, and p38δ) serve non-redundant functions by binding various substrates, including transcription, signal transduction, protein folding, and cytoskeleton maintenance. The inhibition of p38 activity has emerged as a highly appealing therapeutic strategy in multiple neurological disorders, including Alzheimer's Disease (AD). Although many p38 inhibitors have been developed, none of them have been approved as a drug due to a limited selectivity against p38 isoforms and other kinases. The failure of p38 inhibitors in clinical trials highlights the urgent need for new therapeutic approaches to p38 regulation. To address this unmet medical need, we develop a novel approach to control p38 activity by selectively targeting p38 isoform-specific protein-protein interaction (PPI), rather than its kinase activity. The MAPK-activated protein kinase 2 (MK2), coded by MAPKAPK2, is one of the most clinically important p38 substrates. The p38 binding and phosphorylation of MK2 play a critical role in neurotoxicity and neuroinflammation in AD. The discovery of potent p38/MK2 PPI inhibitors may open new avenues for p38-based therapeutic development. Our Time-Resolved Fluorescence Energy Transfer (TR-FRET) and affinity pulldown assays revealed that MK2 has a significantly higher binding affinity to p38α and p38β compared to p38γ and p38δ isoforms. Using computational structural analysis we identified multiple contact sites on the p38/MK2 PPI interface that are critical for p38/MK2 interaction. The calculated druggability scores and contribution of p38 and MK2 residues to the MK2/p38 free binding energy allowed us to prioritize two pockets suitable for small molecule p38/MK2 disruptor binding. Based on the analysis, we experimentally confirmed the p38/MK2 interface and designed short inhibitory peptides to disrupt the p38/MK2 PPI. We have further optimized and miniaturized the TR-FRET assay for the high-throughput screening (HTS) 384- and ultra-HTS 1,536-well plate formats. We have shown that both p38a/MK2 and p38b/MK2 PPIs demonstrated strong TR-FRET signal with >20 signal/background ratio, which was stable for more than 48 hours and tolerated >10 % DMSO. These data provide new critical insights into molecular mechanisms of AD mediated through p38/MK2 PPIs, indicate the p38/MK2 complex druggability, and establish a new robust assay to discover small molecule MK3/p38 PPI inhibitors to facilitate therapeutic discovery in Alzheimer's other neurological diseases.

Disclosures: M. Hu: None. **Y. Du:** None. **H. Fu:** None. **A. Ivanov:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.07/J2

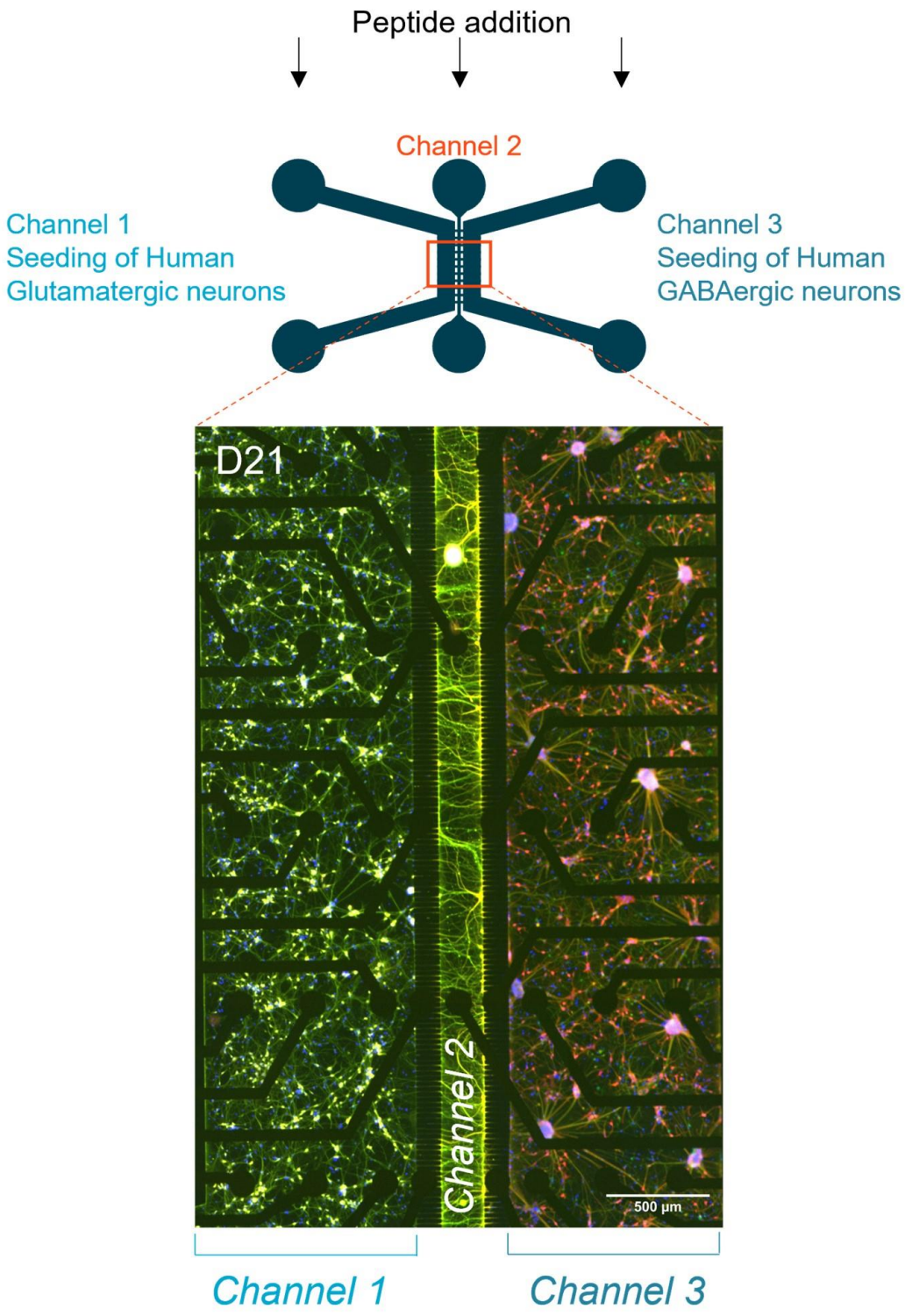
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Innovation en biotherapies et bioproduction - France 2030

Title: Relevant alzheimer's disease models for target validation and drug testing

Authors: L. MINY[1], A. ALLOUCHE[2], J. RONTARD[1], S. KRIDI[2], J. COLIN[2], ***B. MAISONNEUVE**[1], N. VIOLLE[2], T. HONEGGER[1];
[1]NETRI, Lyon, France; [2]ETAP-Lab, Vandœuvre-lès-Nancy, France

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease, leading to decline in memory, cognition, and ultimately all brain functions. Development of new therapies for AD is challenging with high failure rates and long development times. Many factors have been contributing to this global failure of clinical trials including poorly understood etiology and physiological differences between species, leading to a lack of translational preclinical models. A growing body of evidence suggests that oligomeric forms of amyloid beta (AβO) and Tau (TauO) play key roles in the physiopathology of AD. These oligomers are the predominant neurotoxic species in brains during the early stages of AD and provide a suitable effective target to treat it. Brain-on-a-chip platforms, using human-induced pluripotent stem cells (hiPSCs), have the potential to strongly impact and improve the drug screening process and to fill the gap between animal and human physiologies. We introduce here a compartmentalized and fluidically isolated co-culture of hiPSCs-derived glutamatergic and GABAergic neurons, using NETRI's DuaLink microfluidic chips (see Figure). ETAP-Lab produces and uses AβO and TauO for *in vitro* pharmacology. These oligomers are well characterized by various biophysical methods and induce a dose-dependent neurotoxicity *in vitro* as well as memory and learning alterations in mice, associated with synaptic alterations, neuronal apoptosis, and cerebral inflammation. In this poster, we present a full characterization of the neuronal populations, using a wide variety of readouts (immunoassay, immunostaining, and electrophysiological activity), alongside their specific variabilities. This is an essential step toward developing models of AD that are both relevant and up to the industry's standards and could be utilized by a wide range of academic teams and pharmaceutical industries to unravel the mechanisms underlying the pathology and to screen for new and effective therapeutics.



Disclosures: **L. Miny:** None. **A. Allouche:** None. **J. Rontard:** None. **S. Kridi:** None. **J. Colin:** None. **B. Maisonneuve:** None. **N. Violle:** None. **T. Honegger:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.08/J3

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

Support: AG073040
GM139413

Title: Advancing Senescence-Based In Vitro Drug Testing for Alzheimer's Disease: Targeting Aβ and p-Tau Models in a Gut-Liver-Brain Multiorgan System

Authors: *J. COLLINS, H. WONG, G. KATARA, C. J. COLLINS, J. A. J. JOSE, J. KOHANA, A. J. COLLINS;
Biopico Systems Inc, Irvine, CA

Abstract: Preclinical studies have demonstrated the accumulation of senescent cells in multiple organs as a result of aging. In a gut-liver-brain organ model, we have used cellular senescence as a potential mechanism underlying the pathological accumulation of A-beta (amyloid-beta) and p-tau (phosphorylated tau) in AD. Studies have revealed that individuals at risk for AD exhibit gut dysfunction, characterized by changes in gut microbiota, increased intestinal permeability, and inflammation. Additionally, considering the liver's involvement in glucose, insulin, and mitochondrial metabolism, it may play a crucial role in clearing A-beta, p-tau and inflammatory cytokines. Utilizing pharmacotherapies to enhance hepatic clearance of A-beta by targeting the liver shows promise as a potential approach for treating or preventing AD. With the shifted focus from A-beta to p-tau, it has been recognized that the accumulation of tau protein, a key factor in cognitive decline, plays a role in cellular senescence and exhibits mislocalization to the somatodendritic compartment. To investigate the impact of pretreated AD drugs on neurotoxicity, we compared experiments using a multiorgan system consisting of coupled multiorgans model and brain only organ model in culture. We will present our results showing treatment with multiple neurotoxic and senescence causing agents such as A-beta plaques or p-tau fibrils, glyceraldehyde and sodium phenylbutyrate. Such study is conducted with pretreatment by neuroprotective agents such as aspirin, tacrine, curcumin, Acetoacetate. We will compare Aβ 42 levels and total tau and p-tauT181 levels in culture media and also the intracellular levels of total tau, p-tauT181, VEGF, and TGF-β in the organ model, using previously published 2-d culture models. Additionally, we explored the neuroprotective effects of combinational therapy by targeting senescent cells for clearance using senolytic compounds such as dasatinib and quercetin. We will discuss the results of reduction in neuropathological burden and improvement in clinically relevant outcomes. Based on these considerations, we will establish the pathology of cellular senescence associated with p-tau and A-beta accumulation in AD and drug pretreatment utilizing the gut-liver-brain multiorgan system for AD drug discovery strategies.

Disclosures: **J. Collins:** A. Employment/Salary (full or part-time):; Biopico Systems Inc. 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. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); None. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); None. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biopico Systems Inc. F. Consulting Fees (e.g., advisory boards); None. Other; None. **H. Wong:** A. Employment/Salary (full or part-time):; Biopico Systems Inc. **G. Katara:** A. Employment/Salary (full or part-time):; Biopico Systems Inc. **C.J. Collins:** A. Employment/Salary (full or part-time):; Biopico Systems Inc, University of California Irvine. **J.A.J. Jose:** A. Employment/Salary (full or part-time):; Biopico Systems Inc, University of California Irvine. **J. Kohana:** A. Employment/Salary (full or part-time):; Biopico Systems Inc. **A.J. Collins:** A. Employment/Salary (full or part-time):; Biopico Systems Inc, University of California Irvine.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.09/J4

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

Support: AG079141
AG077610

Title: Understanding the Role of BIN1's SH3 Domain in Alzheimer's Disease

Authors: *D. M. BLAZIER[1], E. LEWANDOWSKI[3], S. WANG[4], X. ZHANG[3], J. MCMILLAN[3], L. J. BLAIR[5], Y. CHEN[3], G. THINAKARAN[2];
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Abstract: Alzheimer's disease (AD), a progressive and irreversible neurodegenerative disease, is characterized by the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles that lead to synaptic loss, neuronal cell death, severe cognitive impairment, and eventual death. Genome-wide association studies identified Bridging Integrator 1 *(BIN1)* as the second most significant susceptibility locus for late-onset Alzheimer's disease (AD) development. While BIN1 has been implicated in tau pathology, the pathogenic mechanisms by which BIN1 facilitates AD onset remain unclear and warrant further investigations. Through the identification of novel BIN1 binding partners by structural techniques, including x-ray crystallography and surface plasmon resonance (SPR), we aim to characterize novel BIN1 binding partners and identify small molecule inhibitors. Under homeostatic conditions, BIN1 functions in multiple cellular pathways, including synaptic transmission, endocytosis, cytoskeletal remodeling, and generating membrane curvature, and is

alternatively spliced to form tissue/cell-type-specific and ubiquitous isoforms. A common denominator unites all BIN1 isoforms - the C-terminal Src homology 3 (SH3) domain, a highly conserved functional module that facilitates protein-protein interactions, typically through a combination of hydrophobic and electrostatic interactions of side chains. BIN1's SH3 domain has been shown to bind a class II proline-rich motif on tau, implicating it in tau pathology. We used SPR to ascertain the affinity of BIN1's SH3 domain to full-length 0N4R wild-type tau, and the results showed the equilibrium dissociation constant (K_D) of 1.186 µM. We are using complementary experimental approaches to expand the repertoire of neuronal and non-neuronal proteins interacting with the BIN1 SH3 domain at an affinity similar to or better than tau. Developing a comprehensive interaction map of BIN1's SH3 domain may contribute to advancing the development of therapeutics against AD.

Disclosures: D.M. Blazier: None. **E. Lewandowski:** None. **S. Wang:** None. **X. Zhang:** None. **J. McMillan:** None. **L.J. Blair:** None. **Y. Chen:** None. **G. Thinakaran:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.10/J5

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

Support: Intramural Research Program, National Institute on Aging, NIH: AG000333

Title: Incretin-based multi-agonist peptides demonstrate neuroprotective properties in preclinical Alzheimer's disease models

Authors: *K. O. KOPP[1], Y. LI[1], B. J. HOFFER[2], N. H. GREIG[1];
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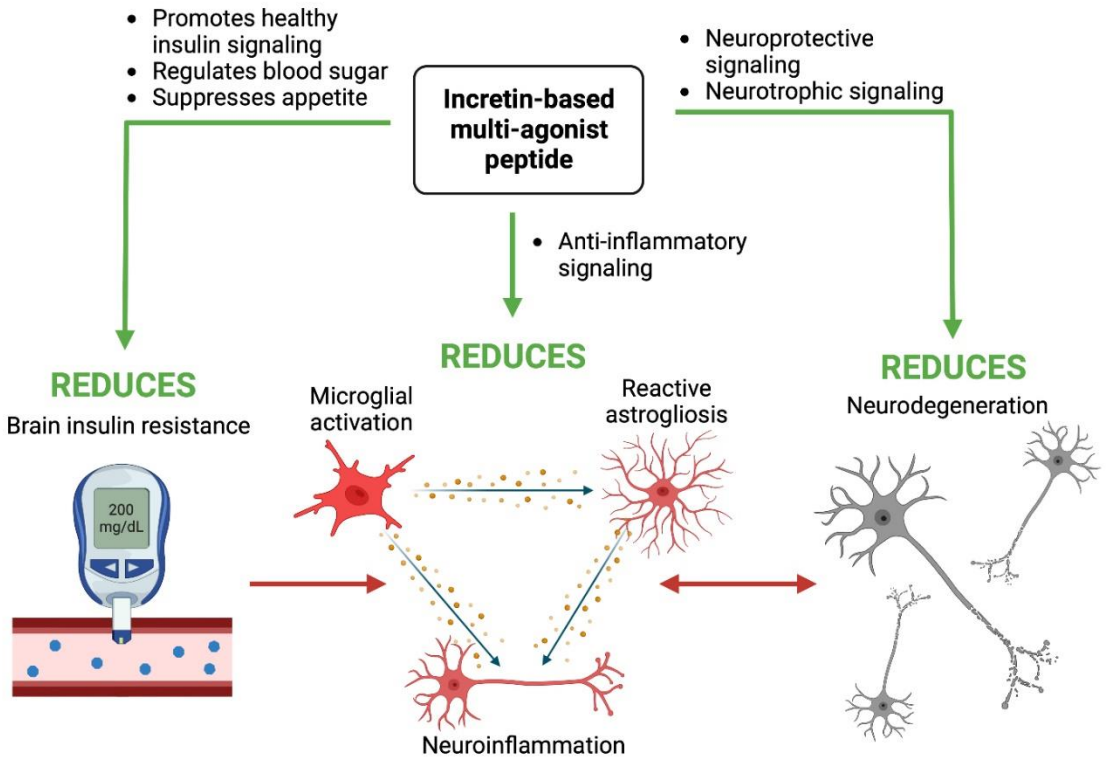
Abstract: Neurodegenerative diseases are accompanied and exacerbated by chronic neuroinflammation, yet no treatments targeting neuroinflammatory pathways have yet been successfully developed for neurodegenerative diseases. Interestingly, type 2 diabetes mellitus and insulin resistance have been linked with increased inflammation and neurodegenerative disease risk, warranting the study of effective diabetes drugs as treatments for neurodegeneration. Incretin receptor agonists are FDA-approved diabetes drugs that stimulate receptors with functions in maintaining healthy blood glucose levels and regulating insulin signaling, such as the glucagon-like peptide-1 (GLP-1) receptor and glucose-dependent insulinotropic polypeptide (GIP) receptor, and recently have combined glucagon (Gcg) receptor activation. Current research has led to the development of unimolecular compounds targeting multiple incretin/secretin receptors ("multi-agonists") to enhance drug benefits. Numerous incretin-based peptides are being investigated in preclinical and clinical studies as treatments for

neurodegenerative diseases, which has revealed anti-inflammatory and neuroprotective benefits of single incretin receptor agonists. However, less research has been conducted on the more novel multi-agonists. We investigate anti-neurodegenerative effects of dual (GLP-1 and GIP receptors) and triple (GLP-1, GIP, and Gcg receptors) agonists in preclinical models. We challenged human neuroblastoma cells with hydrogen peroxide or amyloid-beta 1-42 to simulate conditions of the Alzheimer's disease brain and treated with multi-agonists to evaluate their neuroprotective potential. We quantified markers of cell viability, cell death, oxidative stress, and neuroinflammation and found neurotrophic, anti-oxidative, and anti-inflammatory effects of the dual and triple agonists. Thus, we postulate that repurposing this drug class to treat neurodegenerative diseases may comprise a more efficient strategy to meet the urgent demand for treatments for these life-threatening conditions.



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Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.11/J7

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

Support: NIA Grant R01AG065582

Title: Crispr a/i microglia for screening targets of alzheimer's disease

Authors: *J. M. VICARI, Jr[1], R. KOSOY[2], X. WANG[2], Z. SHAO[2], J. FULLARD[2], C. P. KELLNER[3], P. ROUSSOS[2];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that manifests as memory and language deficits due to neuronal death, accumulation of misfolded proteins and inflammation in the brain. Growing evidence has pointed to microglia (MGs), the brain's immune cells, as a key cell type in the progression of AD. Therefore, targeting MGs may be key to develop new therapeutics for AD. To do this, we will use CRISPR activation and inhibition (CRISPRa/i) systems to screen genetic targets identified in primary human MGs (phMG) isolated from AD cases and controls. The human immortalized MG cell line HMC3 was characterized and used to generate stable lines expressing the CRISPR systems. HMC3s were transduced with dCas9-VPR or -KRAB containing lentivirus to generate stable CRISPR lines. dCas9 expression was determined by qPCR and effector activity evaluated using guide RNAs targeting IL6. Flow cytometry was used to observe MG surface markers and inflammatory activation determined in lipopolysaccharide (LPS) and amyloid-beta (Aβ) stimulated cells. HMC3s were positive for CD11b, Iba1 and TMEM119, but lowly expressed CD45. Following stimulation, both LPS and Aβ showed induction of IL1α and IL1β, while only LPS increased TNFα and IL6 expression. Myelin isolated from human brain tissue was labeled with a pH sensitive fluorophore (pHrodo) and used to assess phagocytic function. When treated with myelin, HMC3s had increased fluorescence after 24 hours compared to the cytochalasin-D negative control. RNA sequencing was performed on stimulated HMC3s, primary human microglia (phMG) and induced-pluripotent stem cell derived microglia (iPSC-MG) to compare transcriptional profiles. Sequence data showed iPSC-MGs were more similar to phMG compared to HMC3s, and that Aβ treatment induced protein targeting and mitochondrial pathways seen in phMG-AD which were not induced with LPS treatment. HMC3s transduced with guides targeting IL6 resulted in increased and decreased mRNA expression in VPR (2480%) and KRAB (24.8%) lines respectively. In conclusion, HMC3s expressed MG markers, demonstrate phagocytic functions and are activated to an inflammatory state when stimulated with LPS but not Aβ. While HMC3s are transcriptionally distinct from phMG, specific molecular pathways enriched by Aβ stimulation are also found in phMG from AD patients. Finally, stable HMC3 lines expressing the CRISPRa/i effectors were established and demonstrated their functionality using guide RNAs targeting IL6. This stable line has been well characterized and will serve as a useful tool for high throughput CRISPR screens to identify potential therapeutic targets for AD.

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Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.12/J8

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

Support: I01 BX004730
I01 BX003527
RF1AG063913

Title: Effect of the Rho-associated coiled-coil kinase inhibitor fasudil in Alzheimer's induced pluripotent stem cell-derived neuro-spheroids.

Authors: *E. GIUNTI[1,3], R. COLLU[1,3], S. A. DALEY[1,3], H. QUERFURTH[4], P. MORIN[2], R. KILLICK[5], R. MALAMED[6], W. XIA[1,3,6];
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Abstract: Alzheimer’s disease (AD) is the most predominant form of dementia. The brain accumulation of phosphorylated tau protein is considered a key contributor in the neuropathology of AD. Previous studies have linked the alteration of several signaling pathways with AD, but the mechanisms driving abnormal phosphorylation of tau remain to be elucidated. The Rho-associated coiled coil kinase (ROCK) inhibitor, fasudil, approved in 1995 in Japan to treat subarachnoid hemorrhage is considered a potential candidate drug against AD progression. In the present study, we aimed to explore the effect of the ROCK inhibitor fasudil in three-dimensional neuro-spheroids (3D neuro-spheroids) derived from AD and healthy control (HC) induced pluripotent stem cells (iPSCs).3D neuro-spheroids were differentiated from peripheral blood mononuclear cells (PBMC) derived from AD and HC human patients. Combining enzymatic immune assay, transcriptomic and mass spectrometry-based proteomic analysis we characterized the profile of 3D neuro-spheroids cultures and investigated the response to fasudil treatment on AD-related makers. Our results showed an evident increase of phosphorylated tau (pTau) at four different residues (pTau-181, -202, -231 and -396), and a decrease of secreted clusterin protein (clu) in AD-derived 3D neuro-spheroids as compared to HC ($P<0.001$), that were reverted in the presence of fasudil.Transcriptomic analysis revealed a reduction in the expression of AKT serine/threonine-protein kinase 1 (AKT1) in AD compared to HC ($P<0.05$), condition that was reverted in the presence of fasudil. Proteomic analysis revealed an up-regulation of the glycogen synthase kinase-3β (GSK3β) protein expression in AD ($P<0.001$) and a down-regulation in the same samples after fasudil treatment. In our AD-derived neuro-spheroids, fasudil appears to enhance AKT1 expression and to modulate clu protein levels through a ROCK dependent mechanism that results in the suppression of AD associated tau phosphorylation. Wnt and PI3K/Akt signaling pathways seems to be strongly involved in the effect of fasudil on tau phosphorylation. Future studies are needed to deeply explore PI3K/Akt and Wnt pathways and fasudil mechanisms as potential AD therapeutic development.

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Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.13/J9

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

Support: The Alzheimer's Society of Alberta and Northwest Territories
The University Hospital Foundation to the SynAD Group at the University of Alberta as a part of the Hope for Tomorrow Project
The Canadian Institutes of Health Research (PS 159746)

Title: Extracellular vesicles enriched in amylin receptors improve amyloid beta-induced reductions in hippocampal synaptic plasticity.

Authors: *R. KIMURA[1], R. N. SOUDY[2], W. FU[3], A. PATEL[3], J. H. JHAMANDAS[3]; [1]Sanyo-Onoda City Univ., Sanyo-Onoda, Japan; [2]Dept. of Med., [3]Med. (Neurology), Univ. of Alberta, Edmonton, AB, Canada

Abstract: Alzheimer's disease (AD) is characterized by accumulation of amyloid-β peptide (Aβ) in the brain regions that subserve memory and cognition. We have previously demonstrated that effects of soluble oligomeric $A\beta_{1-42}$ and human amylin on hippocampal long-term potentiation (LTP) are mediated by the amylin receptor (AMY). In addition, a recent revealed that AMY3 receptor subtype enriched extracellular vesicles (EVs) protect N2a cells against Aß toxicity. EVs are double membrane structures released by all cell types with identified roles in the generation, transportation, and degradation of Aβ oligomers in AD. In the present study, we examined the effects of soluble oligomeric $A\beta_{1-42}$ (50 nM)-evoked depression of LTP at Schaeffer collateral-CA1 hippocampal synapses in the presence of EVs generated from either wildtype (Wt) or AMY3 transfected HEK cells. In mouse hippocampal brain slices, Aβ depressed LTP evoked using 3-theta burst stimulation (TBS) protocols and EVs from either Wt or AMY3 cells were perfused for 5 min prior to applications of soluble oligomeric Aβ. Applications of EVs alone did not demonstrate abnormalities in basal synaptic transmission or LTP. However, in the presence of EVs from AMY3 cells, but not those from Wt cells, $A\beta_{1-42}$-induced reduction of LTP was significantly improved. Our observations support the role of AMY receptors, particularly AMY3, as a potential therapeutic target for AD.

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Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.14/J10

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

Support: SAF2017-88812-R
PID2020-119236RB-I00
PID2020-119386RB-I00
PID2019-108842GB-C21
PID2019-106447RB-I00
FPU18/00194
IBES-2015-072727
FI-B-00378
FPU21/02928
MJFF-000858

Title: Rtp801/redd1 propagates transneuronal toxicity via extracellular vesicles

Authors: *J. SOLANA BALAGUER[1,2,3], N. MARTÍN FLORES[1,2,4], P. GARCIA SEGURA[1,2,3], G. CAMPOY CAMPOS[1,2,3], L. PÉREZ SISQUÉS[1,2,5], A. CHICOTE GONZÁLEZ[1,2,3], J. FERNÁNDEZ IRIGOYEN[6], E. SANTAMARÍA[6], E. PÉREZ NAVARRO[1,2,3,7], J. ALBERCH[1,2,3,8], C. MALAGELADA[1,2,3];
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Abstract: Extracellular vesicles (EVs) play a crucial role in intercellular communication, participating in the paracrine trophic support or in the propagation of toxic molecules, including proteins. RTP801 is a stress-regulated protein, whose levels are elevated during neurodegeneration and induce neuron death. However, whether RTP801 toxicity is transferred trans-neuronally via EVs remains unknown. We overexpressed or silenced RTP801 protein in rat cultured cortical neurons using lentiviral particles, and at DIV13 we isolated the neuron-derived EVs (RTP801-EVs, or shRTP801-EVs respectively) by sequential ultracentrifugation (UC). EVs release was quantified by nanoparticle tracking analysis (NTA) and the protein content was

assessed by mass spectrometry (MS) and western blotting (WB). RTP801-EVs or shRTP801-EVs were used to treat sister neuronal cultures for 24 h and apoptotic neuron death was analyzed by immunofluorescence (IF), by assessing nuclei fragmentation and cleaved-caspase-3 levels. Neuron branching complexity was also assessed with MAP-2 staining using Cell Profiler software. We found that RTP801 is present and released in neuron-derived EVs, suggesting its potential toxic/regulatory function via EVs. We confirmed that RTP801 overexpression increased the number of EVs released by neurons by NTA and the levels of EVs-markers by WB. Increasing evidence shows that intracellular stress can modulate the specific sorting of proteins and other components. In line with these observations, we found that RTP801 expression modulation led to a distinct proteomic signature of neuron-derived EVs. When RTP801 was overexpressed, neuron-derived EVs contained more pro-apoptotic markers. RPS6 was found at low levels in RTP801-EVs, in line with the inhibitory role of RTP801 of the mTOR pathway. In contrast, silencing RTP801 led to an increase in anti-apoptotic proteins in EVs. Interestingly, RTP801-EVs treatment increased the levels of active caspase-3 in neurons and had a visible effect on late apoptosis. In addition, RTP801-EVs significantly impaired dendritic arborization in recipient neurons, further confirming RTP801 transcellular toxicity. In contrast, EVs derived from neurons where RTP801 was silenced were able to increase the arborization of treated neurons. Taken together, these results suggest that RTP801 toxicity is spread via EVs and therefore, it could contribute to the progression of neurodegenerative diseases, in which RTP801 is involved.

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Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.15/K1

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

Support: NIA R56 - The infectious etiology of Alzheimer's disease

Title: Alzheimer's disease pathologies may act as a pathogen-driven neuron protective strategy

Authors: *V. R. HYDE[1], C. ZHOU[1], K. CHATTERJEE[1], J. FERNANDEZ[1], P. RAMAKRISHNA[1], A. LIN[1], G. FISHER[1], O. TUNÇ ÇELIKER[1], J. CALDWELL[2], O. BENDER[4], P. KINCHINGTON[3], D. BAR[4], L. D'AIUTO[2], O. A. SHEMESH[1]; [1]Neurobio., [2]Psychiatry, [3]Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA; [4]The Maurice and Gabriela Goldschleger Sch. of Dent. Med. Res. Labs., Tel Aviv, Israel

Abstract: Alzheimer's disease (AD) is diagnosed via postmortem detection of extracellular amyloid beta (Aβ) plaques or oligomers and intracellular hyperphosphorylation tau. These canonical pathologies are key players in AD etiology. A complementary line of research suggests that common human pathogens serve as the initial seeding agents which facilitate the pathologies of AD. We used expansion microscopy (ExM), a tissue manipulation method which enables the imaging of biological samples at 40nm resolution. For our purposes, we used a novel version of ExM, decrowding expansion pathology (dExPath), which exposes inaccessible protein epitopes to antibody staining while enabling super resolution imaging. Using dExPath, we visualized pathogen-related proteins within human brain samples. We discovered a clear colocalization between AD pathologies and pathogen-derived proteins. Utilizing human brain organoids and rodent cultures as model systems for the human brain, we used pathogen infection to replicate the AD pathologies observed in the AD human brain. Our results suggest that (1) neurons exposed to pathogens produce human AD pathologies, (2) AD pathologies may be antimicrobial, and (3) AD pathologies may be neuron protective. With these important roles in mind, our continued research focuses on isolating the mechanism from which the proposed neuron-protective strategy operates.

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Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.16/K2

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

Support: NIH Grant R01AG067419
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NIH Grant RF1NS130713

Title: Amyloid-beta and tau proteins vary across the lifespan of humans and chimpanzees

Authors: *M. K. EDLER[1], I. MHATRE-WINTERS[2], C. C. SHERWOOD[3], P. R. HOF[4], W. D. HOPKINS[5], J. R. RICHARDSON[2], M. RAGHANTI[1];
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Abstract: Neuropathologic hallmarks of Alzheimer's disease (AD) include amyloid-beta (Aβ) plaques and tau-associated neurofibrillary tangles (NFT). Plaques and NFT are formed when toxic soluble monomers aggregate into insoluble oligomers and fibrils. While AD is unique to humans, elderly chimpanzee brains also exhibit Aβ plaques, NFT, and cerebrovascular amyloid yet lack substantial neuron loss. To investigate this species difference, multiplex immunoassays were used to quantify soluble Aβ40, Aβ42, phosphorylated-tau 231 (p-tau), and total tau protein in the dorsolateral prefrontal cortex (DLPFC), middle temporal gyrus (MTG), and lateral cerebellar cortex (CB) of unaffected control humans (Braak Stage I-II; n = 61, 0-97 y) and chimpanzees (n = 39, 15-58 y) sex- and age-matched across their respective lifespans. Using ultracentrifugation and guanidine hydrochloride (GuHCl) extraction, insoluble Aβ40 and Aβ42 protein levels were also measured. No sex differences were detected for any variable. ANCOVA analyses revealed that humans have higher levels of soluble Aβ40 (DLPFC, MTG), soluble Aβ42 (MTG), and ratios of soluble Aβ42/Aβ40, soluble p-tau/total tau, and insoluble Aβ42/Aβ40 (MTG) than chimpanzees (p's ≤ 0.03). However, soluble p-tau and total tau did not differ between species (p's ≥ 0.09). Soluble Aβ40 (H: DLPFC; C: MTG) and Aβ42 (H: MTG; C: MTG, CB) increased with age in humans and chimpanzees (p's ≤ 0.02). Both species also exhibited age-related increases in insoluble Aβ40 and Aβ42 (DLPFC, MTG), though only chimpanzees experienced a rise in the CB (p's ≤ 0.02). Soluble p-tau and total tau declined with age in humans in all regions (p's ≤ 0.01). Conversely, chimpanzees demonstrated an age-related increase in p-tau in the CB ($p \leq 0.02$). Age correlated with a greater ratio of soluble Aβ42/Aβ40 in chimpanzees (MTG, CB; p's ≤ 0.05), whereas humans exhibited age-associated increases in ratios of insoluble Aβ42/Aβ40 (DLPFC, MTG; p's ≤ 0.01) and p-tau/total tau (DLPFC; $p \leq 0.05$). Unexpectedly, chimpanzees displayed a rise in insoluble Aβ42/Aβ40 levels in the CB during aging ($p \leq 0.04$), while p-tau/total tau ratios in chimpanzees appeared stable (p's ≥ 0.16). Growing evidence suggests that soluble Aβ42 and p-tau are highly toxic to neurons and synapses; therefore, humans having greater ratios of soluble Aβ42/Aβ40 and p-tau/total tau illustrate key species variances that may contribute to reduced NFT and a relative lack of neuron loss in chimpanzees despite high levels of Aβ. Identifying differences that potentially afford protection to chimpanzees from neurodegenerative processes is critical to creating novel translational therapeutic interventions.

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Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

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

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The University of Miami Team Science Funding Program

Muriel, Murray, and Robert Smith Foundation
The University of Miami Miller School of Medicine

Title: Alzheimer's disease-like characteristics in long-COVID

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Abstract: Long-COVID is a debilitating condition that has been observed in a substantial portion of patients who have suffered from severe SARS-CoV-2 infection. However, our current understanding of the impact of mild to moderate SARS-CoV-2 infection in non-hospitalized individuals remains limited. Accordingly, it is postulated that many previously infected individuals will experience at least mild to moderate long-term symptoms, as more than 200 symptoms affecting multiple organs have been identified post-COVID. It is estimated that long-COVID affects approximately 65 million individuals worldwide, with the number of cases growing daily. The major symptoms and conditions associated with long-COVID include stress, fear, loneliness, cognitive impairment known as “brain fog”, myalgic encephalomyelitis/chronic fatigue syndrome, postural orthostatic tachycardia, anxiety, depression, and sleep disorders. Muscular weakness, neurocognitive impairments, and pain syndromes are frequently observed in people who require intensive care during their infection and hospitalization. In a recent study utilizing a murine model of long-COVID (with Murine Hepatitis Virus-1 as the surrogate infection), we reported notable brain findings including the presence of hyperphosphorylated tau and TDP43, activated microglia and astrocytes, along with a reduction in pre-synaptic protein synaptophysin. We now identify alterations in several genes in the brains of these mice that are characteristic of Alzheimer’s disease (AD) long-term post-infection. These include increased mRNA expression of LDL receptor-related protein (1.6-fold vs. Sham), alpha 2 macroglobulin (0.55-fold), USP7 (3.9-fold), SERPINA5 (1.2-fold), FE65, CCNF, BACE1, DCTN1, and RNF130 (1.0, 0.64, 0.81, 2.1, and 1.35-folds, respectively vs. Sham), and decreased levels of sAPP beta and CTF beta mRNA (57.6 and 67.3% vs. sham), presenilin 1/2 (61.4 and 49.1% vs. Sham), and apolipoprotein E (52.8%). Additionally, we detected the presence of neurofibrillary tangles (with silver and thioflavin-S staining), fibrous plaques, B cell depletion, increased sAPP and CTF alpha (0.9 and 1.8-fold vs. Sham) suggestive of AD-like disease progression in these mice. These changes were predominantly observed in the cortex, hippocampal CA1, and dentate gyrus, as well as in the brain stem. Moreover, these changes are associated with deficits in motor coordination as measured by the Rota-rod apparatus, which was observed as early as 150 days post-infection. These findings collectively suggest the possible development of AD in a considerable proportion of hospitalized patients, as well as non-hospitalized individuals.

Disclosures: **M.J. Paidas:** None. **R. Ramamoorthy:** None. **H. Hussain:** None. **A.D. Masciarella:** None. **D.M. Di Gregorio:** None. **A.R. Jayakumar:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.18/K4

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

Support: Key Technologies R & D Programme of Henan 232102311226

Title: Alleviating effects of Isoquercetin-ligustrazine co-polymorph on cognitive impairments in Alzheimer's disease

Authors: *Y. YANG[1], X. LONG[1], L. DU[2], X. XIE[1];
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Abstract: Alzheimer's disease (AD) is one of the most common neurodegenerative disorder characterized by memory loss and cognitive impairments in the elderly. Isoquercitrin and ligustrazine have shown beneficial effects on cognitive dysfunction in AD animal models. Combining isoquercitrin and ligustrazine further enhances autophagy activation and mitigates neuronal injury. In this study, we aim to investigate the underlying mechanism of isoquercetin-ligustrazine co-polymorph (ILCP) in improving cognitive dysfunction in AD mice models. The effects of ILCP on oxidative stress, mitochondrial damage pathology, beta-amyloid pathology, and impaired learning and cognitive capabilities were examined. Neuronal apoptosis and astrogliosis were also assessed in various brain regions. Our findings revealed multiple pharmacological effects of ILCP in ameliorating AD-related cognitive disorders and memory decline. ILCP application effectively reduced cortical and hippocampal beta-amyloid aggregation, neuro-inflammation, and astrogliosis during AD development. Our results suggest that ILCP significantly improves cognitive and memory deficits associated with AD in mouse models, highlighting its potential as a therapeutic agent for the treatment of Alzheimer's disease.

Disclosures: **Y. Yang:** None. **X. Long:** None. **L. Du:** None. **X. Xie:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.19/K5

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

Support: HEAL program at NCATS

Title: Brain region-specific spheroids for modeling Alzheimer's disease and therapeutic evaluation

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Abstract: Alzheimer's disease (AD) continues to be a large public health burden with no curative agents and very few therapeutic options available. The lack of clinically predictive *in vitro* human-based preclinical models is a challenge for the drug discovery and development of effective therapies for AD. 3D organotypic cellular models that better mimic the physiological complexity of the human brain are being developed as clinically predictive assay platforms for drug testing. To create a high throughput (HTS)-compatible, predictive biomimetic *in vitro* assay model for AD, we have established a protocol to generate functional 3D neural spheroids by using human induced pluripotent stem cell (hiPSC)-derived neurons and astrocytes. These neural spheroids are assembled by mixing and aggregating fully differentiated and matured glutamatergic neurons, GABAergic neurons, and astrocytes at a composition that mimics the human prefrontal cortex (PFC, resulting spheroids referred to here as PFC-like spheroids). To assess neuronal activities of the neural spheroids *in vitro*, we use both a calcium dye as a high-throughput compatible functional readout, as well as genetically encoded fluorescent biosensors to further examine cell-type specific activities within the spheroids. We have previously shown that these PFC-like neural spheroids exhibit unique and reproducible baseline calcium activity profiles contributed by both glutamatergic and GABAergic transmission. To generate AD disease models, we either incorporate GABAergic neurons with AD disease-related variants (APOE4/4 or APP A673V) into the neural spheroids or treat the healthy spheroids with oligomeric amyloid beta to induce deficits in baseline calcium activities. These neural spheroid models are produced in 384-well plate format to enable the screening of large compound libraries, including clinically approved drugs, to look for compounds that pharmacologically reverse the AD calcium activity phenotype. In summary, we describe a novel platform of functional neural assays to model Alzheimer's disease using brain region-specific neural spheroids assembled with pre-differentiated iPSC-derived neurons and astrocytes, in a HT-compatible format for drug screening.

Disclosures: J. Zhang: None. **C.E. Strong:** None. **M. Carrasco Carvajal:** None. **E. Lee:** None. **M. Ferrer:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.20/K6

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

Support: NRF Grant 2022R1A2C1011996
NRF Grant 2022K1A3A1A20015190
NRF Grant 2022K2A9A1A01098131
22RB1130

Title: Luteolin improves the congnitive function in the 5xFAD Alzheimer's disease model mice

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Abstract: The cognitive deficits in Alzheimer's disease (AD), are associated with the neuronal loss, abnormal protein processing, cellular oxidative stress in the specific brain regions of AD patients. Flavonoids, the bioactive polyphenolic phytochemicals, are known as the natural product with antioxidative, anti-inflammatory effects. In this study, we determined the effects of luteolin which are one of the flavonoids, obtained from the plant Reseda luteolin, on *in vivo* and *in vitro* AD models. We daily administered luteolin into 5xFAD AD model mice for 21 days (20mg/kg,i.p.). Luteolin showed the anti-depressant effects and anti-anxiety effects on the behavioral tests including tail suspension test and open field test. We also found that luteolin increased cell viability and neurite outgrowth under the amyloid beta associated *in vitro AD* conditions using mouse hippocampal neuronal HT-22 cells. These data suggest that luteolin induces the behavioral recovery, neuroprotection, and neuroregeneration in the AD models.

Disclosures: **S. Sun:** None. **H. Hu:** None. **H. Seo:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.21/K7

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

Support: University of Toronto Fellowship
CAMH Discovery Fund

Title: Characterization of extracellular vesicles derived from neuronal, astrocytic, and microglial cell lines following autophagic-lysosomal modulation

Authors: *S. TAM[1,3], A. SEREGIN[5,3], A. KHANI[2,3], H. YU[1,3,4];
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Abstract: Extracellular vesicles (EVs) are lipid-bound vesicles that contribute to normal physiological function including intercellular communication, immune system activation, cellular homeostasis, and cargo clearance. Emerging evidence indicates that EVs may be involved in neurodegenerative diseases like Alzheimer’s disease (AD) through changes in pathological transmission and altered autophagic-lysosomal system (A-LS) function. Despite this, mechanistic insights on the functions of EVs in both healthy and diseased brain conditions have yet to be thoroughly investigated. Moreover, the link between the A-LS, EVs, and AD

requires elucidation. Based on our lab's previous data showing changes in EV biogenesis and cargo upon autophagic modulation, we hypothesized that inhibition of autophagy in cells would result in increased biogenesis of EVs and altered protein composition and expression. Using major brain cells of human and murine origin such as neurons (SH-SY5Y, mHippoE14, respectively), astrocytes (U87-MG, C8-D1A), and microglia (HMC3, BV-2), we examined the impacts of autophagic modulation on EV biogenesis and cargo through chemical treatment with autophagic activators (KU-0063794, trehalose, TFEB activator 1) and inhibitors (bafilomycin A1, spautin-1). EVs were isolated using ExoQuick-TC and analyzed biochemically through Western blot and flow cytometry, and biophysically through transmission electron microscopy (EM) / cryo-EM and NanoTracker Analysis (NTA). Existing literature indicates that increased levels of autophagy impede EVs release, supporting a hypothesis of elevated EV concentration and size following autophagic inhibition. Interestingly, we found that mean concentration and size of EVs is unchanged by autophagic modulation, as our NTA results revealed no significant differences in mean particle concentration and size between treatment groups. Our Western blot results show differences in protein cargo between cell types upon autophagic modulation, thus aligning with our lab's previous findings demonstrating increased transport of pathogenic proteins in response to inhibition of autophagy. These findings provide novel insights into the role of autophagy on EV biogenesis and cargo and offer essential knowledge into the potential impacts of A-LS dysfunction on the neuropathogenic progression of AD and the translational opportunities of EVs as biomarkers and therapeutics. Future work will employ EVs and changes in autophagic markers as measures of proteostasis and stress, with prospective clinical applications as diagnostic tools.

Disclosures: **S. Tam:** None. **A. Seregin:** None. **A. Khani:** None. **H. Yu:** None.

Poster

PSTR196. Alzheimer's Dementia Models and Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR196.22/K8

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

Support: NSERC Grant: 2016-05964
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University of Manitoba Tri-Agency Bridge: 57289
Alzheimer Society of Manitoba Graduate Student Fellowship: N/A
McCrorie-West Family Fellowship in Alzheimer's Research: 44172
Baxter Bursary: N/A

Title: Predicting cognitively stability in patients with prodromal Alzheimer's disease for the ethical use of anti-amyloid pharmaceuticals

Authors: *J. R. PERRON[1], J. KO[2];
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Abstract: Anti-amyloid drugs now exist for the treatment of Alzheimer's disease (AD), a neurodegenerative disease for which there is no cure, but they are costly and have severe side-effects. These drugs are effective when used early in AD progression and are indicated for patients with early AD dementia, however, post-mortem studies show that AD diagnoses are moderately sensitive, modestly specific and most accurate late in AD progression. Mild cognitive impairment (MCI), defined by greater than expected cognitive decline for age and education, is potentially prodromal to AD because over 3 years about 20% of patients progress from MCI (PMCI) to dementia, but most remain cognitively stable (SMCI). Effective and ethical use of anti-amyloid drugs in a paradigm of early detection for pharmaceutical intervention requires early discrimination between MCI groups. We posit that machine intelligence is able to identify subtle differences in brain metabolism across a spectrum of cognitive and biomarker profiles specific to patients with MCI. Fluorodeoxyglucose positron emission tomography (FDG-PET) is able to precisely measure brain glucose metabolism *in vivo*, and so FDG-PET studies of 432 patients (minimum 3 year follow-up) from the Alzheimer's Disease Neuroimaging Initiative whose amyloid and tau measures were available underwent preprocessing and stratification by biomarker profile. Training and testing sets were generated by randomly splitting 80% and 20% of 169 amyloid and tau positive subjects with MCI balanced at the level of the subject for age, sex, education, APOE ε4, race and mental exam scores. The training data was augmented by including AD_{A+T+} and SHC_{A+T+} subjects into balanced training folds. Densely-connected convolutional neural networks were trained by 5-fold cross-validation. The machine intelligence classified $SMCI_{A+T+}$ vs $PMCI_{A+T+}$ test set subjects with area-under-curve of 87.5% and 100% specificity for $SMCI_{A+T+}$ subjects. It was also accurate in SMCI subjects with other biomarker profiles (86.7% - $SMCI_{A+T-}$, 98.0% - $SMCI_{A-T-}$) and predicted cognitive decline in 90.6% of all $PMCI_{A+T+}$ subjects at baseline. Class probability scores generated by the machine intelligence were statistically significant (two-sample t-testing, $p < 0.001$) between $PMCI_{A+T+}$ and all SMCI groups at baseline. The excellent performance in the holdout set and specificity to the spectrum of SMCI biomarker profiles suggests that the class probability score output by the machine intelligence is a meaningful prognostic marker of cognitive stability that may prevent unnecessary treatment with anti-amyloid drugs and provide a means of enrichment for clinical trials of anti-AD drugs

Disclosures: J.R. Perron: None. **J. Ko:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.01/K9

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

Support: JBP foundation
NIH

Title: Interactions of neuromodulatory brain states with the beneficial effects of 40 Hz gamma stimulation on amyloid pathology

Authors: C. BLANCO[1], ***M. C. KAHN**[2], N. LAVOIE[1], H. NAWAID[1], A. LOON[1], R. RAJU[1], A. DAVISON[1], C.-Y. YANG[1], L.-H. TSAI[1];
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Abstract: Sensory stimulation at 40 Hz is emerging as a promising treatment for Alzheimer's Disease. In particular, several groups have shown that this non-invasive treatment can reduce the burden of pathological amyloid and tau in mouse models and the first clinical trials have begun to suggest beneficial effects in humans. Therefore, it is paramount to understand how gamma stimulation exerts its beneficial effects and what clinically relevant factors can modulate treatment outcomes. The aim of this study is to characterize the bidirectional interactions between gamma stimulation and brain states, such as sleep or neuromodulatory transmitter release (e.g. acetylcholine). We use the 5xFAD mouse model of amyloid accumulation, wherein the amyloid-reducing effects of gamma stimulation are well characterized. In 5xFAD mice, we employ closed-loop electrophysiological recordings in freely moving mice to characterize the effects of gamma stimulation on arousal states. Moreover, we use genetically encoded fluorescent sensors for neuromodulatory neurotransmitters to assess how gamma stimulation affects the brain's neuromodulatory state. Finally, we combine in-vivo pharmacology with biochemical assays (i.e. ELISA) and immunohistochemistry to characterize how arousal state modulates the effects of gamma stimulation on amyloid burden. We find that the beneficial effects of GENUS significantly depend on but do not affect brain state. Interestingly, we find that this effect is unlikely to be mediated by differences in brain entrainment to stimulation at gamma frequency. Our data suggest that the clinical efficacy of GENUS may depend significantly on the brain state.

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Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.02/K10

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

Support: NIH–NCCIH R15 Grant (1R15AT010789-01A1)

Title: Cranial osteopathic manipulation alters Alzheimer's disease phenotype in wild type and transgenic rat models

Authors: *D. HINES[1], H. TOBEY[5], L. KWAPISZ[5], A. INGRAM[5], P. DUGGAN[5], S. BOEHRINGER[2], J. PIWOWARSKI[5], A. RAMANATHAN[5], R. ANANDAKRISHNAN[5,3], P. J. VANDEVORD[1], B. COSTA[5,3,4];
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Abstract: As humans age, fluid circulation decreases in the brain, causing a build-up of macromolecules leading to neuroinflammation which has been linked to Alzheimer's Disease development (Iliff et al.,2013). The CNS lymphatic vessels clear this metabolic waste, including amyloid beta (Aβ), which is associated with AD (Louveau et al., 2015). Cranial Osteopathic Manipulation (COM) is a novel noninvasive treatment that could help alleviate this issue since there is a lack of practical physiological or pharmacological mechanisms to increase fluid circulation. Based on our pilot study, immunoassay analysis and positron emission tomography (PET) showed that COM reduced Aβ levels, activated astrocytes, and improved excitatory neurotransmission in aged rats. This study planned to confirm these findings using Fischer 344 transgenic rats (Tg) and 3-month-old wild type (WT) rats of both sex split into COM and untreated control groups. COM was performed for 7 days with the osteopath wearing FingerTPS's nano sensor gloves to quantify the pressure applied to the occipital squama. Spatial learning and memory were assessed using Morris Water Maze (MWM) for Tg rats and MWM plus Novel Object Recognition (NOR) for WT rats. Handlers were blinded for both assays. To identify differentially expressed genes associated with AD, proteome analysis was performed on Tg hippocampal tissue. The Holm-Šídák multiple comparison T-Test showed significant differences in 7 MWM parameters for Tg and 9 for WT on day 5 (platform hidden), including Shortest Visit to the NW Zone. Numerical differences were present among Tg and WT. The same analysis was used for NOR. COM treated animals spent numerically more time exploring the novel object. The proteome analysis of the Tg hippocampal tissue identified that COM significantly increased the expression of serine-threonine kinase P21-activated kinase 3 (PAK3), and fifty other proteins. These findings indicated that COM improved cognitive function as it induced improvement in spatial learning and memory parameters for Tg and WT rats. Further studies with larger sample sizes are underway to validate current findings. Therefore, our results favor the clinical use of COM therapy as a non-invasive, non-pharmacological treatment for AD patients.

Disclosures: D. Hines: None. **H. Tobey:** None. **L. Kwapisz:** None. **A. Ingram:** None. **P. Duggan:** None. **S. Boehringer:** None. **J. Piwowarski:** None. **A. Ramanathan:** None. **R. Anandakrishnan:** None. **P.J. VandeVord:** None. **B. Costa:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

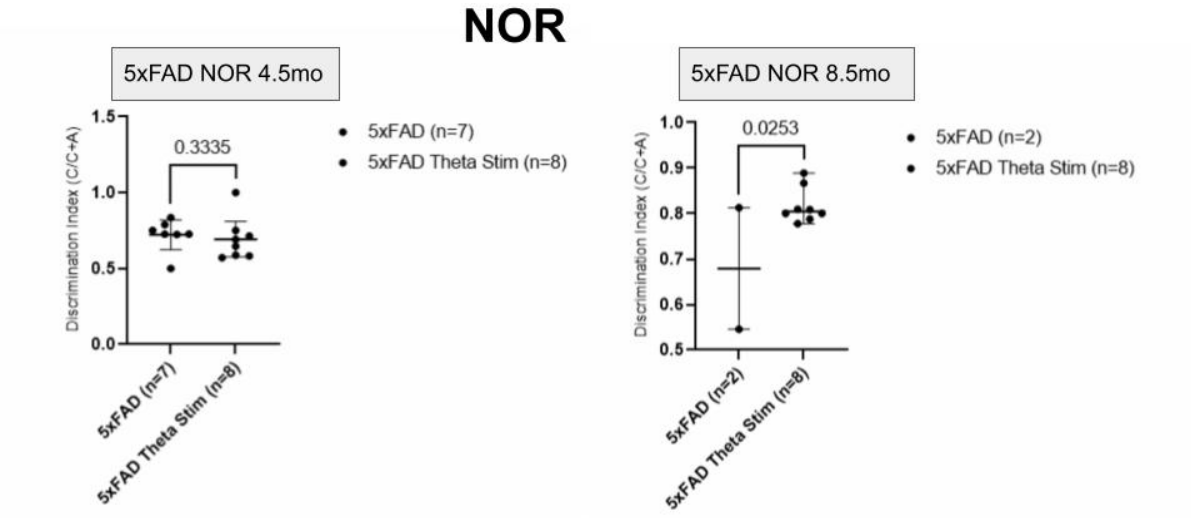
Program #/Poster #: PSTR197.03/L1

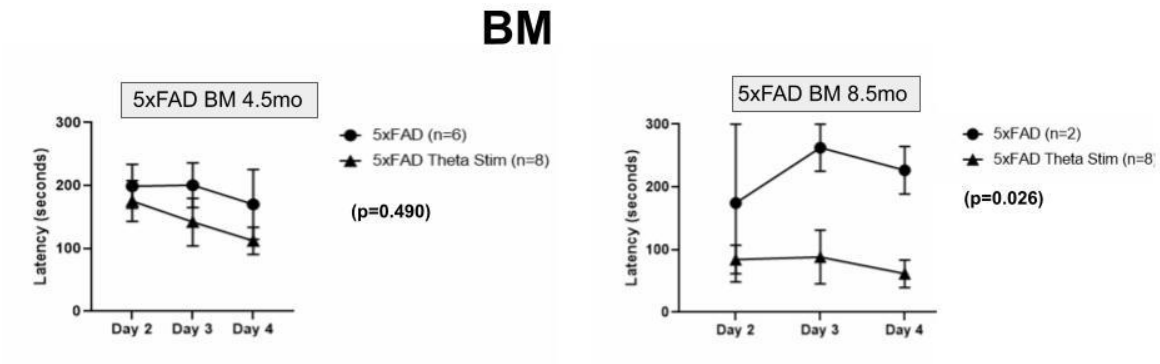
Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neuromodulation of the septohippocampal network through medial septal nucleus theta deep brain stimulation rescues both recognition and spatial memory in a 5xFAD model of Alzheimer's disease.

Authors: *N. C. ZEPEDA, M. B. BERGOSH, W. CHOI, D. J. LEE;
Neurolog. Surgery, KSOM Univ. of Southern California, Los Angeles, CA

Abstract: Recent evidence suggests that neuromodulation, such as deep brain stimulation (DBS), may improve cognitive dysfunction seen in Alzheimer's Disease (AD). Here, we evaluate the potential benefits of medial septal nucleus (MSN) theta frequency (7.7 Hz) DBS on memory in a 5xFAD mouse model of AD. 16 male 5xFAD mice were implanted with hippocampal, nucleus accumbens, nucleus basalis, thalamic, and medial prefrontal cortex recording electrodes and a bipolar MSN stimulating electrode at 3.5 months of age. Mice underwent MSN DBS (n=8) or sham DBS (n=8) during a novel object recognition task (NOR), elevated plus maze (EPM) and Barnes maze (BM) task at two time points (4.5 and 8.5 months of age).
While there was no difference between stimulation and no stimulation groups in the NOR (p=0.334) or BM (p=0.490) at 4.5 months, MSN theta DBS resulted in significant improvements in NOR times (p=0.025) and BM latency (p=0.026) at 8.5 months. No significant difference was found between groups for EPM at any time point (at 4.5 months (p=0.407) or 8.5 months(p=0.186)).
These findings suggest that modulating the septohippocampal network through MSN theta DBS may be able to rescue both recognition and spatial memory in a 5xFAD model of Alzheimer's disease.





Disclosures: **N.C. Zepeda:** None. **M.B. Bergosh:** None. **W. Choi:** None. **D.J. Lee:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.04/L2

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

Support: NSERC Discovery Grant RGPIN/03909-2021
Canada Foundation for Innovation # 41428

Title: Modulating sleep using optogenetic activation of the thalamic reticular nucleus in mice

Authors: *S. TOK[1], M. ARAI[2], T. YILDIRIM[3], B. A. KENT[1];
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Abstract: Title: Modulating sleep using optogenetic activation of the thalamic reticular nucleus in mice
Abstract: Optogenetics is a powerful tool for understanding neural circuitry involved in physiological and behavioral processes. The thalamic reticular nucleus (TRN), composed of GABAergic neurons surrounding the thalamus, regulates sensory information flow, attention, and sleep-wake transitions. Inadequate inhibitory drive caused by interneuronal dysfunction may lead to sleep fragmentation and disruption, including the sleep disturbances common in early-

stage Alzheimer's disease (AD).
Our study explores how optogenetic stimulation of GABAergic TRN neurons affects sleep. Using a two-virus strategy, we expressed channelrhodopsin-2 (ChR2) in GABAergic cells of the TRN in mice. Subsequently, these mice (five- to eleven-month-old, c57BL/6; n= 16) received both tonic and phasic stimulation at rostral or caudal segments of the TRN at ZT 16 for 1 hour. Control mice received a sham injection (i.e., saline). The optogenetic stimulation induced changes in sleep, and sleep architecture, quality, power spectra, and sleep-dependent behaviors, which were assessed via simultaneous electroencephalography (EEG) and electromyography (EMG) recordings.
We are now evaluating these manipulations in a mouse model of AD, the APP-KI NL-F model in order to assess efficacy in terms of ameliorating sleep disruptions, fragmentation, pathology, and improving functional cognitive outcomes. These readouts will be measured using EEG analysis (e.g., sleep staging, power spectra analysis), immunohistological approaches (e.g., amyloid load, neuroinflammation) and functional cognitive touchscreen testing (e.g. trial-unique, delayed non-matching to location (TUNL)).
In conclusion, these findings shed light on the precise mechanisms through which TRN contributes to sleep regulation and highlight the potential of optogenetics as a powerful tool for developing innovative therapeutic strategies for neurodegenerative and sleep disorders. By unraveling the neural circuitry underlying sleep control we hope to identify potential targets for future therapeutic interventions aimed at sleep disruption not only in AD, but other sleep disorders as well.

Disclosures: S. Tok: None. **M. Arai:** None. **T. Yildirim:** None. **B.A. Kent:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

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Program #/Poster #: PSTR197.05/L3

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

Support: R21-MH121723-S1

Title: Retinal regulation of Locus Coeruleus: A chemogenetic approach to treat neurodegenerative disorders.

Authors: *S. DELCOURTE[1,2], G. CROZIER[2], Y. RAKHOLIA[2], G. ASTON-JONES[2];
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Abstract: Alzheimer's Disease (AD) is the most prevalent form of dementia. Clinical studies show that abnormal accumulation of tau protein in the locus coeruleus (LC) may play an early role in AD progression. Using an animal model of AD with this early LC tau pathology, the TG-F344 AD rat, Rorabaugh et al. (2017) showed that specific chemogenetic activation of LC rescued impaired reversal learning. Given its deep location in the brainstem, LC is difficult to

access in humans, limiting this approach for clinical application. Suprachiasmatic nucleus (SCN) provides an indirect input to LC via a relay in dorsomedial hypothalamus (DMH) (Aston-Jones et al., Nat. Neurosci. 2001). SCN is therefore in a key position to integrate light information with LC, via a circuit we denote as the Photic Regulation of Arousal and Mood (PRAM) pathway: retina ->SCN->DMH->LC (Bowrey & Aston-Jones, Anxiety Depress. 2017). Methods: 3-month-old Tg-F344 or WT rats received intravitreal injections of an AAV encoding a Gq DREADD (AAV2-hSyn-hM3D(Gq)-mCherry) or control virus (AAV2-hSyn-EGFP). 6 months later, we assessed the effects of retinal DREADD stimulation on learning and memory in Tg-F344 rats using the Morris Swim Maze (MWM). Rats learned the location of the platform over 4x1min sessions daily for 6 days. They were then subjected to a referral (extinction) session and 4 reversal sessions (new platform location). Injections of the DREADD agonist clozapine-N-oxide (CNO; 2mg/kg, ip) were given 30min before each acquisition referral and reversal session, or before referral and reversal sessions only. Results: Electrophysiological and Fos analyses showed that Gq DREADD retinal stimulation increased retinal ganglion cell, SCN, DMH and LC activities. Tg-F344-AD rats showed poor initial as well as reversal learning. Retinal DREADD stimulation disrupted recall during the referral session and decreased reversal deficits in Tg-F344 rats without affecting WT performance. Conclusions: Dysregulation of the noradrenergic LC, which is associated with behavioral deficits in Alzheimer disease, can be attenuated by PRAM-induced activation of LC. The PRAM pathway is a novel circuit for a relatively non-invasive approach to treating multiple neuropsychiatric disorders linked to LC. Supported by PHS grant R21-MH121723-S1.

Disclosures: **S. Delcourte:** None. **G. Crozier:** None. **Y. Rakholia:** None. **G. Aston-Jones:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.06/L4

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

Support: NIH 4K00AG068428-03
Bluefield Project to cure Frontotemporal Dementia

Title: An increase in interstitial progranulin using AAV gene therapy reduces markers of neurodegeneration

Authors: *S. N. FOX, S. N. KASHYAP, A. E. ARRANT, E. D. ROBERSON;
Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Title: An increase in interstitial progranulin using AAV gene therapy reduces markers of neurodegeneration
Authors: SN Fox[1], S. Kashyap[1], AE Arrant[1], ED Roberson[1]

Affiliations:1. Department of Neurology, University of Alabama at Birmingham, Birmingham, AL
Abstract: Frontotemporal dementia (FTD) is a common form of early-onset dementia and is characterized by neuronal dysfunction in the frontal and temporal lobes leading to progressive language and behavioral impairment. Mutations in the progranulin gene (*GRN*), a lysosomal glycoprotein, can cause FTD. Of the 70 pathogenic mutations in *GRN* identified, the majority are loss-of-function, making AAV-progranulin gene therapy to boost protein levels an attractive approach for FTD. Previous work from our lab has demonstrated the effectiveness of neurotropic AAV-mouse progranulin (AAV-m*Grn*) gene therapy in progranulin insufficient mice. Neurotropic AAV-m*Grn* therapy can reverse lysosomal deficits, lipofuscinosis, and microgliosis observed in mice with complete loss of progranulin ($Grn^{-/-}$). Injecting $Grn^{-/-}$ mice with AAV-m*Grn* boosted parenchymal levels of AAV-derived progranulin as measured by immunohistochemistry. To further explore this, we used microdialysis to measure the levels of progranulin protein in the interstitial fluid (ISF) in the prefrontal cortex. Mice were injected with either AAV-m*Grn* or AAV-*Gfp* as a control and ISF was collected using the zero-flow microdialysis technique. Injecting mice with AAV-m*Grn* increased the total amount of progranulin protein in the ISF using this approach. Young and aged $Grn^{-/-}$ mice have an increase in biomarkers associated with neurodegeneration in both the plasma and cerebrospinal fluid compared to littermate controls. To understand the connection between the increase in interstitial progranulin and neurodegeneration, biomarkers were measured in plasma from aged $Grn^{-/-}$ mice injected with AAV-m*Grn*. A reduction in neurodegeneration biomarkers was found, suggesting an increase in ISF progranulin is protective against neurodegeneration associated with aged $Grn^{-/-}$ mice. These data demonstrate the importance of boosting progranulin as a potential therapeutic for FTD.

Disclosures: S.N. Fox: None. **S.N. Kashyap:** None. **A.E. Arrant:** None. **E.D. Roberson:** 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.; Bluefield Project to Cure Frontotemporal Dementia. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Licensing fees from Genentech. F. Consulting Fees (e.g., advisory boards); AGTC consultant, Lilly data monitoring committee, Editorial board Journal of Neuroscience.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.07/L5

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

Support: Alzheimer’s Disease Association grant 2019-AARG-NTF-644507
NIH R61 HL159948
NIH R01 NS083402

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University of Illinois Campus Research Board RB 19060

Title: Intracerebral Nanoparticle Transport Facilitated by Alzheimer Pathology and Age

Authors: G. C. TRACY[1], K.-Y. HUANG[2], Y.-T. HONG[2], S. DING[2], E. KIM[1], H. NOBLET[3], **K. LIM**[1], *H. CHUNG[4], H. KONG[2];
[1]Mol. and Integrative Physiol., [2]Chem. Engin., [3]Neurosci. Grad. Program, [4]Univ. of Illinois At Urbana Champaign, Urbana, IL

Abstract: Nanoparticles have emerged as promising transporters of Alzheimer's disease (AD)-treating hydrophobic drugs for their potential to enhance therapeutic outcomes. However, particles have been often designed without fully understanding the integrity of the blood-brain barrier (BBB) and extravascular microenvironment of the diseased brain. To this end, this study presents that aging is a significant factor in enhancing the transport and retention of nanoparticles engineered to bind with reactive astrocytes highly enriched in the aged and diseased brain. We assembled 200 nm diameter block copolymer of poly (lactic-co-glycolic acid) (PLGA) and hyaluronic acid (HA) which binds to CD44, a cell surface adhesion receptor found on the surface membrane of reactive astrocytes. The resulting PLGA-b-HA particles displayed increased binding to CD44-expressing reactive astrocytes with minimal neurotoxicity in the primary culture. Upon intravascular injection, PLGA-b-HA nanoparticles were localized to the hippocampi of aged APPSwe/PSEN1dE9 Alzheimer's disease model mice and their control littermates at 13-16 months of age due to enhanced BBB leakage in both genotypes. No particles were found in the hippocampi of young adult mice in both the Alzheimer's disease model and control. These findings demonstrate the brain localization and retention of PLGA-b-HA nanoparticles due to aging-induced BBB breakdown, regardless of the presence of extracellular amyloid beta plaques.

Disclosures: G.C. Tracy: None. **K. Huang:** None. **Y. Hong:** None. **S. Ding:** None. **E. Kim:** None. **H. Noblet:** None. **K. Lim:** None. **H. Chung:** None. **H. Kong:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.08/L6

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

Support: NIH Grant R01AG077829
NIH Grant R01AG071281
NIH Grant RF1AG074543
Alzheimer's Association Grant AARF-21-852175

Title: Restoring the statistical power failure and rigor of research using amyloid-β amyloidosis mouse models for Alzheimer's disease

Authors: *J. PARK[1,2], H. KARAHAN[1], B. KIM[1], M. D. TATE[1], J. KIM[1];
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Abstract: Although the FDA has granted accelerated approval for some Alzheimer's disease (AD) drugs, AD research is still being challenged by the lack of highly effective treatments despite more than hundreds of apparently successful drug discoveries using preclinical animal models. This translational failure has been, in part, attributed to the reproducibility crisis with the poorly established reporting practice of experimental design elements, particularly statistical power and sample size calculation. Power calculation is critical for study design because the required sample size can substantially vary with the intrinsic variability of readouts and the effect size of an intervention. However, only 3% of AD preclinical studies (40 out of 1298) from 2017-2021 have reported power calculations. This underreporting practice has raised the need to determine the variability of widely used AD animal models per different effect sizes of an intervention. We aimed to determine how many samples would be needed to detect the observable effect of an intervention on amyloid beta (Aβ), the main component of the extracellular plaques found in the AD brain. We used the Meso Scale Discovery (MSD) electrochemiluminescent assay to quantify Aβ levels from four widely used amyloidosis mouse models (5xFAD; *N = 70*, APPPS1-21; *N = 27*, APPPS1; *N = 12*, and $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$; *N = 25*) at several ages (from 4-10 months), across sex, and in different brain regions (cortex and hippocampus). We calculated sample sizes using R based on a two-tailed t-test with a 0.05 significance level (α) and 80% power (1-β). We found that the sample size vastly varies by the sample variability per age, sex, and brain region. Younger age groups required a larger sample size than older age groups in 5xFAD and $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ but not in APPPS1-21. Males were more variable than females in 5xFAD but not in APPPS1-21. The hippocampus was mostly less variable than the cortex but not in APPPS1-21. Although transgenic mouse models (5xFAD, APPPS1-21, APPPS1) required a larger sample size than the $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ knock-in model, in general, they cannot be directly compared due to different genetic backgrounds and ages. This benchmarking study provides an important guideline to the research community when their primary readout is the quantitative analysis of amyloid levels in Aβ amyloidosis models. Rigorous reporting practices will lead to better translation of preclinical success to human AD treatment.

Disclosures: J. Park: None. **H. Karahan:** None. **B. Kim:** None. **M.D. Tate:** None. **J. Kim:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.09/L7

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

Support: NRF MSIT 2020M3E5D9080660

Title: Suppression of hypoadiponectinemia-induced NLRP3 inflammasome activation by osmotin rescues Alzheimer's disease pathology

Authors: J. PARK, K. CHOE, A. KHAN, H. LEE, M. KANG, ***M. KIM**;
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Abstract: Several hypothesis regarding the pathophysiology of Alzheimer’s disease have been proposed so far. One of the known mechanism is deficiency in the level of circulating adiponectin in the brains, which may cause the detrimental effects in the brains. Hypoadiponectinemia conditions with either decreased production or a deficiency in circulating adiponectin levels exert detrimental effects on different organs, particularly the brain by disrupting several physiological functions in the brain, as energy imbalance and disturbance in glucose metabolism. Similarly, the functional NLRP3 inflammasome (a multiprotein complex) contributes to several inflammatory and neurodegenerative diseases, including Alzheimer’s disease (AD). The link between Hypoadiponectinemia-induced energy deprivation and NLRP3 inflammasome activation in the brain remains elusive. Here, we report for the first time that osmotin (adiponectin structural homologue) potentially inhibits NLRP3 inflammasome activation in young adiponectin knockout (AKO), aged AKO and APP/PS1 mice brain via the AdipoR1/AMPK pathway. Osmotin prevented NLRP3 inflammasome-mediated amyloidogenic amyloid beta (Aβ) production and aggregation and memory impairments and suppressed long term potentiation (LTP). Moreover, both *in vitro* and in *in vivo* osmotin treatments ablated NLRP3 inflammasome activation and the associated AD pathology. Based on our current findings, both osmotin and adiponectin (used as a gold standard) potentially inactivate the Hypoadiponectinemia-induced NLRP3 inflammasome function and associated dementia.

Disclosures: J. Park: None. **K. Choe:** None. **A. Khan:** None. **H. Lee:** None. **M. Kang:** None. **M. Kim:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.10/L8

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

Support: Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture Food and Rural Affairs (MAFRA) (318027-04)
Basic Science Research Program through the National Research

Foundation of Korea (NRF) funded by the Ministry of Education (2022R1I1A01071886)

Title: A therapeutic approach using herbal medicine and probiotics for metabolic disruption-induced neurotoxicity and inflammation in 5xFAD Alzheimer's disease mouse model

Authors: *I. JU[1], S. SON[2], S. LEE[1], H. IM[1], E. HUH[1], J. CHOI[1], M. SOHN[3], S.-V. YIM[1], S. KIM[4], D.-H. KIM[1], C. LEE[2], M. OH[1];
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Abstract: Alzheimer's disease (AD) is the most prevalent type of dementia characterized by the accumulation of amyloid plaques and neurofibrillary tangles, neuronal degeneration and excessive brain inflammation. Metabolic disturbance appears in AD and the altered metabolites show a strong association with beta-amyloid (Aβ) neuropathology and impaired behavior. Accordingly, managing various neuropathological mechanisms and metabolic disruptions can be an effective therapeutic approach for treating AD. This study aimed to assess the impacts of CCL01, an optimized combination of Cuscuta seeds and *Lactobacillus paracasei*, on AD neuropathologies and altered metabolism. When treated to five familial AD (5xFAD) transgenic mice, CCL01 ameliorated memory decline, inhibited synaptic and neuronal degeneration, and tau phosphorylation. The 5xFAD mice exhibited notable changes in the metabolite profile compared to wild-type, specifically involving disrupted phospholipid metabolism, whereas CCL01 treatment partially reversed these alterations. The major metabolite of which the level was higher in the brains of 5xFAD mice induced neuronal cell damage when treated *in vitro*, however, CCL01 protected the neuronal cells. Moreover, CCL01 down-regulated inflammation in the brain and colon of 5xFAD mice. Gut inflammation was revealed to be related with the brain pathological features. Taken together, these results indicate that CCL01 ameliorated the AD neuropathologies appeared in 5xFAD mice by protecting neuronal cells against metabolic disruption-induced toxicity and excessed inflammation, suggesting the therapeutic potential of CCL01 for AD treatment.

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Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.11/M1

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

Title: Effect of Photobiological Modulation Therapy on Cognitive Impairment, Cardiovascular Structure and Cortex Gene Expression in an APP/PS1 Mouse Model of Alzheimer's Disease

Authors: M. HAGHKAR[1], R. A. MCDEVITT[1], Y. N. GRIGOROVA[1], P. ARANY[2], V. I. ZERNETKINA[1], C. ROCHA DOS SANTOS[1], W. WEI[1], S. B. SYED[1], O. JUHASZ[1], S. FOX[1], C. H. MORRELL[1], E. G. LAKATTA[1], ***O. V. FEDOROVA**[1];
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Abstract: ***Introduction:*** Photobiological modulation (PBM), a form of low-dose light therapy, is beneficial in treating Alzheimer's disease (AD). Double-transgenic AD mice (APPswe/PS1dE9; APP/PS1) develop cognitive impairment (CI) and alterations in cardiovascular (CV) structure and function due to amyloid-β (Aβ) accumulation in brain and CV system. PBM therapy reduces Aβ in neocortex and hippocampus in the AD mice. The aim of this study was to investigate the effects of PBM on CI and CV structure and function in this AD mouse model. ***Methods:*** Six-month old female AD (APP/PS1; AD-PBM, n=8) and wild type mice (WT-PBM, n=8) were exposed to near-infrared light (wavelength 850 nm, 25 mW/cm^2 for 3 min, 1.5 Einstein or 6.75 p.J/cm^2, 5 days/week) or sham-treated (control; AD-C, n=6; WT-C, n=6) for 6-mo. CV parameters (echocardiography), cortical gene expression (qPCR), gait analysis (DigiGait), home cage activity (HCA), and spatial working memory (indicated by alternation rate in cross maze, CM) were assessed after 6-mo of treatment. Data analysis: by 2-way ANOVA; a 2-sided $p<0.05$ was considered significant. ***Results***: Both AD-PBM and WT-PBM had lower relative wall thickness of left ventricle (LV) and LV mass, and higher LV volume at the end of diastole vs. correspondent control groups. At 12-mo of age, cortical *TGFβ1, FN1* and *AQP4* gene expression was higher in AD-C vs WT-C; *TGFβ1* expression was lower in AD-PBM vs AD-C. In HCA test, AD-C exhibited circadian locomotor hyperactivity at night-time vs WT-C, which was reduced by PBM. AD-C had spatial working memory impairment vs WT-C, i.e., lower alternation rates in CM; PBM had minimal effect on this parameter. A lower fore propel time in gait test in AD-C vs WT-C was normalized by PBM in AD-PBM to the level of WT-C (Table). ***Conclusion***: Beneficial effect of PBM on CV system was associated with better cognitive/behavioral functions in the APP/PS1 mouse model. Upregulation of cortical cytokine *TGFβ1* in AD-C may indicate an activation of anti-inflammatory mechanisms as a response to Aβ-induced neurodegeneration, which may be counterbalanced by the beneficial effect of PBM.

Table 1. Physiological, Cardiovascular, Behavioral Parameters and Pro-frontal Cortex Gene Expression in WT and AD mice treated with PBM

	WT		AD		Two-way ANOVA		
	CNT, n=6	PBM, n=8	CNT, n=6	PBM, n=8	Strain effect	PBM effect	Interaction effect
Body weight (g)	27.1 ± 3.0	26.6 ± 5.3	27.9 ± 4.3	30.1 ± 3.3	P = 0.18	P = 0.59	P = 0.39
HR (bpm)	365±42	389 ± 48	403 ± 47	374 ± 46	P = 0.52	P = 0.90	P = 0.15
PWV (m/s)	2.05 ± 0.37	2.25 ± 0.97	2.15 ± 0.41	2.27 ± 0.78	P = 0.83	P = 0.59	P = 0.88
RWT	0.51 ± 0.09	**0.36 ± 0.05 #**	0.60 ± 0.17	**0.45 ± 0.07 #**	**P < 0.05**	**P < 0.01**	P = 0.98
LV Mass (mg)	102.2 ± 19.1	82.9 ± 14.4	104.0 ± 20.9	90.7 ± 16.2	P = 0.49	**P < 0.05**	P = 0.66
LV Vold (uL)	38.9 ± 13.7	45.7 ± 13.7	39.3 ± 4.7	50.4 ± 11.0	P = 0.58	**P = 0.06**	P = 0.63
Home Cage Activity, first 3 hours of Night-time, Activation (%)	0.72 ± 0.16	1.07 ± 0.32	**2.09 ± 0.63 ****	**1.44 ± 0.39 #**	**P < 0.01**	P = 0.38	**P < 0.05**
Gait Test, Fore Propel (s)	0.12 ± 0.02	0.12 ± 0.03	**0.09 ± 0.02 ***	**0.12 ± 0.03 #**	P = 0.11	**P = 0.05**	**P < 0.05**
Gait Test, Fore Brake (s)	0.08 ± 0.03	0.09 ± 0.03	0.11 ± 0.03	0.09 ± 0.03	P = 0.23	P = 0.37	**P < 0.05**
Cross Maze Test, % 4 Alternation (%)	43.6 ± 12.1	35.2 ± 11.7	28.5 ± 11.0	21.2 ± 4.4	**P < 0.01**	**P = 0.05**	P = 0.89
Cortical gene expression vs. WT-CTN:							
TGFβ1 (Transforming Growth Factor beta 1)	1.00 ± 0.10	0.93 ± 0.15	**1.73 ± 0.03 ****	**1.47 ± 0.11**#**	**P < 0.01**	**P < 0.05**	P = 0.17
FN1 (Fibronectin 1)	1.00 ± 0.16	0.84 ± 0.18	**1.38 ± 0.25 ***	**1.16 ± 0.30***	**P < 0.01**	**P < 0.05**	P = 0.87
AQP4 (Aquaporin 4)	1.00 ± 0.19	0.88 ± 0.23	**1.68 ± 0.41****	**1.44 ± 0.29****	**P < 0.01**	**P = 0.10**	P = 0.58

Values are mean ± SD. P value was estimated by 2-way ANOVA followed by post-hoc Sidak's multiple comparison test: *p<0.05, ** p<0.01, AD-CNT vs. WT-CNT and WT-PBM vs. AD-PBM; # p<0.05, PBM vs. CNT in both WT and AD. In red color: P < 0.05; in blue color 0.05 ≤ P ≤ 0.1. HR, heart rate; bpm, beats per minute; PWV, pulse wave velocity, a measure of aortic stiffness; RWT, relative LV wall thickness; LV, left ventricle; Vold, volume in diastole.

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Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.12/M2

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

Title: Bioenhanced Phloretin formulation: A holistic approach in the management of Alzheimer's disease

Authors: *N. CHIMTHANAWALA, S. SATHAYE;
Inst. of Chem. Technology, Matunga, Mumbai, India

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that plagues the elderly and has a detrimental impact on society. Falling within the spectrum of dementias, AD is multifactorial in origin with a heterogenous presentation. It leads to a progressive loss of orientation and memory along with cognitive deficits. Existing drugs in the market show symptomatic relief at best, by counterbalancing the neurotransmitter disturbances, but fail to capture the complex pathophysiology of AD; hence a multi-faceted treatment approach is needed. The rationale of the study was to develop an adjunct therapy that would specifically enhance cognition, offer neuroprotection, and facilitate neurogenesis. The project involved formulating and developing an oral dosage form of phloretin (PHL), an antioxidant flavonoid, obtained from apple leaves (Malus domestica), that displays enhanced bioavailability and distribution within the brain and attenuates oxidative stress, combats inflammation, and offers neuroprotection; thereby enhancing the quality of life of AD patients. For this, hot-melt fusion technique with a selection of polymers was employed which is fast, continuous, solvent-free, and economical that enhances the solubility and prolongs the shelf-life of the formulation.PHL has previously shown to have an anti-inflammatory, anti-cancer, and neuroprotective role along with being effective in ischemia-reperfusion and cardiovascular diseases. In this study, male Wistar rats were subjected to intrahippocampal $A\beta_{1-42}$ administration in N=12 per group using stereotaxic apparatus. The efficacy of the developed formulation vs. its API was assessed in-vivo performing neurobehavioral tests like Barnes maze test (BMT), Open field test (OFT), and T-maze along with per-oral treatment of PHL formulation for a period of 6 weeks. Biomarkers like anti-oxidants [Superoxide dismutase (SOD), reduced glutathione (GSH), Catalase], membrane peroxidation [Lipid peroxidation (LPO)], inflammatory parameter (TNF-α), and immunohistochemistry for $A\beta_{1-42}$ peptide using Congo red staining was measured. Lastly, various patient-compliant dosage forms of PHL will be developed for their nutraceutical potential and optimized for bringing a finished product into the market.

Disclosures: **N. Chimthanawala:** None. **S. Sathaye:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.13/M3

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

Support: FAPESP 2022/00249-8
CAPES Finance Code 001
CNPq 312904/2021-5

Title: Brain and behavioral changes after a long-term program of resistance exercise in APP/PS1 animal model of Alzheimer's disease

Authors: ***B. LONGO**[1], C. V. AZEVEDO[2], M. BRAGA BARNABÉ[1], L. DRAGONI[2], E. FIGUEIREDO[2], H. C. CAMPOS[2], V. O. BOLDARINE[3], L. OYAMA[3], R. M. ARIDA[4];

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Abstract: INTRODUCTION: Alzheimer disease (AD) is characterized primarily by the presence of amyloid-beta (Ab) plaques and neurofibrillary tangles manly formed in the pre-frontal cortex and hippocampus and can lead to cognitive and behavioral deficits. Resistance exercise (RE) has been proposed as a non-pharmacological therapy, that may have beneficial effects on the central nervous system, such as improving memory and cognitive function, modulating the inflammatory response, and inducing neuroplasticity. To investigate the effects of RE on cognitive function as well as amyloid plaques and Aβ protein and interleukin 10 (IL-10) levels in the hippocampus. **METHODS:** In this study, transgenic APP/PS1 mice, male and female at 6 months of age and their respective wild-type (WT) controls were divided into four groups: WT-SED (sedentary), WT-RE, APP/PS1-SED and APP/PS1-RE. Animals of the RE groups were submitted to a long-term resistance exercise program, during which they climbed a ladder with progressive load and moderate weights, 3x/week, for 16 weeks. After the exercise period, all animals were tested for exploratory behavior (locomotor activity and anxiety) and short- and long-term memories (object recognition and T-maze tests). At the end of the experiment, the hippocampi were collected and ELISA essay for Ab and IL-10 was performed. **RESULTS:** The APP/PS1-SED group showed hyperlocomotor activity, which is a sign of agitation and anxiety, whereas it was decreased in the APP/PS1-RE group ($P<0.0001$). No significant difference was detected in the object recognition test for short- and long-term memories between the groups, although long-term memory improved in the WT-RE animals compared to WT-SED ($P=0.0245$). Hippocampal memory, assessed by the T-maze test, indicated that both APP/PS1-SED and APP/PS1-RE groups took longer to reach the correct arm compared to WT-SED and WT-RE. Considering the influence of RE, no difference was detected between groups. ELISA analyses indicated that hippocampal levels of Ab protein decreased in APP/PS1-RE group when compared to APP/PS1-SED ($P=0.0372$). Also, the levels of IL-10 in the hippocampus increased in the WT-RE and APP/PS1-RE compared to SED groups ($P=0.0137$ and $P=0.0061$, respectively). **CONCLUSIONS:** This study suggests that a 4-month program of RE helps preserve memory and reduce agitation and anxiety in APP/PS1 mice. Furthermore, RE increases IL-10 and reduces Aβ, leading to anti-inflammatory effects in the hippocampus. For this reason, the long-term RE program can be proposed as a supportive therapy to control and reduce the progression of AD.

Disclosures: B. Longo: None. **C.V. Azevedo:** None. **M. Braga Barnabé:** None. **L. Dragoni:** None. **E. Figueiredo:** None. **H.C. Campos:** None. **V.O. Boldarine:** None. **L. Oyama:** None. **R.M. Arida:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.14/M4

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

Support: Seelos Therapeutics Inc.

Title: Trehalose treatment in an AAV-tau model of Alzheimer's disease

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Abstract: Non-human primate (NHP) models represent some of the most relevant animal models to human disease and are vital to the development and assessment of novel therapeutics. We present a model of tau pathology in cynomolgus macaques based on two tau pathogenic mutations, P301L and S320F, both associated with Frontotemporal Dementia (FTD) linked to chromosome-17 and characterized by FTD and parkinsonism. This model brings together P301L, the most common microtubule-associated protein tau (MAPT) mutation associated with FTD-17, and S320F which is known to induce tau aggregation spontaneously. Both mutations are expressed within the context of the human *MAPT* 4R/0N transcript using an AAV9 vector injected bilaterally into the entorhinal cortex of the NHP brain. Six months post injection, tau phosphorylated paired helical filaments marked by AT8 immunostaining were seen throughout the entorhinal cortex and hippocampus. This model was utilized to test the therapeutic potential of SLS-005 (trehalose, a disaccharide sugar molecule), which has previously been shown to ameliorate disease phenotypes in both cell culture and rodent models of numerous neurodegenerative diseases. The improvements induced by trehalose have been attributed primarily to its ability to induce autophagy, but trehalose also has chaperone like capabilities, and is able to aid in cellular responses to inflammation and oxidative damage. SLS-005 (Trehalose) has an impressive safety profile, including in human clinical trials across numerous routes of administration. In the current study, trehalose was administered as a combination of oral trehalose in drinking water and IV trehalose. Preliminary results from the first cohort of animals treated with oral and IV trehalose show a trend towards improvements in two biomarkers: neurofilament light chain (NFL) and brain derived neurotrophic factor (BDNF). NFL is a neuronal specific cytoskeletal protein released into the CSF upon cellular damage and/or death and is used as a surrogate biomarker for neuronal damage. Trehalose treatment in animals expressing mutant tau decreased the levels of NFL in the CSF. BDNF is a neurotrophic factor with a wide range of roles in neurogenesis, synaptic plasticity and neuronal survival, and levels of BDNF have been shown to be reduced in Alzheimer’s patients. BDNF levels are increased in tau expressing animals treated with trehalose compared to controls. Together this data further strengthens the potential role of SLS-005 (trehalose) in ameliorating neurodegeneration and supports its use as a potential therapeutic option for Alzheimer’s disease and tauopathies.

Disclosures: **A. Boehringer:** None. **S. Muller:** None. **J. Molina:** None. **K. Subramanian:** A. Employment/Salary (full or part-time):; Seelos Therapeutics Inc. **R. Mehra:** A. Employment/Salary (full or part-time):; Seelos Therapeutics Inc.. **J. Morrison:** None. **J. Kordower:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.15/M5

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

Title: Microfluidic perfusion of ex-vivo tissue slices for response analysis in the presence of amyloid beta and tau

Authors: *V. NORMAN, R. A. PROSSER;
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Abstract: Alzheimer’s disease (AD) is a progressive brain disorder pathologically defined as the accumulation of amyloid-B (AB)peptides (plaques) and strings of hyperphosphorylated tau proteins (tangles). Whether these plaques and tangles cause the neuronal loss associated with AD is unknown. In this study, we are investigating initial responses of brain tissue slices to AB and/or tau exposure. We are also comparing the effects of AB and tau across the suprachiasmatic nucleus (SCN), anterior hypothalamus (AH) and entorhinal cortex (EC). Additionally, we are developing a novel microfluidic bubble perfusion device to allow for observations of real-time response with high spatial and temporal resolution in ex-vivo tissues when exposed to stimulus. Coronal brain tissue slices containing the SCN/AH or EC were prepared from adult male C57/Bl mice and maintained in interface tissue chambers for 12 h. Media samples collected every 2 h showed increased release of lactate dehydrogenase (LDH; a measure of cell death) in response to AB vs. other conditions in SCN/AH slices. At the end of incubation, the slices were incubated with propidium iodide, then fixed, sectioned, and imaged for histological quantification of cell damage. PI staining showed a significant difference in cell damage/death between tau vs. AB:tau conditions in the EC.
To more accurately assess the effects of AB and tau, we are shifting to the microfluidic device. Currently we are optimizing the perfusion conditions for maintaining brain slices in the device to allow for a minimum of 12 h perfusion time. Tissue health is being assessed using LDH, PI, and intracellular Ca^{2+} imaging using a fluorescent marker. Details of the microfluidic device functionality and effects of AB and tau will be presented.

Disclosures: V. Norman: None. **R.A. Prosser:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.16/M7

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

Support: NIH AG067473

Title: Early and chronic treatment of lithium in the sporadic Alzheimer's disease model of GluN3A knockout mice

Authors: *S. POURKHODADAD, M. JIANG, X. GU, L. WEI, S. YU;
Emory Univ., Atlanta, GA

Abstract: Early and chronic treatment of lithium in the sporadic Alzheimer's disease model of GluN3A knockout mice

Soheila Pourkhodadad[1,2], Michael Qize Jiang[1,2], Xiaohuan Gu[1,2], Ling Wei[1], Shan Ping Yu[1,21]Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA; [2]Center for Visual & Neurocognitive Rehabilitation, Atlanta VA Medical Center, Atlanta, GA.

Activation of N-methyl-D-aspartate receptors (NMDARs) mediates calcium influx into neurons and overactivation of NMDARs can cause excitototoxicity. According to the Ca^{2+} hypothesis of Alzheimer's disease (AD), slight but persistent increases in Ca^{2+} influx and chronically disrupted Ca^{2+} homeostasis contribute to AD pathophysiology. Our recent study revealed that the NMDAR subunit GluN3A (NR3A) deficiency caused persistant neuronal hyperactivity and chronic Ca^{2+} dyshomeostasis, leading to age-dependent degenerative excitotoxicity and AD-like pathological alterations in GluN3A knockout mice. Lithium is a clinical drug for psychological disorders and has been tested as a potential therapy of AD and dementia. Lithium has a broad spectrum of actions including an inhibitory effect on the NMDAR activity and regulatory effects on the Ca^{2+}-dependent kinase CaMKII that plays a critical role in synaptic plasticity and cognitive function. The present investigation is a longitudinal examination of early and chronic treatment of lithium against the AD phynotype in the GluN3A KO mouse. Young adult wild type (WT) and GluN3A KO mice were fed with a scheduled diet plan containing 4% lithium for a duration of six months (from 3-month age to 9-month age). Western blot analysis and immunocytochemistry were applied to measure reactive astrocytes and microglia activation as well as Ca^{2+}-dependent signaling pathways. The aging GluN3A KO brain showed a significantly higher level of phosphorylated CaMKII (pCaMKII) compared to WT mice. GluN3A KO mice receiving 4% lithium diet remained near normal level of pCaMKII in the brain. The lithium treatment also prevented increased activation of astrocytes and microglia cells. Animals were subjected to various behavioral tests to assess psychological and cognitive functions. Available data indicate lithium may offer some therapeutic benefits by retaining inflammatory cells and Ca^{2+}-dependent signaling.

Disclosures: S. pourkhodadad: None. **M. Jiang:** None. **X. Gu:** None. **L. Wei:** None. **S. Yu:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.17/M8

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

Support: National Key R&D Program of China grant (2021YFE0203000)
Collaborative Research Fund [C6027-19GF]
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Hong Kong RGC Postdoctoral Fellowship Scheme

Title: Long-term persistence of AAV-mediated genome editing on familial Alzheimer's disease mutation in mice

Authors: *Y. DUAN[1,2], J. XU[1,2], T. YE[1,3,4], H.-Y. LAU[1,2], Y. JIANG[1,2], Y. CHEN[1,3,4], Y. CHEN[1,2,3,4], A. FU[1,2,3], N. IP[1,2,3];
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Abstract: Familial Alzheimer's disease (AD) is caused by autosomal dominant mutations in the genes encoding amyloid precursor protein (APP), presenilin 1 and presenilin 2, which result in the excessive production of amyloid-beta (Aβ) peptides. Previously, we demonstrated that an AAV-mediated CRISPR/Cas9-based strategy can selectively and efficiently edit a familial AD mutation *in vivo*, ameliorating AD-associated pathologies and improving cognitive functions. While the effect of genome editing is theoretically considered "once and for all", whether the beneficial effect of this AAV-mediated CRISPR/Cas9-based strategy could persist as mice age has not been demonstrated. Here we show that the CRISPR/Cas9-mediated genome editing of familial AD mutations is sustained for around two years in a familial AD transgenic mouse model after a single virus administration. We also observed a decrease of amyloid plaque deposition and a reduction of neuronal loss in aged genome-edited AD transgenic mice (around 30 months old). Moreover, the levels of AD-associated blood protein biomarkers, including neurofilament light chain, decreased in the familial AD transgenic mouse following the AAV-mediated genome-editing treatment. Together, this study demonstrates the long-term persistence

of AAV-mediated genome editing on familial AD mutation. Our findings also provide important insights for future translational development in disease-modifying treatments for familial AD.

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Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.18/M9

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

Support: NIH Grant R01AG064078

Title: Roles of Hippo Signaling in Neuronal Resilience and Alzheimer’s Disease Pathogenesis

Authors: ***R. C. EVANS**[1], L. CHEN[2], R. NA[2], Q. RAN[3];
[1]Univ. of Texas Hlth. San Antonio, Dallas, TX; [2]UT Hlth. Sci. Ctr., San Antonio, TX; [3]UT Hlth. Sci. Ctr., San Sntonio, TX

Abstract: The death and degeneration of neurons is the primary cause of the cognitive impairments observed in Alzheimer’s Disease (AD). Recent studies have indicated that ferroptosis, a form of regulated cell death driven by iron-dependent accumulation of lipid peroxides, likely plays a significant role in the neurodegenerative pathology observed in AD. Additionally, new evidence has suggested that the Hippo signaling pathway, which is known to regulate cell growth and proliferation, also regulates the expression of pro-ferroptotic genes in some cell types. While the effects of Hippo signaling activity on neurons and their sensitivity to ferroptosis are unknown, we have shown that markers of Hippo signaling activity are elevated in brain tissue taken from a mouse model of AD, implicating the pathway as a potential new target for therapeutic intervention. The current study investigates whether direct inhibition of Hippo signaling activity in neurons is protective against ferroptosis-driven neurodegeneration in AD. To test this, we developed a mouse model that allows for conditional, forebrain neuron-specific ablation of LATS1 and LATS2, key regulators of Hippo pathway activity, and crossed this model with an AD mouse model. Our findings indicate that inhibiting Hippo signaling in neurons protected against the development of cognitive deficits in AD mice, and reduced signs of cortical neurodegeneration. Additionally, we generated primary neuronal cultures from the LATS1/LATS2 knockout mice to test the effects of Hippo signaling inhibition on neuronal viability. Our results indicate that genetic ablation of LATS1 and LATS2 increases neuronal resilience against chemical inducers of ferroptosis, but not other forms of cell death. The present findings suggest that inhibition of Hippo signaling activity may help to ameliorate AD pathogenesis, potentially through increased neuronal resilience against ferroptosis.

Disclosures: **R.C. Evans:** None. **L. Chen:** None. **R. Na:** None. **Q. Ran:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.19/M10

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

Support: NIH NINDS grant R01NS105971
NIH NIA grant RF1AG079318
The Alzheimer's Drug Discovery Foundation (ADDF) and the Association for Frontotemporal Degeneration (AFTD)
The Bluefield Project to Cure Frontotemporal Dementia,
The BrightFocus Foundation
A New Vision Research Investigator Award (CCAD)
Emory School of Medicine (SOM) Dean's Imagine, Innovate, and Impact (I3) Wow! Research Award
NIH F31NS117129

Title: A single granulin is sufficient to rescue inflammation, lysosome dysfunction, and neuropathology in a mouse model of complete progranulin deficiency.

Authors: J. ROOT[1], A. MENDSAIKHAN[1], S. NANDY[2], G. TAYLOR[1], L. TROIANO ARAUJO[1], P. MERINO[1], M. WANG[3], D. RYU[3], C. HOLLER[4], B. THOMPSON[4], G. ASTARITA[4], J.-F. BLAIN[4], ***T. KUKAR**[1];
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Abstract: Progranulin (PGRN) deficiency is linked to multiple neurodegenerative diseases including frontotemporal dementia (FTD), Alzheimers disease (AD), Parkinsons disease (PD), and neuronal ceroid lipofuscinosis (NCL). Proper PGRN levels are critical to maintain brain health and neuronal survival, however the function of PGRN is not well understood. PGRN is composed of 7.5 tandem repeat domains, called granulins, and is proteolytically processed into 7 individual granulins (1-7; A- E) and a half-granulin inside the lysosome. The neuroprotective effects of full-length PGRN are well-documented, but the role of granulins is still unclear. Here we report, for the first time, that expression of single granulins is sufficient to rescue the full spectrum of disease pathology in mice with complete PGRN deficiency (*Grn*$^{-/-}$). Specifically, rAAV delivery of either human granulin-2 or granulin-4 to *Grn*$^{-/-}$ mouse brain ameliorates lysosome dysfunction, lipid dysregulation, microgliosis, and lipofuscinosis similar to full-length PGRN. These findings support the hypothesis that individual granulins are the functional units of PGRN and that granulins likely mediate neuroprotection within the lysosome. Moreover, our data suggest that pre-clinical development of therapeutics to treat FTD-*GRN* need to consider how they impact lysosomal granulins levels, which are critical to prevent neuroinflammation and neurodegeneration.

Disclosures: **J. Root:** None. **A. Mendsaikhan:** None. **S. Nandy:** None. **G. Taylor:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent related to use of granulins. **L. Troiano Araujo:** None. **P. Merino:** None. **M. Wang:** None. **D. Ryu:** None. **C. Holler:** A. Employment/Salary (full or part-time):; Arkuda Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent related to use of granulins. **B. Thompson:** A. Employment/Salary (full or part-time):; Arkuda Therapeutics. **G. Astarita:** A. Employment/Salary (full or part-time):; Arkuda Therapeutics. **J. Blain:** A. Employment/Salary (full or part-time):; Arkuda Therapeutics. **T. Kukar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent related to use of granulins.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.20/N1

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

Title: Deletion of endothelial nitric oxide synthase increases alpha5 integrin expression and neuroinflammation in mouse model vascular dementia

Authors: *S. ISMAEL[1], Z. WANG[3], F.-F. LIAO[3], G. BIX[2];
[2]Clin. Neurosci. Res. Ctr., [1]Tulane Univ., New Orleans, LA; [3]Dept. of Pharmacology, Addiction Science, Toxicology, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Vascular dementia (VaD) is the second most common cause of dementia, behind Alzheimer’s disease. Cerebral hypoperfusion and/or hypoxia leads to the BBB disruption, aberrant compensatory angiogenesis and, ultimately, white matter lesions and cognitive impairment. We hypothesize that the extracellular matrix and its integrin cellular receptors play a critical role in regulating this compensatory angiogenesis (efforts to increase diminished CBF) that precedes VaD. Previously we have found that genetic deletion of this integrin in endothelial cells or by using the clinically-validated α5β1 integrin function-blocking pentapeptide ATN-161 profoundly protects against BBB disruption, inflammation (decreased edema and leukocyte infiltration), size of the ischemic infarct, and improved cognitive impairment in an animal stroke and bilateral common carotid artery stenosis (BCAS, another model of VaD that reduces CBF). Using the endothelial nitric oxide synthase (eNOS)-deficient mouse that models VaD through cerebral small vessel disease we investigated whether alterations in alpha 5 integrin (and other markers of neuroinflammation and BBB disruption) were associated with the development of VaD. 6-month-old (an age where cerebrovascular pathology is known to occur in these mice) eNOS+/- and age-matched WT mice were used for the study. RNA and protein were isolated whole-brain and were analyzed for quantitative PCR and immunoblotting and target mRNA and protein levels were normalized to those of GAPDH. The observations were further confirmed by

immunofluorescence. We found that 6-month-old eNOS+/- mice had increased levels of alpha5 integrin expression relative to age-matched controls. Real-time PCR analysis showed that eNOS+/- has elevated inflammatory mediators such as TNFα, CCL2, and NLRP3 transcripts. This is associated with increased BBB permeability represented by reduced claudin 5 mRNA. The eNOS deficient mice express elevated alpha 5 integrin along with increased neuroinflammation and BBB permeability. In addition, the deletion of endothelial specific deletion α5 increases the brain expression of eNOS. These observations suggest that alpha 5 integrin expression coincides with neurocognitive symptoms associated with eNOS deficiency and progressive vascular dementia. Furthermore, our preliminary data suggest that alpha 5 integrin could also be a neuroprotective and pro-angiogenic target in this model of VaD worthy of further study.

Disclosures: S. Ismael: None. **Z. Wang:** None. **F. Liao:** None. **G. Bix:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.21/N2

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

Support: R21AG059157
NCI CCSG P30 CA060553

Title: Overexpression of Nrf2 in 5XFAD mouse model of Alzheimer's disease

Authors: *K. R. SADLEIR[1], K. P. GOMEZ[1], M. LEY[1], J. GUO[1], S. CHANDRA[1], A. KHATRI[1], Y. XUE[2], C. L. CEPKO[2], R. VASSAR[1];
[1]Neurol., Northwestern Univ., Chicago, IL; [2]Harvard Med. Sch., Harvard Univ., Boston, MA

Abstract: The Alzheimer’s disease brain contains amyloid plaques consisting of the β-amyloid protein and neurofibrillary tangles containing hyperphosphorylated, aggregated tau protein, which cause activated, inflammatory microglia and astrocytes. These pathologies cause synaptic loss, neuronal dysfunction and neuronal loss. The goal of neuroprotective therapies is to protect neurons from plaques, tangles and inflammatory factors. One type of stress experienced by neurons is oxidative stress, which is observed in degenerative conditions and normal aging, leading to damaged proteins, nucleic acids, and lipids. Nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor is a key regulator in redox balance and signaling. Nrf2 regulates the expression of many antioxidant and detoxification genes by binding to antioxidant response elements (AREs). Nrf2 mRNA is decreased in AD brains and deletion of the Nrf2 gene increased BACE1 and Aβ production, and worsened cognitive deficits in amyloid mouse models. We hypothesized that neuronal Nrf2 overexpression would be neuroprotective by reducing BACE1 levels and therefor amyloid production in 5XFAD mice, and would reduce neuronal loss. To overexpress Nrf2 in 5XFAD mouse brains, AAV8 hSyn-Nrf2 and AAV8 hSyn-GFP or AAV8

hSyn-GFP alone (control) were injected into the ventricles of day-old mouse pups. At 9.5 months of age, the mice were perfused, and half the brain fixed for sectioning and immunofluorescence analysis, the other half dissected into cortex and hippocampus and frozen for mRNA and protein analysis. Sections were strained with antibodies to Aβ42, or amyloid binding dyes to measure plaques, NeuN to quantify neuronal loss, Iba1 and GFAP to assess microglial and astrocytic activation respectively. Overexpression of Nrf2 did not prevent neuronal loss or decrease neuroinflammation or amyloid deposition. Nrf2 expression did reduce BACE1 protein levels, especially in dystrophic axons that develop around plaques and accumulate many vesicles and proteins. We confirmed the decrease in dystrophic neurites in general using the dystrophic neurite marker LAMP1. Dystrophic neurites accumulate phospho-tau and play a role in tau spreading, so we immunostained for ptau-181 that reduced as well. To understand how Nrf2 overexpression decreases dystrophic neurites, and confirm activation of Nrf2 transcriptional targets, we performed bulk mRNA sequencing on hippocampi. Targets of Nrf2 such as Hmox1 and Txnrd1 were elevated in Nrf2 overexpression mice confirming effective activation of antioxidant pathways, and further analysis will address neuronal changes that could affect dystrophic neurite formation.

Disclosures: **K.R. Sadleir:** None. **K.P. Gomez:** None. **M. Ley:** None. **J. Guo:** None. **S. Chandra:** None. **A. Khatri:** None. **Y. Xue:** None. **C.L. Cepko:** None. **R. Vassar:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.22/N3

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

Support: NIH R01 AG071859-01A1
NIH R01 AG073826-01A1

Title: Repurposing a classic developmental mouse model: Validation of RARE-LacZ mice for the study of retinoic acid signaling in Alzheimer’s disease

Authors: *A. HINDLE[1,2], M. HERNANDEZ[1,2], J. MEDINA[1,2,6], A. BAKER[1,3], J. J. LAWRENCE[1,2,4,5];
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Abstract: A disruption in balance between amyloidogenic and non-amyloidogenic pathways leads to cognitive decline in Alzheimer’s disease (AD). However, upstream molecular mechanisms that alter this balance are poorly understood. We previously found evidence that hippocampal retinoic acid (RA) signaling is impaired in human AD. Several lines of evidence

suggest that vitamin A (VA) supplementation favors the non-amyloidogenic pathway through upregulation of α-secretase. Originally used to map embryonic RA signaling via xGal staining, RARE-lacZ mice express E. coli beta-galactosidase (lacZ) under the control of retinoic acid response elements (RAREs) on a CD1 background. Because Jackson Laboratory reports that xGal staining is lost on a C57BL/6 background, we tested the utility of crossing RARE-lacZ mice with C57BL/6-based strains for behavioral and immunohistochemistry (IHC) studies. RARE-lacZ mice possess multiple transgene copies of unknown locus. This draws into question whether progeny of RARE-LacZ mice inherit equal copy numbers of the transgene. We first performed qPCR on gDNA from RARE-LacZ mice and on F1 progeny of RARE-lacZ and wildtype (WT) crosses. We found a copy number ratio of ~2:1 between these cohorts. Importantly, within cohorts copy number values were within ± 26% (N=5) of the mean supporting inheritance from one block of tandem repeats rather than multiple loci. Second, we used a water T maze (WTM) to examine simple discrimination (9 trials, platform on left) and reversal learning (9 trials, platform moved to right) in 4-month-old RARE-lacZ mice and in crosses with WT C57BL/6J, -NJ, and CD1 mice (respective strains of J20, hAβ-KI, and RARE-lacZ mice). Strain affected latency to platform during simple discrimination and reversal learning ($N = 11\text{-}12$, $P < 0.05$, Friedman) but did not affect total distance traveled. Taken together, hippocampal-mediated learning was intact in all crosses. Finally, IHC staining in dorsal hippocampus of RARE-LacZ$^{+/+}$ mice found LacZ expression localized mostly to a subset of dentate gyrus granule cells, extending into their dendrites as well as axons (mossy fiber terminals) in CA3. Consistent with zygosity, LacZ expression was higher in homozygous RARE-LacZ$^{+/+}$ mice than in the heterozygous RARE-lacZ$^{+/-}$ from the CD1 background. In contrast to a previous report, F1 progeny from crosses onto the C57BL/6J background showed higher lacZ expression than the CD1 background, implying that the C57BL/6 background enhances RA signaling. In conclusion, RARE-lacZ mice have appropriate characteristics for testing VA-mediated interventions in C57BL/6-based AD models, although comparisons across backgrounds should be avoided.

Disclosures: **A. Hindle:** None. **M. Hernandez:** None. **J. Medina:** None. **A. Baker:** None. **J.J. Lawrence:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.23/Web Only

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

Support: 5R01AG069433-03
1T32AG067952-01
R01NS128808

Title: Continuous wave 670nm LED light therapy improves short- and long-term object recognition, improves neurogenesis in aging 3xTg-AD mice

Authors: *K. J. JOHNSON[1], A. C. GRANT[2], K. M. JOHNSON[3], G. TAGLIALATELA[4], M.-A. MICCI[5];
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Abstract: BACKGROUND Non-invasive therapeutic alternatives for Alzheimer's Disease (AD) are highly sought after due to their accessible nature and sustainability of use. Photobiomodulation (PBM) therapy uses 620-1100nm red to near-infrared light to modulate a variety of biological processes including neuroinflammation, amyloid and tau oligomerization, and apoptosis. 670nm red light has been shown to reduce AD pathology and improve cognitive function in various mouse models, but to our knowledge, PBM effects on neurogenesis in the 3xTg-AD mouse have not been investigated. The 3xTg-AD mouse model develops an abundance of amyloid plaques and neurofibrillary tangles, and early depletion of hippocampal neural stem cells, contributing to progressive cognitive dysfunction starting around 12 months of age. Here we sought to determine if 670nm light therapy could rescue cognitive and motor dysfunction and improve neurogenesis in 14-to-18-month-old 3xTgAD mice.
METHODS14-to-18-month-old 3xTgAD mice were treated with continuous-wave 670nm LED light applied directly to the head for 90s/day, 5 days/week for 4 weeks. 3xTg-AD sham and WT control animals were held under the LED device with the light turned off for the same amount of time. During the fourth week of treatment, animals were tested for short-and long-term memory in the novel object recognition task. At the completion of behavioral testing on treatment day 20, brains were collected for western blot analysis of hippocampal total protein and synaptic proteins, and immunofluorescence analysis of the hippocampal neurogenic niche.
RESULTSLight-treated 3xTg-AD animals showed significant short-term object recognition (DI 21.83 ± 7.96, one sample t-test $p<0.05$) at 2 hours post-training, whereas sham animals did not. The subgranular zone of the dentate gyrus showed increased Sox2+ neural stem cells in PBM-treated 3xTg mice compared to sham (58.94 ± 2.89 cells/mm vs. 50.05 ± 2.60, Welch's t-test $p<0.05$).
CONCLUSIONSHere we show rescue of hippocampal-dependent memory and preservation of the hippocampal neural stem cell population in 3xTg-AD mice using a chronic, non-invasive transcranial treatment with a commercially available 670nm LED light source. Clinical trials have shown short-term improvements in cognition, sleep, and memory performance following PBM therapy in dementia patients. Hippocampal neural stem cells have been shown to have neuroprotective effects in the context of AD pathology, independent of their capacity for neurogenesis. Our data suggest that treatment with 670nm red light could significantly improve cognitive function in AD patients via the preservation of hippocampal neural stem cells.

Disclosures: K.J. Johnson: None. **A.C. Grant:** None. **K.M. Johnson:** None. **G. Taglialatela:** None. **M. Micci:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.24/N4

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

Support: Cure Alzheimer's Fund Investigating the role of tau protein in neuronal senescence induction and maintenance.
NIA R37AG013925-17S1, Effect of Aging on Preadipocyte Differentiation
5IK2BX003804 Alzheimer's Disease Associated Tau Toxicity Induces Cellular Senescence in Brain

Title: Comparison of senolytic therapies fisetin versus dasatinib plus quercetin in tau transgenic mice

Authors: *J. M. ERICHSEN[1], V. GARBARINO[2], H. YODER[1], Y. IKENO[3], T. ORR[1], M. ORR[1];
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Abstract: Senolytic therapy, which involves the clearance of senescent cells, is currently being explored as a treatment for Alzheimer's disease (AD). Cellular senescence is defined as a stress response that results in a stable cell cycle arrest and resistance to apoptosis; it is often accompanied by a pro-inflammatory senescence-associated secretory phenotype (SASP). The clearance of senescent cells has been associated with reduced AD pathology, inflammation, white matter hyperintensities, and improved cerebral blood flow and cognitive function. In this study, the senolytics fisetin (FIS) and the combination of dasatinib plus quercetin (D+Q) were explored. Twelve- to fourteen-month-old male and female tau transgenic (rTg4510) and wildtype (CKtTA) mice were treated intermittently for 12 weeks with FIS, D+Q, or vehicle by oral gavage. Physical, functional, and behavioral outcomes were measured before and after treatment. We found that both senolytics ameliorated neurodegeneration, but also had effects on frailty, gait, nerve conduction velocity, and limb grip force/duration. Nanostring GeoMx Digital Spatial Profiling was also used to assess differentially expressed proteins in rTg4510 and CKtTA mice following senolytic treatment. We found that, in rTg4510 female mice, senolytic treatment downregulated expression of apoptotic markers BCL-XL, Cleaved Caspase 3, and BAD in comparison to vehicle-treated controls. GFAP expression, an astrocytic marker, was also downregulated in rTg4510 mice following senolytic treatment, as were proteins involved in the Ras/Raf/MEK/ERK pathway. We did note greater downregulation of tau protein with D+Q compared to FIS. Changes in protein expression following senolytic treatment in CKtTA mice appeared very different to that seen in rTg4510 mice, with substantially more protein upregulation in the CKtTA mice. This suggests that the mechanism of action for these senolytics may be dependent on disease state. More studies are needed to investigate FIS and D+Q as treatment strategies for AD and to fully understand their side effect profiles. It is important to thoroughly examine protein expression following senolytic treatment to gain mechanistic insight into how these therapies can effectively treat AD.

Disclosures: **J.M. Erichsen:** None. **V. Garbarino:** None. **H. Yoder:** None. **Y. Ikeno:** None. **T. Orr:** None. **M. Orr:** None.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.25/N5

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

Title: Pharmacodynamic, pharmacokinetic, and GLP-tox studies of ISU203: a novel antibody therapeutics which targets ASM in blood for Alzheimer’s disease.

Authors: ***H. LEE**[1], M. PARK[2], J.-I. CHO[1], H.-Y. LEE[1], H. JIN[2], J.-S. BAE[2], J.-J. PARK[1]; [1]Drug Discovery Divison, ISU ABXIS, Seongnam-si, Korea, Republic of; [2]Kyungpook Natl. Univ., Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: ISU203 is a novel antibody therapeutics which targets acid sphingomyelinase (ASM) in plasma for Alzheimer's disease (AD). Given that the activity of ASM in blood is elevated in patients with AD and the suppression of ASM activity ameliorates AD pathology, we developed the monoclonal antibody targeting ASM in plasma for the treatment of AD patients. To understand the efficacy of ISU203, we tested the cognitive function, removal of amyloid-β plaques, and the neuroinflammation in AD mouse model (APP/PS1). To do this, repeated dose of ISU203 was administered to AD mice for two months (50mg/kg/day, twice a week). As expected, suppression of ASM activity in plasma by ISU203 treatment showed significant improvement of cognitive function. In Morris water maze test, the escape latency time was decreased by approximately 30% upon treatment of ISU203. Moreover, we also observed the increased freezing ratio in ISU203-treated group by using Fear conditioning test. ISU203 treatment also facilitates the clearance of amyloid-β plaque by enhancing microglial phagocytosis function. In addition, ISU203 could alleviate neuroinflammation by suppression of immune response in blood, especially by inhibition of Th17 differentiation. Thus, ISU203 could be novel therapeutics to AD via suppression of the inflammation in blood. To further characterize the ISU203, we demonstrated pharmacokinetic study in two animal species, Sprague Dawley rat and Cynomolgus monkey. ISU203 was administered in animals via intravenous route by considering the dosing route for further clinical study. Three dose level of ISU203 was administered, and we observed that mean terminal half-life ($t_{1/2}$) were 73.9, 85.6, 89.8 hours at 2, 10, and 50mg/kg, respectively, for rat. For monkey, mean terminal half-life ($t_{1/2}$) values were 250, 401, and 359 hours at 2, 10, and 50mg/kg, respectively. To further understand possible toxicity, we also carried out GLP-tox study. Three dose levels (10, 50, and 200mg/kg) were administered in rat and monkey through intravenous injection. As a result, there is no severe toxicity like as death observed even in the high dose group in both two animal species. Therefore, pharmacodynamic, pharmacokinetic, and toxicity studies might indicate ISU203 is sufficient for clinical trial. Taken together, ISU203 is suggested as a potent, safe, and novel

antibody therapeutics for AD patients with a novel mechanism of action, which alleviates AD pathologies indirectly by inhibition of elevated activity of ASM in blood.

Disclosures: **H. Lee:** A. Employment/Salary (full or part-time):; ISU ABXIS. **M. Park:** 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.; ISU ABXIS. **J. Cho:** A. Employment/Salary (full or part-time):; ISU ABXIS. **H. Lee:** A. Employment/Salary (full or part-time):; ISU ABXIS. **H. Jin:** 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.; ISU ABXIS. **J. Bae:** 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.; ISU ABXIS. **J. Park:** A. Employment/Salary (full or part-time):; ISU ABXIS.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.26/N6

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

Support: Edward N. and Della L. Thome Memorial Foundation Awards Program in Alzheimer's Disease Drug Discovery Research
Alzheimer's Association
Appia Pharmaceuticals

Title: OA57, a novel histone acetyltransferases activator against Alzheimer's Disease

Authors: *E. CALCAGNO[1], E. ZUCCARELLO[2,3], A. STANISZEWSKI[2], H. ZHANG[2], E. K. ARGYROUSI[2], A. HAMMI[2,4], S.-X. DENG[3], D. W. LANDRY[3], J. FIORITO[3,5], O. ARANCIO[2,3];
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Abstract: Epigenetic regulation of gene expression is involved in memory formation through histone acetylation. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) control the acetylation status of chromatin, regulating gene expression. Acetylation of histones opens the chromatin structure; thereby the DNA becomes accessible to transcription factors involved in memory formation (p300, CBP, PCAF, Tip60 and Src3). We and others have demonstrated that reduction of histone acetylation is present both in animal models of

Alzheimer's disease (AD) and in human specimens derived from AD patients. Thus, we formulated the hypothesis that enhancing acetylation through drugs that increase HAT activity might be beneficial against AD. A drug discovery program aimed at developing HAT activators led to the small molecule, OA57, which was characterized through biochemical, pharmacological, electrophysiological, and behavioral techniques. A technology platform using a cell-free assay aimed at determining histone acetylation by gene transcription factors, demonstrates OA57-induced enhancement of acetylation of histone lysines H3K4 (EC_{50}=146.60±0.32 nM), H3K18 (EC_{50}=124.91±0.28 nM), H3K27 (EC_{50}=88.10±0.23 nM), but not H3K23 and H3K36 via p300, whereas in the presence of CBP, OA57 enhances acetylation of H3K14 (EC_{50}=46,727±5,28 nM), H3K18 (EC_{50}=3.86±0.59 nM), and H3K27 (EC_{50}=0.03±0.001 nM), but not H3K4 and H3K9. Consistent with these findings, administration of OA57 (i.p., 20 mg/kg, 30 min prior to an electric shock that induces memory) to adult mice enhances acetylation of memory-related histone lysines (H3K4, H3K9, H3K14). Moreover, OA57 increases the expression of various components of the memory related gene machinery, Arc, pCREB, CREB, c-Fos, and BDNF. Pharmacokinetic studies demonstrated that OA57 is rapidly absorbed into the brain, exhibiting a highly favorable brain/blood ratio (5.7) after oral administration. Notably, chronic i.p. administration of OA57 (5 mg/kg) to the APP/PS1 mouse model of amyloid deposition and the hTau/Mapt-KO mouse model of tau elevation shows rescues of the defects in associative and spatial memory, as well as in hippocampal long-term potentiation, a cellular surrogate of memory formation. In conclusion, OA57 represents a very promising drug against AD.

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Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.27/N7

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

Support: Centene Personalized Medicine Initiative

Title: Enhancing proteasomal activity and tau clearance by antisense oligonucleotide-mediated Usp14 reduction in a tauopathy mouse model

Authors: *M. HU, K. M. SCHOCH, T. MILLER;
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Abstract: A primary pathological feature of several dementias, including Alzheimer's disease, is the accumulation of neurofibrillary tau tangles. Tau accumulation best correlates with clinical

AD symptoms of cognitive decline and neuronal loss. Therefore, strategies that seek to interrupt tau deposition and propagation may be effective treatments. Regions associated with tau accumulation in postmortem AD brains show less active proteasome systems, suggesting impaired protein degradation may contribute to tau pathology. Increasing tau degradation through proteasome activation may be an efficient therapy to reduce tau burden and neurodegeneration in disease. The ubiquitin-proteasome system is negatively regulated, in part, by enzymes that remove the ubiquitin side chains from proteins targeted to the proteasome. Among these enzymes, the ubiquitin-specific protease 14 (Usp14) selectively acts on substrates, including tau, to reduce or prevent their degradation. We developed an antisense oligonucleotide (ASO) to reduce Usp14 within the central nervous system to achieve selective Usp14 inhibition and increase proteasomal activity.We tested Usp14-lowering ASO candidates in vitro for their ability to reduce Usp14 mRNA and protein levels. The top Usp14-lowering candidate decreased Usp14 mRNA by 90% and protein expression by 50-80% compared to a non-targeting control ASO. Treatment with this Usp14-lowering ASO also led to a decrease in tau protein and appeared to increase proteasome activity compared to non-targeting ASO in primary wild-type neurons. When Usp14-lowering ASOs were injected into the lateral ventricle of wild-type and tau transgenic (P301S) PS19 mice, Usp14 gene expression decreased by 50% and protein levels by 80% compared to control ASO treatment. In wildtype mice, Usp14-lowering ASO treatment decreased mouse tau by 50% via sandwich ELISA. Similarly, total human tau in PS19 mice decreased by 20%. In summary, we have successfully generated an ASO that lowers Usp14 gene and protein expression in vitro and are the first group to use an ASO-mediated Usp14-lowering strategy in vivo. Our initial findings support Usp14 targeting to increase proteasome activity to lower tau protein which may enhance tau clearance in disease. Our future work will test the ability of Usp14-lowering ASOs to alter phosphorylated tau pathology and neuronal death in tau transgenic mouse models.

Disclosures: **M. Hu:** None. **K.M. Schoch:** None. **T. Miller:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Ionis Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); Ionis Pharmaceuticals, Biogen, Disarm, UCB, Cytokinetics.

Poster

PSTR197. Preclinical Therapeutic Strategies for Alzheimer's Disease

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.28/N8

Topic: H.08. Learning and Memory

Support: NIH Grant RF1AG061824
BrightFocus grant A2017443S

Title: Spatiotemporal expression of membrane-tethered APP intracellular domain plays a crucial role in cognitive behaviors of non-transgenic and Alzheimer's disease mouse models

Authors: *A. SADANAND[1], A. TORRELLI-DILJOHN[3], T. SABBLAH[2], N. COLEMAN[2], M. WEINRICH[2], V. SPRUILL[4], G. BESANT[4], P. KULKARANI[1], N. BORAD[1], R. WANG[1], A. PARENT[1];
[1]Mol. Med., [2]Univ. of South Florida, Tampa, FL; [3]Univ. of Alabama, Alabama, AL; [4]Univ. of Chicago, Chicago, IL

Abstract: Alzheimer's disease (AD) is an age-associated disease pathologically defined by the deposition of Aβ amyloid peptide in the brain. Aβ is generated from amyloid precursor protein (APP). The APP undergoes sequential cleavage by beta and gamma-secretases to release Aβ and the APP intracellular domain (AICD). Alpha or beta-secretase cleavage produces membrane-tethered AICD (mAICD), the C83 and C99 metabolites, respectively. We previously reported that expression of mAICD could rescue memory impairment in an amyloidogenic AD mouse model (5XFAD) if expressed since birth. We showed that the mAICD interaction site with Gαs was necessary for this outcome, suggesting that APP-mediated cAMP/PKA-dependent signaling was a significant contributor. Here, we aim to explore if the expression of mAICD and mAICD variant lacking Gαs interaction in the hippocampus of young and older adult mice with advanced AD condition is sufficient to preserve their cognition and spatial memory. The 5XFAD transgenic mice present an aggressive accumulation of Aβ at an early age. We injected recombinant adeno-associated virus (AAV) of mAICD constructs in the dentate gyrus (DG) or CA3 area of 5XFAD and non-transgenic (NTg) littermate mice at three and nine months of age. Several anxiolytic and spatial memory tests were performed three months later. The 5XFAD mice (n=15-21) did not improve their cognitive behaviors when expressing mAICD in the DG, whereas they showed memory improvement if injected in the CA3 area (n=8-16). However, we observed that the NTg mice expressing mAICD in the DG at a young age (n=21-23) exhibited cognitive impairment three months after AAV injection. This effect was not seen in older cohorts or mice expressing mAICD in the CA3 area (n=10-20). Altogether, our results suggest that mAICD overexpression in select brain areas at a particular time during lifespan might have consequences on cognitive function and AD etiology. Funded by NIH and the Bright Focus Foundation

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.01/O1

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

Support: This research project was supported by CONAHCYT Grant: 319578. We are grateful for the Postdoctoral Fellowships from CONAHCYT (Dr.María del Carmen Silva-Lucero) and DGAPA (Dr. Laura Gómez-Virgilio).

Title: Relationships between olfactory dysfunction, Alzheimer's disease, and type 2 Diabetes mellitus

Authors: *A. GUTIERREZ-MALACARA, L. GOMEZ-VIRGILIO, M. SILVA-LUCERO, O. LORA-MARIN, M. CARDENAS-AGUAYO;
Physiology, Sch. of Med., UNAM, Mexico City, Mexico

Abstract: Introduction: Alzheimer's Disease (AD) is a neurodegenerative disease characterized by de dysfunctionof cognitive functions and is the most common type of dementia worldwide.Recent studies point to an association between AD and Type 2 Diabetes (T2DM)due to the shared pathophysiologic mechanisms. There are studies that reportolfactory dysfunction in people with Mild Cognitive Impairment (MCI) and AD,this is probably because of the affection of the neuroanatomic structures whichplay an important role in olfactory function, such as the olfactory bulb,hippocampus, and entorhinal cortex. Recently, biomarkers detection in blood wasproposed as a less invasive approach to support the diagnosis. There areseveral cell culture models for studying AD, and the most recent ones propose a3D approach that recapitulates tau and amyloid pathology present in AD, whichcan't be modeled in the single-layer cell culture approach. In this study, weevaluate the relationship between cognitive impairment, olfactory dysfunction,and diabetes in the Mexican population older than 50 years old and link theseresults to the levels of the biomarkers in the studied group. We also aim todevelop a 3D cell culture model from isolated human olfactory neuroepitheliumneural precursor cells (hONE NPCs) to generate a more feasible cell culturemodel without induced pluripotent stem cells (iPSCs) or embryonic stem cells(ESC). **Methods**: We divided our study groups into apparently healthysubjects (AHS), subjects with cognitive impairment (SCI), subjects with ST2DM,and subjects with type 2 diabetes and cognitive impairment (ST2DM-SCI).We obtained hONE NPCs from the volunteers which were characterized byWestern Blotting and immunocytofluorescence. We obtained blood samples in EDTAtubes and without additives for HbA1c and AD biomarkers (tau, Aβ1-42, and Aβ1-40)tests. We also performed a Montreal Cognitive Assessment Test (MoCA Test) by acertified rater (MXGUTAN710628566-01) and a medical interview and an olfactorytest for each volunteer. **Results and conclusions**: We obtained bloodsamples, performed medical evaluation, cognitive and olfactory assessment from35 volunteers. Subjects were 67.6% womenand 32.4% men, with an age between 20 and 84 yo. Diabetes/prediabetesprevalence was 22.9%. The mean HbA1c was 5.81%. Average BMI was 25.71 kg/m2.40% of the participants were overweight and 14.3% were obese. One subject wasdiagnosed with AD. Wb and ICC of the hONE show that these cells express Ki67,Nestin and β-III tubulin, and GFAP. We conclude that in our cohort the diabetesproportion was 22.9% and the cognitive decline was 17.14% and hONE cells are agood model to study neurodegeneration.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.02/O2

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

Support: University of Kentucky Lyman T. Johnson Postdoctoral Fellowship
R56 AG074613 To Ann Stowe

Title: Sex and age effects on IL-1b in subjects with cardiovascular diseases and at risk for Alzheimer's disease

Authors: *J. LUTSHUMBA[1], C. J. MCLOUTH[2], E. L. ABNER[3], D. M. WILCOCK[3], A. M. STOWE[1];
[1]Neurol., [2]Biostatistics, [3]Sanders-Brown center on aging, Univ. of Kentucky, Lexington, KY

Abstract: Cardiovascular diseases (CVD) are a major risk factor for Alzheimer's disease (AD) and Vascular contributions to Cognitive Impairment and Dementia (VCID), the two most common forms of dementia associated with aging. Systemic inflammation is a common etiologic factor associated with both CVD and dementia. Despite the disproportionate distribution in the prevalence of dementia, with women representing two-thirds of patients with dementia, sex-based differences remain understudied. Furthermore, the effects of sex and age on inflammation and the pathogenesis of dementia are still unknown. Method**:** Data derived from the University of Kentucky Alzheimer's Disease Research Center (UK-ADRC) cohort. Participants were selected based on their first visit to UK-ADRC from 2012 to 2022; the following inclusion criteria were applied: normal cognition, history of hypertension, hypercholesterolemia, or type 2 diabetes. Exclusion criteria were a history of stroke and traumatic brain injury. Multiple linear regression was used to understand how age, sex, and the interaction of age and sex were related to plasma AD/VCID biomarkers and cytokines, measured using Quanterix HD-X. Results**:** We identified 413 subjects (146 men and 267 women) that fit our criteria. Their characteristics were as follows: history of hypertension (72% men, 67% women), hypercholesterolemia (81.5% and 65%), and type 2 diabetes (20.5% and 14.2%). The average age was 74 years in men and 73 years in women. When predicting IL-1β, there was a significant three-way interaction between pTau181/Aβ_{42} ratio, age, and sex ($F_{(1, 182)} = 8.02$, $p = .032$). Follow-up tests revealed that the relationship between pTau181/Aβ_{42} ratio and IL-1β was qualitatively different in men than in women ($b_{men} = 2.02$, $b_{women} = -0.78$, $p_{interaction} = .026$). Additionally, the effect of age on IL-1β was also different in men than in women ($F_{(1, 182)} = 11.40$, $p < .001$). This relationship was not significant within women ($b = 0.015$, $p = 0.02$, $p = .447$). In men, however, there was a negative relationship such that older ages were associated with lower IL-1β expression ($b = -0.09$, $p = .005$). None of the other interactions of inflammatory cytokines and AD/VCID biomarkers, sex, and age showed any significance. However, inflammatory cytokines (IL-6, IL-8, and IL-10), A$\beta_{42/40}$, neurodegeneration marker (NfLight), and neuroinflammation marker (GFAP) showed an effect with age but not sex. Conclusion**:** Pro-inflammatory cytokine IL-1β associates with amyloid beta burden (pTau181/Aβ_{42} ratio) in subjects with cardiovascular risk factors and at risk for Alzheimer's disease. This association is age dependent and differs between men and women.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.03/O3

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

Support: Morton Plant Mease Foundation

Title: Ptau217 mediates relationship between cognition and sleep in preclinical alzheimer's disease

Authors: A. PRICE[1], P. J. SNYDER[1], J. STRENGER[2], L. I. THOMPSON[2], N. RIERA[2], A. JEROMIN[3], S. SINOFF[4], *J. ALBER[5];
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Abstract: Recently, plasma phosphorylated tau 217 (ptau217) has emerged as a biomarker for identification of preclinical Alzheimer's disease (AD). The relationships between ptau217 and other known changes in preclinical AD (cognition, circadian rhythm) have yet to be characterized. We examined the relationships between circadian rhythm, ptau217, and cognition in a cohort of cognitively unimpaired (CU) older adults to determine: a) whether circadian rhythm changes are related to ptau217 pathology and whether or not the APOE E4 allele, a risk factor for AD, mediates this relationship, and b) whether ptau217 influences the relationship between sleep and cognition. 158 older adults aged 55-80 (70 male, 88 female) were identified as CU based on clinical dementia rating scale (CDR) of 0 and a score >= 24 on the Montreal Cognitive Assessment (MoCA). pTau217 was measured with a validated SIMOA assay, developed by one of us (A.J.) for ALZPath, Inc. Cognitive assessments included the Free and Cued Selective Reminding Test (FCSRT), digit-symbol substitution test (DSST), Repeatable Battery for the Assessment of Neuropsychological Status - Update (RBANS U), and MoCA. Philips actigraphy watches were worn for 14 days and nights to objectively measure sleep patterns (outcome variables: average sleep time, average duration, average efficiency, % of time awake, % of time asleep, onset latency, average wake time). There was a significant correlation between ptau217 and average sleep time and average duration ($p<.05$). ANCOVA showed no significant group differences in objective sleep measures between APOE E4 carriers and non-carriers (all $p>.05$). APOE E4 non-carriers showed a significant partial correlation between ptau217 and average duration, average efficiency, % wake, and %sleep (all $p<.05$). In E4 carriers, partial correlations controlling for age showed significant correlations between ptau217 and average onset latency ($p<.05$), with trend level correlations between ptau217 and average duration and average sleep and wake time ($p=.06-.08$). ptau217 level had a significant mediation

effect on the relationship between FCSRT learning and average sleep duration (moderate effect size, p<.05). ptau217 was found to be related to objective sleep changes in this sample of CU older adults. APOE E4 carriers and non-carriers showed different profiles with respect to relationship between ptau217 and objective sleep measures, but there were no significant group differences on these measures, meaning that APOE E4 may be indirectly related to sleep changes in CU older adults. AD pathophysiology (ptau217) may exacerbate the effects of poor sleep on cognition in CU older adults.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.04/O4

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

Support: MOST109-2113-M-006-015
MOST110-2113-M-006-014
MOST111-2113-M-006-011
CMNCKU 11013

Title: Comparative assessment of the hair metabolome in two animal models of Alzheimer's disease using high-resolution mass spectrometry

Authors: *P.-C. LIAO;
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Abstract: *Background:* In modern society, with an aging population, dementia has emerged as a significant concern in the 21st century, with Alzheimer's disease (AD) being a leading cause. Diagnosing AD in clinical practice involves PET scanning or cerebrospinal fluid extraction to detect Aβ42, which enables accurate diagnosis. However, these methods' high cost and invasive nature limit their widespread use. Metabolites as potential diagnostic biomarkers for Alzheimer's disease have been reported. Among various sample types, hair has attracted attention in metabolomics research due to its non-invasive, easy storage and accumulation of metabolites. *Research objective:* This study aimed to investigate the diagnostic potential of hair metabolomes as biomarkers for Alzheimer's disease. *Methods:* Two standard AD animal models: Aβ1-42-induced AD model (eight-week-old male Wister rats were an infusion of Aβ1-42 into the bilateral cerebral; N=12) and 5XFAD AD model (male mice harboring the 5XFAD transgene; N=10) were used simultaneously to investigate differences in hair metabolite expression between each model's AD and sham groups. Homogenized and extracted hair samples were subjected to Ultra-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS) for metabolite signal detection, while data processing was performed using MS-DIAL.

Statistical analysis using two-tailed unpaired student t-tests to detect significant difference signals. Signals showing statistical differences were identified using secondary mass spectrometry and the MoNA database. *Results:* In the Aβ1-42 induction model, 30 different metabolites were significantly upregulated in the AD group, particularly those associated with sphingolipid metabolism. In contrast, metabolites associated with unsaturated fatty acid and arachidonic acid metabolism were downregulated. In the gene transfer model, 35 differential metabolites were observed, with downregulation of metabolites related to steroid hormone biosynthesis and arginine and proline metabolism in the AD group. Interestingly, abnormal expression of phenylalanine-related metabolites was found in both models. *Conclusions:* This study highlights the alterations in hair metabolites in an AD animal model and suggests a potential link between abnormalities in the phenylalanine pathway and AD development.

Disclosures: P. Liao: None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.05/O5

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

Title: Development and characterization of a novel monoclonal antibody to Tau p217 in Alzheimer’s disease murine models and human disease tissue

Authors: *A. AIELLO, F. LIN, M. STUMP, S. SINGH, T. WIEDERHOLD, R. W. CHO; Cell Signaling Technology, Inc., Danvers, MA

Abstract: Phosphorylated tau have emerged as potential fluid-based biomarkers for Alzheimer’s disease (Hansson, 2021). Establishing a fluid-based biomarker, which measures analytes from patient cerebrospinal fluid (CSF) or plasma that associates with disease, would accelerate clinical research in treating this devastating neurodegenerative disease. Tau is phosphorylated at multiple sites. Amongst several specific tau phosphorylation sites, phosphorylation at threonine 217 (pTau217) has recently emerged as a potential reliable biomarker as levels of pTau217 increase in autosomal dominant AD patients (Barthélemy *et al*., 2020) and can discriminate AD patients from non-AD patients compared to other tau phosphorylation sites (Karikari *et al*., 2021; Palmqvist *et al*., 2020). Measurement of fluid-based biomarkers largely leverage immuno-based technologies, which include enzyme-linked immunosorbent assay (ELISA) assays, immunoassays with electrochemiluminescence detection (ECL), single molecule arrays, and immunoprecipitation mass spectrometry (IP-MS). These assays require antibodies to the target protein of the highest specificity and sensitivity. In order to improve on these assays, we sought to develop a rabbit monoclonal antibody to pTau217. Screening of rabbit monoclonal antibody libraries identified a clone, E9Y4S, that exhibited properties specific to pTau217. By western, we detected bands consistent with tau from WT mice that were absent in lambda phosphatase-treated tissue as well as tau KO brain lysates. The E9Y4S clone also specifically detected

phosphorylated tau 217 peptide without reactivity to the corresponding non-phosphorylated tau. Using the E9Y4S clone, we developed a sandwich ELISA-compatible antibody pair and plate assay that detected pTau217 in rodent brain tissue. Moreover, we were able to detect elevated p217 levels in human AD brain tissue compared to non-diseased controls. Finally, we used the pTau217 ELISA to detect elevated levels of pTau217 in plasma from the TauP301S transgenic mouse model compared to WT controls, suggesting that our identified pTau217 ELISA pair and assay could be used to detect pTau217 in patient biofluids. Together, our data suggest the newly identified E9Y4S pTau217 monoclonal rabbit antibody is highly specific and sensitive to pTau217 in human and rodent AD tissue as well as rodent AD biofluids.

Disclosures: **A. Aiello:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **F. Lin:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **M. Stump:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **S. Singh:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **T. Wiederhold:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **R.W. Cho:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc..

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.06/O6

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

Support: NIH (R21AG078538 and R01 AG078796)
Stiftelsen Gamla Tjänarinnor
Gun och Bertil Stohnes Stiftelse

Title: Delineating amyloid peptide signatures among heterogenous plaque population in Alzheimer’s Disease

Authors: *S. KOUTARAPU;
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Abstract: Delineating amyloid peptide signatures among heterogenous plaque population in Alzheimer’s disease Srinivas Koutarapu[1], Junyue Ge[1], Durga Jha [1] , Sneha Desai[1], Wojciech Michno [1,3] and Jörg Hanrieder [1,2]* 1) Department of Psychiatry and Neurochemistry, Sahlgrenska Academy, University of Gothenburg, Mölndal, Sweden 2) Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, United Kingdom 3) Department of Pediatrics, Stanford University School of Medicine, Stanford University **Aim:** Alzheimer’s disease (AD) poses an immense societal challenge and personal suffering, particularly as there were still no curative treatments as the pathogenic mechanisms of AD remain elusive. One of the main challenge would be to understand the chemical basis of

heterogeneity involved in the formation of variety of plaque polymorphs. In this study we aim to investigate the chemical nature of structuraly diverse plaque polymorphs in patient samples with sporadic AD, familial AD and Cognitively unimpaired pathological ageing. **Methods:** We employed a novel chemical imaging strategy combining microscopy with matrix assisted laser desorption/ioninsation - mass spectrometry imaging (MALDI-MSI) to delineate AB signatures of heterogenous plaque polymorphs. These plaques are then grouped into sAD-diffused plaques (DP), sAD-cored plaques (CP), sAD coarse grain plaques (sAD-CG), CU-AP diffused plaques(DP of CU-AP), fAD cored plaques (fAD-CP) and fAD coarse grain plaques (fAD-CG) based on the fluorescent microscopy images combined with multivariate image analysis performed on MALDI-MSI data. These plaques are then investigated for differences in the AB signatures. **Results:** Our results show large amounts of ABx-38 in less aggregated diffused plaques of CU-AP than cored plaques of sAD, along with shorter, truncated peptides including AB1-31, AB4-25, AB4-33 and AB6-36. In turn, AB1-40 is more abundant in more compact plaques like CG and CP in comparison to DP and DP of CU-AP. **Conclusion:** Our results imply possible role of N-terminal and C-terminal truncations in driving the aggregation kinetics of AB peptides. This data would be helpful in understanding the possible regulatory mechanism involved in extra cellular AB deposition.

Disclosures: S. Koutarapu: None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.07/O7

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

Support: National Center for Research Resources Clinical & Translational Science Award UL1TR001439 (National Center for Advancing Translational Sciences, NIH)
UTMB Mitchell Center for Neurodegenerative Diseases NIH T32 Training Grant AG067952-01

Title: Microbial determinants of dementia risk in type 2 diabetics of Mexican descent living in south Texas: A pilot study

Authors: *L. M. MATZ[1,2], N. S. SHAH[4], L. PORTERFIELD[5,6], O. M. STUYCK[3], M. D. JOCHUM[3], R. KAYED[7], G. TAGLIALATELA[7], R. J. URBAN[4], S. A. BUFFINGTON[1,2]; [1]Ctr. for Precision Envrn. Hlth., [2]Dept. of Neurosci., [3]Dept. of Obstetrics and Gynecology, Baylor Col. of Med., Houston, TX; [4]Dept. of Intrnl. Med., [5]Dept. of Family Med., [6]The Sealy institute for Vaccine Sci., The Univ. of Texas Med. Br., Galveston, TX; [7]Dept. of Neurology, Mitchell Ctr. for Neurodegenerative Dis., Univ. of Texas Med. Br., Galveston, TX

Abstract: Type 2 diabetes (T2D) is a common antecedent of pathological neurodegeneration and accompanying dementia, including Alzheimer's Disease (AD). However, relatively little is known regarding mechanistic links between metabolic and neurodegenerative disorders. We recently completed a pilot clinical study focusing on individuals of Mexican descent living in Southern Texas. As this population has increased prevalence of co-morbid T2D and early onset AD, we hypothesized that diet-driven shifts in gut microbiome composition contribute to T2D and AD susceptibility and pathophysiology. To test this, we recruited twelve Mexican American subjects aged 50-70 years old, six with and six without T2D, and performed metataxonomic 16S rRNA gene amplicon sequencing of stool samples at a single timepoint. Subjects with T2D (sT2D) had significantly higher BMI and A1C values compared to healthy controls without T2D (HC). Interestingly, both cumulative and category-averaged gastrointestinal symptom rating scale (GSRS) scores were significantly higher in sT2D compared to HCs, despite no differences in food preferences, suggesting an altered gut microbial ecology in sT2D. Analysis of alpha diversity metrics from 16S metataxonomic sequencing revealed lower diversity in sT2D gut communities compared to HCs. Furthermore, linear discriminant analysis (LDA) effect size (LEfSe) identified significant abundance differences in four taxa between sT2D and HCs. Specifically, we observed a significant decrease in the abundance of the immunomodulatory short-chain fatty acid-producing taxa *Lachnospiraceae* and *Alistipes* and increased abundance of pathobiont *Escherichia-Shigella* in the gut microbiome of sT2D. We are currently running KEGG pathway analysis to identify changes in microbial metabolic pathways between cohorts. Further studies are needed to better differentiate between gut microbiota changes due to the conditions themselves (e.g., diabetes) and the various medications used to treat the conditions (e.g., metformin). Our results suggest that characterization of the gut microbiome of individuals with T2D could be used to identify key actors among "disease state" microbiota which may exacerbate or accelerate neurodegenerative disorders and may point toward novel microbiome-targeted immunotherapeutic approaches for treating neuroinflammation in AD.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.08/O8

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

Support: U19AG069701

Title: Csf inflammatory molecules and alzheimer's disease biomarkers for predicting cognitive impairment risk in the elderly

Authors: *F. SHUE[1], L. WHITE[1], R. D. HENDRIX[8], J. D. ULRICH[12], R. HENSON[9], B. KNIGHT[10], Y. MARTENS[2], N. WANG[1], B. ROY[3], S. C. STARLING[1], C. XIONG[11], Y. W. ASMANN[4], Y. W. ASMANN[4], M. VASSILAKI[5], D. M. HOLTZMAN[13], G. BU[1], R. C. PETERSEN[6], M. G. HECKMAN[7], T. KANEKIYO[3];
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Abstract: The immune system is substantially involved in the development and progression of age-related cognitive decline and Alzheimer's disease (AD), while multiple genetic and environmental factors might contribute to pathogenesis. Therefore, we investigate how risk factors such as *APOE* genotype, age, sex, education, obesity, smoking, hypertension, diabetes, and dyslipidemia influence inflammatory molecules and AD biomarkers in cerebrospinal fluid (CSF) in a longitudinal cohort of the Mayo Clinic Study of Aging. Among cognitively unimpaired subjects over 65 years old at baseline visit (N=298), we measured the CSF levels of 365 inflammatory molecules using the proximity extension assay. We found that age, sex, and diabetes status predominantly influenced CSF levels of inflammatory molecules independent of other factors. In particular, age was a strong factor increasing the inflammatory molecules, where TNFRSF11B, CXCL9, PDLIM7, SCGB1A1, and LGALS9 were top ranked. We observed significant positive correlations of age with CSF levels of total tau, phosphorylated tau-181 (p-tau181), neurofilament light (NfL), and YKL40 among AD biomarkers. *APOE4* was also associated with lower Aβ42 and higher SNAP25 in CSF, despite modest inflammatory molecular effects. In addition, we examined whether the variables at baseline visit can predict cognitive decline, conversion from CDR=0 to CDR≥0.5. We found that age and CSF Aβ42, NfL, and REG4 were independently correlated with CDR conversion risk. When the cohort was dichotomized by their median values, 81.2 % of cases with old age, low Aβ42, high NfL, and high REG4 at baseline developed cognitive impairment during the follow up over 10 years. Our results suggest that assessing CSF inflammatory molecule composition and AD biomarkers can predict the cognitive impairment risk in the elderly in a more sensitive and accurate manner.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.09/P1

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

Title: Brain-derived toxic tau oligomeric conformers in plasma brain-derived extracellular vesicles as a predictive biomarker for Alzheimer’s disease

Authors: *M. MARCATTI, R. KAYED, G. TAGLIALATELA;
UTMB, Galveston, TX

Abstract: Alzheimer's disease (AD) represents a pressing global healthcare issue, and thus establishing reliable predictive biomarkers becomes critical to identify individuals at a high risk of developing AD, enabling the potential initiation of treatments during the preclinical stage. Recent investigations have focused on blood-based biomarkers, such as plasma brain-derived extracellular vesicles (pl-BDEVs) content, to detect alterations within the central nervous system (CNS). While blood-based biomarkers for amyloid proteins (Aβ42/Aβ40 peptide, tau, and phosphorylated tau) demonstrate promising diagnostic accuracy and correlation with cerebrospinal fluid (CSF) and neuroimaging biomarkers in AD, the urgency of identifying predictive biomarkers remains an absolute imperative. Blood total-tau primarily originates from non-brain sources, underscoring the importance of analyzing brain-derived tau (BDT) in pl-BDEVs as an AD and other neurodegenerative diseases biomarker. Longitudinal studies, which involve collecting repeated samples from a single patient over time, possess the potential to identify specific biomarker patterns during the preclinical stage of individuals who may develop AD. However, investigations have yet to focus on the role of oligomers, the most toxic species in AD. In this study, we enriched pl-BDEVs from CNS cell types (neurons, microglia, astrocytes, oligodendrocytes) from plasma samples longitudinally collected from participants enrolled in the Texas Alzheimer's Research and Care Consortium (TARCC), who were initially cognitively normal or displayed mild cognitive impairment (MCI), and later either progressed to AD (termed "converters") or remained cognitively normal/MCI (termed "non-converters"). We evaluated the isolated pl-BDEVs by nanoparticle tracking analysis (size, number, and distribution), and western blot (expression of extracellular vesicles markers: CD63, CD9, CD81). Moreover, we demonstrated the successful detection of brain-derived toxic tau oligomers (BDTOs) conformers in pl-BDEVs derived from MCI plasma samples and showed differences between converters and non-converters. This study addresses the need for predictive AD biomarkers by exploring previously unexplored BDTOs conformers in pl-BDEVs. Discovering distinct BDTOs conformers in peripheral brain derived extracellular vesicles could enable preclinical forecasting and advance early-stage AD treatments.

Disclosures: **M. Marcatti:** None. **R. Kayed:** None. **G. Taglialatela:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.10/P2

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

Title: Detection of miRNAs associated with Alzheimer's disease by electrochemiluminescence

Authors: *Y. MACHIDA, N. MASWANGANYE, T. J. BREAK, S. B. HARKINS, J. DEBAD, J. N. WOHLSTADTER;
Meso Scale Discovery, Rockville, MD

Abstract: Identification of biomarkers for studying Alzheimer's disease is of high importance, and microRNAs (miRNAs) have been implicated as potential targets. miRNA quantification is almost exclusively conducted through sequencing or singleplex measurements that require amplification. In this study, we sought to develop a simple-to-use, multiplexed assay panel for measuring miRNAs associated with Alzheimer's disease from brain tissue across multiple species. The detection of miRNAs was conducted through probe hybridization followed by binding to unique spots on the 96-well, 10-spot N-PLEX platform. Capture and detection probes were designed for each miRNA that were partially complementary to the target. The capture probes contained unique 5′ leader sequences complementary to the plate-bound oligonucleotides, and the paired detection probes contained biotin on their 3′ ends. To detect miRNAs of interest, multiplexed mixtures of capture and detection probes were hybridized to their corresponding miRNAs in 20 µL of brain lysate. These products were then hybridized to spot-specific capture oligonucleotide arrays in N-PLEX plates and detected using SULFO-TAG labeled streptavidin; quantitation was achieved by including multiplexed miRNA calibrators in each run. Plates were analyzed with an MSD electrochemiluminescence reader. A multiplexed probe set was designed against 8 miRNAs associated with Alzheimer's disease. The selected miRNAs displayed high sequence similarity across species, with 96%-100% concordance among human, mouse, rat, and cynomolgus monkey. The assay was first qualified using synthetic miRNA and then assessed in brain tissue lysates from mouse (N = 4), rat (N = 2), and cynomolgus monkey (N = 1). The assay was able to specifically identify target miRNAs with a limit of detection of ≈100 fM or lower. Two NF-kβ-sensitive pro-inflammatory miRNAs, miR-125b-5p and miR-9-5p, were highly expressed across all three species. miR-342-3p and miR-146a-5p, associated with β-amyloid peptide accumulation, were detected at a quantifiable level in all samples. The remaining miRNAs (miR-34a-5p, miR-93-5p, miR-107-3p, and miR-132-3p) could also be directly measured in mouse, rat and cynomolgus monkey brain lysates without extraction or amplification. A multiplexed miRNA assay has been demonstrated that uses a simple, rapid workflow (<4 hours to result). It does not require RNA extraction and does not use a polymerase-based detection system. In a single 96-well plate run, the assay can detect eight miRNAs in 40 tissue lysate samples, providing a method that is both quantitative for multiple miRNAs and that has the potential for high-throughput screening.

Disclosures: Y. Machida: A. Employment/Salary (full or part-time):; Meso Scale Discovery, LLC. **N. Maswanganye:** A. Employment/Salary (full or part-time):; Meso Scale Discovery, LLC. **T.J. Break:** A. Employment/Salary (full or part-time):; Meso Scale Discovery, LLC. **S.B. Harkins:** A. Employment/Salary (full or part-time):; Meso Scale Discovery, LLC. **J. Debad:** A. Employment/Salary (full or part-time):; Meso Scale Discovery, LLC. **J.N. Wohlstadter:** A. Employment/Salary (full or part-time):; Meso Scale Discovery, LLC.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.11/P3

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

Support: National Key R&D Program of China (2021YFE0203000)
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Fundamental Research Program of Shenzhen Virtual University Park (2021Szvup137)

Title: Development of a high-performance blood-based biomarker panel for the early screening and classification of Alzheimer's disease

Authors: *L. OUYANG[1,2], Y. JIANG[1,2], H. UHM[1,2], F. IP[1,2,3], E. CHENG[1,2], R. LO[1,2], X. CAO[1,2], C. TAN[1,2], V. C. MOK[4], T. C. KWOK[5], M. SUÁREZ-CALVET[6,7,8,9], H. ZETTERBERG[2,10,11,12,13], A. FU[1,2,3], N. IP[1,2,3];
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Abstract: Alzheimer's disease (AD) is currently diagnosed through cognitive assessments, brain imaging or measurement of biomarkers in the cerebrospinal fluid. However, the existing diagnostic methods are either subjective, expensive or invasive, while patients are often diagnosed after the manifestation of symptoms, which is already well past the optimal management or intervention window. Therefore, early diagnosis of AD is important, and blood proteins, which can be easily and accurately measured, are emerging as candidate biomarkers for AD diagnosis. We previously performed a systematic profiling of plasma proteome in AD patients to identify novel AD blood biomarkers. Accordingly, we developed a biomarker panel for AD classification based on the levels of 21 proteins. Here we showed that this panel achieves consistently high accuracy in the classification of AD (AUC = 0.941-0.987) and mild cognitive impairment (MCI) (AUC = 0.843-0.895) in three independent cohorts from the populations of Chinese or European descent. Moreover, we developed an AD risk scoring system based on this 21-protein biomarker panel, which correlates well with the features of AD progression including cognitive decline and the development of Aβ and tau pathologies in the brain. Furthermore, we showed that this biomarker panel outperforms existing AD-associated blood biomarkers such as plasma Aβ42/40, NfL and p-Tau181. Thus, this 21-protein biomarker panel achieves an earlier identification of AD cases from cognitively normal populations and also provides information on the status of various biological processes. Collectively, this study demonstrates the feasibility of a blood-based biomarker panel for the early screening and classification of AD in clinical settings.

Disclosures: **L. Ouyang:** A. Employment/Salary (full or part-time):; Cognitact Limited. **Y. Jiang:** F. Consulting Fees (e.g., advisory boards); Cognitact Limited. **H. Uhm:** None. **F. Ip:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognitact Limited. F. Consulting Fees (e.g., advisory boards); Cognitact Limited. **E. Cheng:** None. **R. Lo:** None. **X. Cao:** None. **C. Tan:** None. **V.C. Mok:** None. **T.C. Kwok:** None. **M. Suárez-Calvet:** None. **H. Zetterberg:** None. **A. Fu:** None. **N. Ip:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.12/P4

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

Support: This research project was supported by CONAHCYT Grant: 319578. We are grateful for the Postdoctoral Fellowships from CONAHCYT (Dr.María del Carmen Silva-Lucero) and DGAPA (Dr. Laura Gómez-Virgilio).

Title: Validation of neurodegeneration biomarkers in olfactory neuroepithelial cells from Mexican patients with Mild Cognitive Impairment, selected by bioinformatic analysis.

Authors: *M. SILVA-LUCERO, L. GÓMEZ, A. GUTIÉRREZ-MALACARA, O. LORA-MARIN, M. CARDENAS-AGUAYO;
Physiology, Sch. of Med., UNAM, Ciudad de México, Mexico

Abstract: Introduction. Mild cognitive impairment may increase the risk of dementia from Alzheimer's disease (AD) or another brain disorder. AD is the most common cause of dementia, substantial neuronal loss, and neuropathological lesions can damage many brain regions, symptoms of the disease begin with mild memory difficulties and evolve towards cognitive impairment, dysfunctions in complex daily activities, and several other aspects of cognition. The discovery of biomarkers that confer high confidence in AD diagnosis would be a valuable tool to study the disease's etiology, find risk factors, and improve treatments. **Methods.** Older adult with signs of Mild Cognitive Impairment and healthy individuals of the same age were recruited as controls. A nasal exudate was performed to obtain, isolate, and characterize Olfactory Epithelial Precursor Cells. Once the cultures were established, different biomarkers were evaluated using the Western Blot technique. We selected the biomarkers studied by through a bioinformatic analysis previously carried out by our research group. The intersection of the DEGs in the two databases showed 3 genes shared between the brain and blood of AD patients. **Results.** A cohort of 3 patients with cognitive impairment and 3 apparently healthy controls was recruited, we obtained from all volunteers their clinical history, a MOCA-cognitive test, blood samples, and the culture of the Olfactory Epithelial Precursor Cells of each patient was established. The markers analyzed by Western Blot were Aβ42/Aβ40, total tau (T-tau) and phospho-tau217 (p-tau217), as well as the AD biomarkers selected by our bioinformatics analysis: **calcineurin subunit**, a serine/threonine phosphatase under the control of Ca2+/calmodulin; **β-synuclein**, which is associated with synaptic degeneration; **a-synuclein**; and **FKBP** prolyl isomerase 1B (FKBP1B), a chaperone involved in age-related Ca2+ dysregulation in the brain. **Conclusion.** The search for altered genes in mild cognitive impaired patient-derived peripheral cells will allow us to identify potential early biomarkers of AD that could be validated in blood samples from patients with AD, as a possible diagnostic tool.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.13/P5

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

Title: A microfluidic immunoassay method for analyzing blood glial fibrillary acidic protein (GFAP) across neurodegenerative disorders

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Abstract: The study of neurodegenerative disease requires the establishment of precise, specific, and sensitive biomarker assays. Elevated circulating levels of the astrocytic protein GFAP in CSF and blood have shown promise as a potential diagnostic and prognostic biomarker in various neurodegenerative conditions. However, while detection of GFAP in blood offers the advantage of minimally invasive biomarker analysis, low levels of GFAP products in plasma and serum pose a challenge for the development of an assay that is both robust, sensitive, and easy to implement in a laboratory setting. Here, we report a novel assay for analyzing GFAP in CSF and plasma/ serum samples. Utilizing the Ella^{TM} platform, we employed an automated, microfluidic strategy by forming a sandwich immunoassay within glass nanoreactors, allowing synchronized application of assay reagents within a closed microfluidic circuit. Assay performance was evaluated by applying 25 ul of sample to 72-well microfluidic cartridges, with each well yielding triplicate results (leading to 216 readings in <90 minutes). The Limit of Detection of the novel GFAP assay was determined at 0.64 pg/mL, with a Limit of Quantitation range of 2.5 - 9,600 pg/mL. Intra- and inter-assay precision testing yielded coefficient of variance (CV) of <10%, and <12% respectively, indicating good precision within and across runs. Supporting the overall accuracy of the assay in blood and CSF, spike/recovery and spike/linearity experiments in these human matrices yielded satisfactory recovery across sample types and individual samples, with mean recovery values between 80 and 120%. We next evaluated the biomarker utility of the assay using a cohort of serum samples (n=220) which included clinically characterized patients of various neurodegenerative and neuroinflammatory disorders. Elevated blood GFAP levels were observed in Alzheimer's disease (AD; $p<0.001$) upon comparison with controls of the same age range (Kruskal-Wallis with Dunn's post-hoc comparison). In contrast, no significant increase was measured in frontotemporal dementia, multiple sclerosis, encephalitis, and meningitis patients. Notably, analyzing the same patient cohort using a commercially available bead-based assay yielded equivalent results and demonstrated good correlation between the two assays (Spearman $r = 0.92$, 95% CI = 0.89-0.94, $p<0.0001$). Taken together, these results further establish the utility of GFAP as a blood-based, astrocytic biomarker for neurodegeneration and support the applicability of a microfluidic GFAP immunoassay as a sensitive and easy-to-use benchtop strategy for measuring GFAP protein in biofluids.

Disclosures: M. Connor: A. Employment/Salary (full or part-time):; Bio-Techne. **B. Fazeli:** None. **D. Perregaux:** A. Employment/Salary (full or part-time):; Bio-Techne. **D. Unruh:** A. Employment/Salary (full or part-time):; Bio-Techne. **H. Tumani:** None. **S. Halbgebauer:** None. **Y. Noam:** A. Employment/Salary (full or part-time):; Bio Techne.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.14/P7

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

Support: VA Merit

Title: Unraveling the molecular landscape of neurodegeneration: a comprehensive assessment of 6k-plex RNA panel across whole brain tissue in ALS, AD, and healthy controls

Authors: *G. HODGE[1], L. WU[2], T. ORR[3], C. S. LATIMER[4], C. KEENE[5], M. ORR[6];
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Abstract: We present an unprecedented in-depth spatial transcriptomic analysis of molecular alterations in neurodegenerative diseases. We utilized a robust 6k-plex RNA panel to scrutinize region-matched formalin-fixed paraffin-embedded (FFPE) human brain tissues. This study compares patients diagnosed with Amyotrophic Lateral Sclerosis (ALS), Alzheimer's Disease (AD), and age-matched healthy controls, thereby providing a comprehensive molecular landscape across disease conditions.

The 6k-plex RNA panel enabled the examination of RNA profiles at a molecular resolution, covering a wide range of coding and non-coding RNAs that may contribute to disease pathogenesis or serve as potential biomarkers. We employed advanced computational methods to account for heterogeneity of the region-matched brain samples, ensuring that our analysis is directly comparable across distinct disease conditions and healthy controls.

Our preliminary findings reveal distinct molecular signatures associated with ALS and AD, characterized by differential expression of numerous RNA species across gray and white matter regions of the primary motor cortex. Interestingly, some RNA alterations were common to both diseases, suggesting shared molecular pathways in neurodegeneration. The RNA signatures also clearly demarcated the disease samples from the healthy controls, hinting at their potential utility as novel disease biomarkers. Analysis of various ligand clusters revealed differential degrees in co-localization across AD, ALS, and control. Of particular interest are ligand clusters 4 and 28 within the white matter. Cluster 28 was enriched with genes that relate to extracellular matrix and both clusters differed between ALS and control subjects. These data illuminate the broad and intricate RNA landscape in neurodegenerative disease at an unparalleled resolution.

This study highlights the importance of comprehensive and region-matched molecular profiling in neurodegenerative research, underscoring the significance of molecular resolution and precise tissue comparison for unraveling the complexities of diseases like ALS and AD. The information given from this analysis can be used to formulate future studies that examine the intricacies of the RNA landscape and how RNA can be used to further research in various neurodegenerative diseases.

Disclosures: G. Hodge: None. **L. Wu:** None. **T. Orr:** None. **C.S. Latimer:** None. **C. Keene:** None. **M. Orr:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.15/P8

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

Support: The University of Tennessee Medical Center Alzheimer's Research Initiative

Title: Multidomain Event Related Potentials as Functional Biomarkers of Early Stage Alzheimer's Disease

Authors: *R. FERNANDEZ ROMERO[1], K. L. DEAN[2];
[1]Univ. of Tennessee Med. Ctr., Knoxville, TN; [2]UTMCK, Knoxville, TN

Abstract: ***Objective***: To assess the differences in event-related potentials (ERPs) in normal aging and early-stage Alzheimer's disease (AD) as they relate to multiple cognitive domains.***Background***: Biomarkers of AD can significantly improve diagnostic accuracy and play an increasingly important role as disease modifying therapies become available. Imaging, CSF and blood biomarkers are accurate in detecting structural and pathological changes associated with AD, but provide no information about brain function and may not be directly associated with cognitive decline. This highlights the need for functional and dynamic markers that may improve early detection, help monitor disease progression and objectively assess new treatment interventions. Neurophysiologic tests such as ERPs are good candidates as biomarkers because they directly assess cortical function and may detect the synaptic dysfunction that has been observed years prior to the clinical diagnosis of AD. ERP paradigms measure functionally distinct cortical responses related to a specific type of stimulus or cognitive event. For this study, we developed three ERP paradigms that assess cortical function related to cognitive domains commonly affected in AD, including visuospatial perception, working memory, language, and attention. We hypothesized that waveform amplitudes would be significantly decreased in AD, and that waveform latencies would not significantly differ between AD and healthy older controls (OC).***Methods:*** We recruited 13 OC and 13 AD subjects with Mild Cognitive Impairment from a memory disorders clinic. Subjects completed a neuropsychological test battery and ERP procedures which included Optic Flow motion onset (OF-MO) and direction of motion-onset discrimination (OF-DMD), Delayed Match-to-Sample (DMS) with word recognition, and Change Detection (CD) with color changing shape arrays. Behavioral responses to OF-DMD, DMS and CD were recorded by button-press (BP).***Results:*** All paradigms evoked the expected ERPs (N200 responses for OF-MO and P3b responses for OF-DMD, DMS and CD). N200 and P3b amplitudes for all paradigms were significantly reduced in AD, while N200 and P3b latencies did not differ significantly between groups. Significantly lower BP accuracy was observed in the AD group for all three paradigms.***Conclusion***: These results demonstrate that ERPs can detect selective deficits in cortical responsiveness in early stage AD and that these ERP differences also correlate with cognitive domain specific behavioral responses. Our work highlights the potential of ERPs as early functional markers of AD.

Disclosures: R. Fernandez Romero: None. **K.L. Dean:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.16/P9

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

Title: Multiplex biomarker screening for neuro-inflammation associated with cognitive decline

Authors: *G. KHAYRULLINA, D. CHEO, T. BARVIR, J. SLEZAK, M. ARANA, E. DENNISON, T. PARIRA, T. GIRTSMAN, M. MORELLI, P. KRAI, S. B. HARKINS, J. DEBAD, J. N. WOHLSTADTER;
MSD - Meso Scale Diagnos., Rockville, MD

Abstract: Cytokines and chemokines are known mediators of neuroinflammation and have been correlated with an increased cognitive impairment risk observed in neurodegenerative diseases. Early detection of these factors before symptom onset may be critical for successful treatment and prevention. However, the current screening of asymptomatic patients is invasive and costly. As the prevalence of neurodegenerative diseases continues to rise, there is a need for improved, easy-to-use screening tools. A challenge in the development of multiplex assays for all analytes of interest is mitigating dilution and matrix incompatibilities. Previously developed immunoassays and electrochemiluminescent detection on the Meso Scale Discovery platform were used to multiplex up to 10 analytes, thus conserving precious samples. The three panels reported here target biomarkers associated with various diseases: Alzheimer’s disease (AD; Eotaxin-1, IL-1α, IL-12p40, IL-16, IL-17A, MCP-1, MDC, MIF, SDF-1α, YKL-40), multiple sclerosis (MS; CCL27, CXCL1, CX3CR1, IL-7, IL-12p40, IP-10, MCP-1, MIP-1β, MIP-3α), and traumatic brain injury (TBI; CD40L, CX3CR1, IFN-γ, IL-1β, IL-6, IL-18, MCP-1, RANKL, TNF-α, VEGF-D). Serum samples from patients with observed cognitive dysfunction and samples from unaffected patients (serum, plasma, and cerebrospinal fluid) were assessed with these panels. Our results show elevated levels of Eotaxin-1, CCL27, CD40L, CX3CR1, CXCL1, IL-12p40, IL-16, MCP-1, MIP-1β, VEGF-D, and YKL-40 and downregulation of MIP-3α, RANKL, and TNF-α in patients with cognitive decline. Taken together, our data demonstrate that these multiplex panels can detect biomarkers that potentially are involved in neurodegeneration. Disturbance in homeostasis is a highly dynamic and time-dependent process that warrants further study with well-defined, multi-analyte panels.

Disclosures: **G. Khayrullina:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **D. Cheo:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **T. Barvir:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **J. Slezak:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **M. Arana:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **E. Dennison:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **T. Parira:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **T. Girtsman:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **M. Morelli:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **P. Krai:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **S.B. Harkins:** A.

Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **J. Debad:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **J.N. Wohlstadter:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC..

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.17/P10

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

Support: NIH grant R01 AG057658

Title: Serine 616 phosphorylation of insulin receptor substrate-1 (IRS-1 pS616) pathology in hippocampal field CA1 as a biomarker of Alzheimer’s disease verified in 82 cases from 3 brain banks using artificial intelligence-based image analysis

Authors: X. GU[1,2], T. DISTEL[2], K. TALBOT[1,2,3];
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Abstract: IRS-1 pS616 is almost exclusively limited to cell nuclei in hippocampal field CA1 of normal humans but accumulates prominently in neuronal cytoplasm in Alzheimer’s disease (AD) dementia (ADd, Talbot et al., JCI 122: 1316-1338, 2012). We reconsidered if such IRS-1 pS616 pathology is a biomarker of preclinical AD and ADd when quantified using an artificial intelligence (AI)-based image analysis system on case cohorts from 3 brain banks.
Methods: Formalin-fixed, paraffin-embedded blocks from sex- and age- (within 5 y) matched normal (n = 81), mild cognitively impaired (MCI, n = 57), and ADd (n = 82) cases were selected from 3 brain banks (University of Pennsylvania [UPenn], Religious Order Study [ROS] of Rush University, and Banner Health Research Institute). A subset of the normal cases in each cohort displayed uncommonly high densities of neurofibrillary tangles (NFTs) and sometimes Aβ plaques, for which reason they were re-classified as preclinical AD cases. The ADd cases were not comorbid for other neurodegenerative disorders.
Coronal 6 um sections cut from each tissue block were reacted immunohistochemically for IRS-1 pS616 (antibody 44-550G), Aβ (NAB228), or p-tau (AT8) as in Talbot et al. (2012). The complete area of each CA1 section was imaged at 100X and its borders marked in pen blind to diagnosis. For AI-based image analysis (Visiopharm, Medicon Valley, Denmark), samples of IRS-1 pS616 pathology, Aβ plaques, or NFTs were outlined for supervised U-Net deep learning of properties uniquely identifying each of these tissue features with preset limits on object area and diameter to exclude glial and neuronal nuclei. One-way and two-way ANOVA were used to compare density of neurons with IRS-1 pS616 across diagnostic groups within and across case cohorts.
Results: The density of CA1 neurons (number/sq um) with IRS-1 pS616 pathology in any given diagnostic group was not significantly different across the 3 case cohorts. There were no cohorts

with a significant difference in density of such neurons in MCI vs. normal cases. In contrast, the density of CA1 neurons with IRS-1 pS616 pathology was significantly greater in ADd than normal cases in each cohort and in the combined data set ($p < 3.1 \times 10^{-13}$). Preclinical cases also showed a significant elevation in density of such neurons in the combined data set ($p < 0.004$). Greater than 90% of the ADd cases had greater densities of neurons with IRS-1 pS616 pathology than their matched pairs.
Conclusions: These findings show that an elevated density of CA1 neurons with IRS-1 pS616 may be a biomarker of preclinical and dementia stages of AD, though not at its MCI stage. This suggests ADd can sometimes develop without a clear MCI stage.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.18/Q1

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

Support: National Key R&D Program of China (2021YFE0203000)
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Title: Comprehensive profiling of plasma proteome in mild cognitive impairments identifies high-performance blood biomarkers for early screening and classification of Alzheimer's disease

Authors: *Y. JIANG[1,2], H. UHM[1,2], F. IP[1,2,3], X. CAO[2], C. TAN[2], R. LO[1,2], E. CHENG[1,2], V. C. MOK[4], T. C. KWOK[5], M. SUÁREZ-CALVET[6,7,8,9], H. ZETTERBERG[2,10,11,12,13], A. FU[1,2,3], N. IP[1,2,3];
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Hong Kong; [5]Therese Pei Fong Chow Res. Ctr. for Prevention of Dementia, Div. of Geriatrics, Dept., The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong; [6]Barcelonaβeta Brain Res. Ctr. (BBRC), Pasqual Maragall Fndn., Barcelona, Spain; [7]IMIM (Hospital del Mar Med. Res. Institute), Barcelona, Spain; [8]Ctr. de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable, Inst. de Salud Carlos III, Madrid, Spain; [9]Servei de Neurologia, Hosp. del Mar, Barcelona, Spain; [10]Dept. of Neurodegenerative Dis., UCL Inst. of Neurol., London, United Kingdom; [11]UK Dementia Res. Inst. at UCL, London, United Kingdom; [12]Dept. of Psychiatry and Neurochemistry, Inst. of Neurosci. and Physiol., The Sahlgrenska Acad. at the Univ. of Gothenburg, London, United Kingdom; [13]Clin. Neurochemistry Lab., Sahlgrenska Univ. Hosp., Mölndal, Sweden

Abstract: The importance of the early detection of Alzheimer's disease (AD), especially at the stage of mild cognitive impairment (MCI), is increasingly recognized for the early management and intervention of the disease. However, the existing diagnostic methods, through cognitive assessments, brain imaging or measurement of biomarkers in the cerebrospinal fluid, are either subjective, expensive or invasive, while patients are often diagnosed after the manifestation of symptoms and miss the golden window for intervention. We previously showed that the plasma proteome is altered in patients with AD, suggesting the feasibility of developing a robust blood test for screening and staging of AD. Therefore, to further develop a blood test that can achieve high performance in detecting the early stage of the disease, here we conducted a comprehensive profiling of the plasma proteome of MCI by measuring 1,160 proteins in a Hong Kong Chinese cohort, to identify novel blood biomarkers for MCI and early AD. Accordingly, we identified 496 proteins that were dysregulated in MCI plasma, and we showed that those MCI-associated plasma proteins, involved in different biological processes such as innate immune response, cell adhesion and inflammation, exhibited distinct dysregulating patterns upon the disease progression. We further categorized those plasma proteins into six different groups based on their dysregulating patterns, and by performing co-expression network analysis, we identified a panel of 18 plasma proteins that can capture the profile changes of plasma proteome in MCI and AD. We showed that this 18-protein panel achieves highly accurate classification of MCI (AUC = 0.913-0.925) and AD (AUC = 0.970-0.993) in two independent cohorts. Moreover, this 18-protein panel correlates well with the features of AD progression including cognitive decline and the development of Aβ and tau pathologies in the brain, which outperforms the existing AD-associated blood biomarkers including plasma Aβ42/40, NfL, p-Tau181 and p-Tau217. Taken together, this study comprehensively profiled the MCI plasma proteome and demonstrates the feasibility of a blood-based biomarker panel for the early screening and classification of MCI and AD in clinical settings.

Disclosures: **Y. Jiang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognitact Limited. **H. Uhm:** None. **F. Ip:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognitact Limited. **X. Cao:** None. **C. Tan:** None. **R. Lo:** None. **E. Cheng:** None. **V.C. Mok:** None. **T.C. Kwok:** None. **M. Suárez-Calvet:** None. **H. Zetterberg:** None. **A. Fu:** None. **N. Ip:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.19/Q2

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

Support: NIA Intramural Research Program

Title: Sex based trajectory differences in longitudinal biomarker, cognitive, and structural measures after onset of amyloid β accumulation

Authors: C. M. JOYNES, M. BILGEL, Y. AN, *L. BEASON-HELD, K. A. WALKER, S. M. RESNICK;
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Abstract: Alzheimer's disease (AD) is projected to affect 8.5 million people by 2030 and women are disproportionately affected by AD, in part due to their greater longevity. Previous studies have found sex differences in the trajectories of measures associated with AD in cognitively unimpaired (CU) older adults; in particular, studies of CU Baltimore Longitudinal Study of Aging (BLSA) participants have shown faster cognitive decline and brain volume loss in older men compared to women. To support early detection and treatment of AD it is important to more completely characterize the prodromal phase of AD, marked by the initiation of accumulation of amyloid β (Aβ) plaques in the brain, a process that can occur decades before the onset of dementia. Given the link between amyloid onset, the accumulation of pTau, and the progression of neurodegenerative processes and neurocognitive symptoms, as well as the evidence for sex-based differences in AD prevalence and disease progression, here we investigate sex-specific longitudinal trajectories of plasma biomarkers of AD and neurodegenerative pathology, cognition, and MRI-defined regional brain volumes as a function of time since amyloid onset. We center the observations from 76 amyloid positive BLSA participants (n=43 male; n=41 CU at study end) on each person's age of amyloid onset estimated using their longitudinal Pittsburgh compound B (PiB) positron emission tomography scans. To investigate trajectory differences between men and women, linear mixed effects models included an interaction term between sex and time since amyloid onset. Of the 5 biomarkers investigated ($A\beta_{42/40}$, pTau181, pTau231, glial fibrillary acidic protein, neurofilament light), we found a significant interaction between sex and time since amyloid onset on plasma levels of pTau181 ($\beta=0.44\pm0.15$, $p=0.004$) and pTau231 ($\beta=0.87\pm0.26$, $p=0.0009$). For the 8 cognitive measures, the sex by time interaction was significant for measures of executive function (Trails B (ln(s)); $\beta=1.30\pm0.48$, $p=0.007$) and visuospatial abilities (Card Rotations Test; $\beta=-0.89\pm0.25$, $p=0.0005$). Lastly, for the 6 MRI measures we found significant interactions for total brain volume ($\beta=-816.4\pm264.12$, $p=0.002$), total grey matter ($\beta=-747.01\pm198.84$, $p=0.0002$), total white matter ($\beta=-230.81\pm117.1$, $p=0.049$), and ventricular volume ($\beta=336.57\pm78.24$, $p=0.00002$). These results indicate that after amyloid onset, men have faster rates of soluble pTau accumulation and steeper declines in cognition and brain volume. These findings suggest that sex influences the longitudinal trajectory of AD pathogenesis after amyloid onset, as well as symptomatic progression.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.20/Q3

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

Support: NIH/NIA Grant P30AG066512

Title: Microstructural Changes of the Hippocampus in Early-Stage Alzheimer’s Disease

Authors: ***M. LI**[1], Y. SUI[1], A. V. MASURKAR[2], K. D. MARSH[2], T. M. WISNIEWSKI[2], H. RUSINEK[1], M. LAZAR[1];
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Abstract: Subjective cognitive decline (SCD) and mild cognitive impairment (MCI) are defined as states of cognitive decline that may progress to Alzheimer's Disease (AD). Identifying brain changes at these early stages may inform treatment approaches and help identify individuals at risk. Previous work has suggested hippocampal microstructural differences in MCI versus comparison cognitively normal control (NC) participants; however, data remains limited. Even fewer studies have assessed the earlier SCD stage. Here, we examined hippocampal microstructural changes in both SCD and MCI versus NC participants using both diffusion tensor imaging (DTI) and an advanced diffusion imaging technique, the neurite orientation dispersion and density imaging (NODDI). Participants 52 to 92 years old were assessed using the Global Deterioration Scale (GDS), a behavioral metric that distinguishes between different AD stages. Multi-shell diffusion-weighted images were acquired on a 3T Siemens Prisma scanner and used to derive three-dimensional maps of two DTI metrics, fractional anisotropy (FA) and mean diffusivity (MD) and three NODDI metrics, neurite density index (NDI), orientation dispersion index (ODI), and isotropic volume fraction (ISO) for each subject. Average values of each of these metrics were extracted from left and right hippocampus, segmented using FreeSurfer and T1-weighted images. Border voxels affected by partial volume averaging with nearby CSF were identified using diffusion metrics thresholds and were not included in the analyses. After quality assurance, the analysis included data from 36 NC (GDS=1), 105 SCD (GDS=2), and 41 MCI (GDS=3) participants. Between-groups differences (NC versus SCD and NC versus MCI) in diffusion metrics were examined using multiple linear regression models that controlled for age and gender. Group differences were considered significant for p-values <0.01. Both the SCD and MCI groups showed significantly higher MD in both left and right hippocampus compared to the NC group. Significantly lower NDI in the left hippocampus was noted for both the SCD and MCI groups compared to the NC group. Furthermore, in the SCD group, a significantly lower NDI was noted in the right hippocampus. Decreased ISO was noted only in SCD for the left hippocampus. In summary, our data suggest decreased neurite density as

a primary microstructural change in hippocampus in both SCD and MCI. This change could potentially serve as a biomarker for the early detection and prevention of AD and may relate to the memory deterioration that is the hallmark of AD. Future work will directly examine the relationships between NDI and memory and cognition.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.21/Q4

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

Support: NIGMS Grant P20GM109025

Title: A Linear Model of Neuroinflammatory Blood-Based Biomarkers in Early Detection of Alzheimer's Disease

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Abstract: One of the core features of Alzheimer’s disease (AD) is neuroinflammation, which is driven by the chronic activation of neuroimmune defense cells. Epidemiological and biological research have shown a strong link between neuroinflammation and cognitive decline. The great challenge in AD research is understanding how neural pathology maps to cognitive testing. There is a significant need to develop more efficient and cost effective measures of neuroinflammation that are sensitive enough to delineate the early stages of AD development. One possible avenue is blood-based biomarkers, though concrete targets have yet to be identified. The present study is a preliminary investigation into potential neuroinflammatory biomarkers for early detection that may lead to clinical intervention. We analyzed data collected from participants at the Center for Neurodegeneration and Translational Neuroscience (CNTN) who are enrolled in a longitudinal, natural history study of neurodegeneration and cognitive function. Using a linear model, we examined the predictive capability of several inflammatory blood-based biomarkers to determine outcomes on the sum of boxes of the Cognitive Dementia Rating (CDR) assessment. Biomarker levels were assessed via Luminex multiplex assay in participants’ first year of data collection. Our analysis identified seven significant markers of inflammation, all participating in macrophage and eosinophil immune activity and chemical signaling. In our model, increased levels of eotaxin, GM-CSF, GRO-alpha, IL-18, MIP-1alpha,

PDGF-BB, and MCP-1 together were predictive of higher scores on the CDR sum of boxes, an indication of cognitive and functional decline. More analysis is needed, however given that neuroinflammation has been shown to indicate fast progression of cognitive impairments in AD patients, these preliminary results may be a glimpse into the early detection of AD, and a precursor to intervention. These results point toward a future where pathological changes associated with neuroinflammation can be identified through blood plasma, leading to better, more cost-effective and widely available treatments early in AD pathogenesis.

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Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.22/Q5

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

Support: Ministry of Health and the Welfare Republic of Korea (grant number: HU21C0222)
Future Medicine 2030 Project of the Samsung Medical Center [#SMX1220101]

Title: Cortical Thickness and Volume Alterations as Biomarkers for Cognitive Impairment and Alzheimer's: Insights from MRI Analysis

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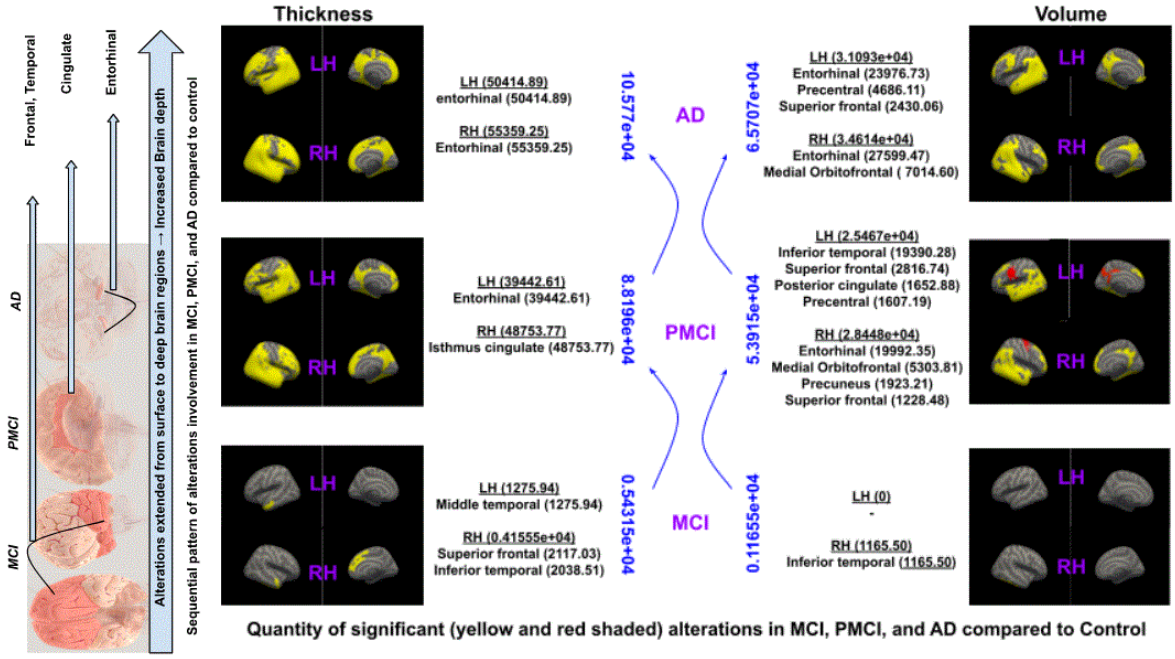
Abstract: This study aimed to investigate cortical thickness and volume alterations in Mild Cognitive Impairment (MCI), Progressive MCI (PMCI), and Alzheimer's disease (AD) compared to control subjects, with objectives of identifying alternative biomarkers for AD and elucidating patterns of hemispheric dominance and deep brain involvement. A cohort of 200 subjects, comprising 50 individuals per group from ADNI (T1 weighted MRI acquired at 1.5 T, with necessary corrections applied for 3D gradwarp and B1 non-uniformity), were utilized for analysis. FreeSurfer was employed to obtain cortical thickness and volume measurements. A General Linear Model, followed by Cluster Correction with a smoothness parameter set at fwhm10, was utilized to compare cortical thickness and volume measurements among all groups, using a significance threshold of p-value less than 0.05.
Results (illustrated in the accompanying figure) demonstrated that MCI, PMCI, and AD show significant cortical thickness and volume alterations compared to controls. These alterations

extended from surface to deep brain regions, following a sequential pattern of involvement. There has been a progressive impact on both hemispheres, extending from the surface to the deep regions of the brain. While both hemispheres have been affected, the right hemisphere has shown a predominant influence, with consistent and significant alterations observed in the right hemispheric regions. Thickness alterations progress middle and inferior temporal and superior frontal regions followed by Isthmus cingulate to the entorhinal cortex. Volume alterations progress from inferior temporal regions, followed by involvement of the posterior cingulate, medial orbitofrontal, precuneus, precentral, and entorhinal regions. These findings suggest potential of cortical thickness and volume alterations as non-invasive biomarkers for AD and provide insights into neurodegenerative processes. Future research aims to develop early detection biomarkers and enhance understanding of disease mechanisms.



Disclosures: **V. Gonuguntla:** None. **B. Chikondra:** None. **J. Kim:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.23/Q6

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

Support: NRF-2019R1A5A2026045
NRF-2020R1A2C1010399

Title: Potential dermal fibroblast biomarkers for the diagnosis of Alzheimer's disease

Authors: *J. KIM, M. LEE, H. HEO, J. CHANG;
Ajou Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract: The accumulation of abnormal intracellular substances plays a crucial role in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD). Autophagy is a fundamental intracellular process involved in the clearance of these accumulated materials. Dysregulation of autophagic activity has been observed in various AD model systems and patient brains. Dermal fibroblasts have the potential to serve as peripheral biomarkers reflecting the overall system status. In this study, we investigated potential biomarkers associated with the autophagy-lysosomal pathway (ALP) in dermal fibroblasts derived from AD patients. To identify relevant biomarkers, we analyzed the expression profiles of ALP-associated proteins and ALP-related activities in dermal fibroblasts from both AD patients and healthy control subjects. Our analysis revealed significant changes in ALP-associated protein levels in dermal fibroblasts derived from AD patients compared to healthy controls. Furthermore, ALP-related activities were found to be altered in the patient cells. Our integrated multivariable logistic regression model, utilizing profiles of ALP-associated protein levels, exhibited excellent performance (AUC > 0.9) in distinguishing AD patients from the healthy control group. These findings indicate that the alterations in ALP-associated protein levels have the potential to serve as reliable peripheral biomarkers for the diagnosis of AD.

Disclosures: **J. Kim:** None. **M. Lee:** None. **H. Heo:** None. **J. Chang:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.24/Q7

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

Support: LIFE R01AG062167
WRAP R01AG027161
ADRC P30AG062715

Title: Investigating the relationship between rate pressure product and cerebrospinal fluid biomarkers of Alzheimer's disease in cognitively unimpaired late-middle-aged adults at risk for Alzheimer's disease

Authors: C. C. ODO[1,2], J. STRONG[1,2], S. R. LOSE[1,2], Y. MA[1,2], C. L. GALLAGHER[3,4], B. B. BENDLIN[1,2,3,5], H. ZETTERBERG[6], K. BLENNOW[6], C. M. CARLSSON[1,2,3,5], G. KOLLMORGEN[7], M. CARBONI[8], S. ASTHANA[1,2,3,5], S. C. JOHNSON[1,2,3,5], *O. C. OKONKWO[1,2,3,5];
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Center, William S. Middleton Mem. Veterans Hosp., Madison, WI; [4]Dept. of Neurology, Univ. of Wisconsin Sch. of Med. and Publ. Health, Madison, Madison, WI; [5]Wisconsin Alzheimer's Institute, Univ. of Wisconsin Sch. of Med. and Publ. Hlth., Madison, WI; [6]Dept. of Psychiatry and Neurochemistry, Inst. of Neurosci. and Physiol., Mölndal, Sweden; [7]Roche Diagnostics GmbH, Penzberg, Germany; [8]Roche Diagnostics Intl. Ltd, Rotkreuz, Switzerland

Abstract: Rate pressure product (RPP) is a simple, indirect assessment of resting cardiac workload and myocardial oxygen consumption that incorporates basal systolic blood pressure (SBP) and heart rate (HR). Elevated RPP has been shown to predict cardiovascular mortality and is associated with poor cognitive test performance among older adults. There is evidence to show a relationship between RPP and cognitive function among persons with neocortical amyloid aggregation, but it is unclear how RPP is related to the cerebrospinal fluid (CSF) biomarkers for Alzheimer's Disease (AD). This study aimed to evaluate RPP as a predictor of CSF biomarker levels in a cohort of cognitively unimpaired late-middle-aged adults at risk for AD. We utilized data from 310 cognitively unimpaired late-middle-aged adults (mean age = 64.7; 68.7% female) enrolled in the Wisconsin Alzheimer's Disease Research Center (WADRC) and the Wisconsin Registry for Alzheimer's Prevention (WRAP). The CSF outcomes, measured using Elecsys assays run on Roche Cobas machines, were $A\beta_{42}$, t-tau, p-tau, alpha-synuclein, $A\beta_{40}$ GFAP, neurogranin, NFL, sTREM2 and YKL40. A Shapiro-Wilk test of normality was done for all biomarkers, and a Blom transformation was applied to non-normally distributed biomarkers to remedy the violation of normality. Linear regression was used to examine the relationship between RPP and CSF biomarkers of AD, while adjusting for age, gender, and APOE ε4. A secondary analysis was performed to investigate whether any observed associations differed by age, gender and APOE ε4 status. All analyses were conducted using IBM SPSS, version 29. RPP was positively associated with NFL (B=.006, SE=.002, p=.012) but not with other CSF biomarkers. Posthoc analyses revealed that the observed relationship was more marked in female participants (NFL: B=.007, SE=.003, p=.017), APOE ε4 positive participants (NFL: B=.010, SE=.004, p=.022), and younger participants, (<65 years of age; NFL: B=.009, SE=.004, p=.018). Our findings indicate that high myocardial oxygen demand at rest may be related to neuronal death and axonal degeneration in cognitively unimpaired late-middle-aged adults. Longitudinal studies would be needed for further establishing causality in these associations.

Disclosures: C.C. Odo: A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA. **J. Strong:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA. **S.R. Lose:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA. **Y. Ma:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA. **C.L. Gallagher:** A. Employment/Salary (full or part-time):; Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, WI, USA, Department of Neurology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. **B.B. Bendlin:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA, Geriatric Research Education and Clinical Center, William S. Middleton

Memorial Veterans Hospital, Madison, WI, USA, Wisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. **H. Zetterberg:** A. Employment/Salary (full or part-time):; Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden. **K. Blennow:** A. Employment/Salary (full or part-time):; Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden. **C.M. Carlsson:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA, Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, WI, USA, Wisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. **G. Kollmorgen:** A. Employment/Salary (full or part-time):; Roche Diagnostics GmbH, Penzberg, Germany. **M. Carboni:** A. Employment/Salary (full or part-time):; Roche Diagnostics International Ltd, Rotkreuz, Switzerland. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche Diagnostics International Ltd, Rotkreuz, Switzerland. **S. Asthana:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA, Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, WI, USA, Wisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. **S.C. Johnson:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA, Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, WI, USA, Wisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. **O.C. Okonkwo:** A. Employment/Salary (full or part-time):; Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA, Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, WI, USA, Wisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.25/Q8

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

Title: A multimodal neuroimaging-based risk score for Alzheimer's disease by combining clinicaland large N>37000 population data

Authors: *E. ZENDEHROUH[1], M. SENDI[2], V. CALHOUN[1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Harvard Med. Sch. and McLean Hosp., Boston, MA

Abstract: Background: Alzheimer's disease (AD) is the most common type of dementia among people over 65. With no effective treatment available, a preventive approach is crucial. We aim to develop a new AD risk score based on brain imaging phenotypes, known as the brain-wide risk score (BRS). This research contributes to identifying individuals at high risk of developing AD and potentially enables targeted preventive measures.Methods: We utilized the OASIS-3 cohort, consisting of 1302 imaging samples, as a base dataset to generate control (CN) and mild cognitive impairment (MCI) groups. For the target population, we accessed cognitive scores and neuroimaging data from the UK Biobank study, including resting-state fMRI, sMRI, and demographic information of 37,784 individuals. Preprocessing was performed using SPM12, and functional network connectivity (FNC) and gray matter (GM) were extracted. In the OASIS-3 dataset, we removed the mean of neuroimaging features and calculated the mean across CN and MCI groups. Similarly, in the UK Biobank dataset, we removed the mean of neuroimaging features and computed the distance between target data and CN/MCI reference data using correlation distance. Each participant in the UK Biobank dataset had two distance values, dist_CN and dist_MCI, which were used to calculate Δdiff = dist_CN - dist_MCI as an AD risk score (BRS). With two imaging modalities, we obtained two BRS values for each UK Biobank participant. Furthermore, we determined the 10th percentile of BRS for each modality and calculated the mean of neuroimaging features and their distance from the reference dataset within each percentile.
Results:We found that there was a higher sensory FNC in the CN group compared to the MCI group. By utilizing the 10th percentile of the AD BRS estimated from FNC, we identified 10 distinct biotypes. Notably, lower percentiles corresponded to the MCI group, while higher percentiles closely resembled the CN group. Similarly, we identified 10 MCI biotypes based on GM. Again, lower percentiles represented the MCI group, while higher percentiles were more closely aligned with the CN group.Conclusion:In this study, we developed a new multimodal neuroimaging-based BRS. Based on the proposed BRS from each modality, we identified 10 MCI biotypes based on 10th percentile of BRS. In the next step, we would explore the link between neuroimaging-based BRS and cognition while exploring different distance metrics.

Disclosures: E. Zendehrouh: None. **M. Sendi:** F. Consulting Fees (e.g., advisory boards); NIJI Corp. **V. Calhoun:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.26/R1

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

Title: Developing cryptic ACTL6B as novel TDP-43 loss of function biomarker

Authors: *M. CHEN[1], K. E. IRWIN[1], K. CHANG[1], P. JASIN[1], K. E. BRAUNSTEIN[3], I. SINHA[1], J. C. TRONCOSO[5], J. P. LING[2], P. C. WONG[4];
[2]Johns Hopkins Univ., [1]Johns Hopkins Univ., Baltimore, MD; [3]Div. Neuropathology, Dept. of

Pathology, [4]Johns Hopkins Sch. of Med., Sch. of Med., Baltimore, MD; [5]Johns Hopkins University, Sch. of Med., Johns Hopkins University, Sch. of Med., Baltimore, MD

Abstract: Introduction
The nuclear clearance and cytoplasmic aggregation of splicing repressor TAR DNA/RNA-binding protein-43 (TDP-43) occur in amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) and approximately 50% of Alzheimer's disease (AD) cases, driving neuron loss (Ling et.al., 2015; Meneses et.al., 2021; Neumann et.al., 2006; Cairns et.al., 2007). However, it is not clear how early such loss of function occurs in human disease as there is no method of detecting TDP-43 dysregulation in living individuals. Since the loss of TDP-43 leads to cryptic exon inclusion, we propose that cryptic exon-encoded peptides may be detected in patient biofluids to indicate TDP-43 loss of function. We developed antibodies against a TDP-43-dependent cryptic peptide within actin-like protein 6B (ACTL6B) and showed that cryptic ACTL6B is detectable in human brain neurons depleted of TDP-43. We also developed a sandwich ELISA for cryptic ACTL6B detection in biofluids.
Methods
We characterized ACTL6B cryptic antibodies through protein blot analysis using TDP-43-knockdown (KD) SH-SY5Y neuroblastoma cell lysates. To test the antibody's sensitivity and specificity for cryptic ACTL6B in human brains, we used immunofluorescence staining on control, ALS, FTLD, and AD brain tissues. We then developed a Meso Scale Discovery (MSD) ELISA using our cryptic ACTL6B antibody.
Results
While the wild-type (WT) ACTL6B antibody detected an expected band in both WT and KD SH-SY5Y lysates, the cryptic ACTL6B antibody detected a band in only KD SH-SY5Y. Using this antibody, we detected ACTL6B signal in FTLD and AD, but not control, brain tissues. The cryptic ACTL6B staining colocalizes with neurons that display TDP-43 nuclear depletion and/or cytoplasmic aggregation. Using transfected SH-SY5Y cells to overexpress either cryptic or WT ACTL6B, the MDS ELISA was sensitive and specific for cryptic ACTL6B, with 0.5 micrograms of lysate from SH-SY5Y overexpressing cryptic ACTL6B showing MSD signal 5-fold higher than 0.5 micrograms of lysate from SH-SY5Y overexpressing WT ACTL6B (1546 vs. 290 units).
Conclusions
Our findings provide evidence that our cryptic ACTL6B antibody is sensitive and specific for the ACTL6B cryptic peptide in both SH-SY5Y cells and human brain tissues. This antibody could be used to determine TDP-43 nuclear clearance/loss of function upon pathological staining, as our cryptic ACTL6B antibody stains some non-immunoreactive neurons for cytoplasmic phosphorylated TDP-43 but show TDP-43 nuclear depletion. In the future, we will use our novel MSD ELISA to test the presence of cryptic ACTL6B in patients' CSF and blood, which may aid in early-stage disease detection.

Disclosures: M. Chen: None. **K.E. Irwin:** None. **K. Chang:** None. **P. Jasin:** None. **K.E. Braunstein:** None. **I. Sinha:** None. **J.C. Troncoso:** None. **J.P. Ling:** None. **P.C. Wong:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.27/R2

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

Title: Automated diagnostic for Alzheimer's disease at 30 minutes validated by the gold standard

Authors: *F. V. CHIRILA, M. M. CHIRILA;
Spot Dx LLC, Morgantown, WV

Abstract: Diagnosing late-onset Alzheimer's disease (AD), representing 95% of cases remains elusive despite intensive research. Most diagnostic studies used samples from clinically diagnosed AD patients and compared the signal with healthy controls. Given the common AD pathways with other dementias and the inaccuracy of clinical diagnosis in the early AD stages, these measures are not AD-specific. Our novel approach automatically analyzed live human skin fibroblasts in images taken 30 minutes after plating on Matrigel. Previously we have demonstrated that cellular aggregates correctly identify AD from Non-AD demented patients at 48 hours (1-3) in gold-standard samples, suggesting that their building blocks, the cells, carry a similar dysregulated morphometric signal even earlier at 30 minutes. Gene expression and protein studies confirmed single-cell dysregulation in AD patients compared to non-AD demented(4). Here we determined the biomarker's cutoff as the 99% percentile of the range for the Healthy Control's (n=13) signals, then applied it to the validation set of 30 samples. The biomarker accurately distinguished 16 AD and 14 non-AD demented patients validated by gold-standard autopsy or autopsy equivalent (genetic), with a minimal overlap probability ($P<1.45x10-7$; Two-tailed, unequal variance, T-test). The 30-minute test is highly accurate(100%; 95% CI >88%), sensitive (100%; 95% CI >79%), and AD-specific(100% 95% CI >77%). When used in conjunction with the 48-hours test(1) on the same samples, the 30-minute automated diagnostic elevates the lowest limits of the 95% confidence intervals (CI) for sensitivity above 96% and specificity above 95%. The 30-Minute Alzheimer's disease diagnostic offers an earlier, more precise, and more accurate alternative to the 48 hours measure (1-4). The diagnostic confidence at 30 minutes is higher than at 48 hours due to better statistics (a more significant number of cells at 30 minutes than the number of aggregates at 48 hours). Due to automation, the analysis time of 30-minute images is cut down to 2 min **1.** Chirila, F.V., Xu, G., Fontaine, D. *et al.* "Morphometric imaging biomarker identifies Alzheimer's disease even among mixed dementia patients." *Sci Rep* 12, 17675 (2022). .**2.** Chirila, F. V. *et al.* 'Spatiotemporal Complexity of Fibroblast Networks Screens for Alzheimer's Disease.' 1 Jan. 2013: 165 - 176. **3.** Chirila, F. V., Khan, Tapan K., and Alkon, D. L.' Fibroblast Aggregation Rate Converges with Validated Peripheral Biomarkers for Alzheimer's Disease." 1 Jan. 2014 : 1279 - 1294. **4.** Chirila, F.V and Alkon, D. L., "Synchronized Cell Cycle Gene Expression Test for Alzheimer's Disese." CTAD 2019.

Disclosures: F.V. Chirila: None. **M.M. Chirila:** None.

Poster

PSTR198. Alzheimer's Disease: Biomarkers and Risk Factors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.28/R3

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

Support: NIH Grant K99AG065645
NIH Grant R00AG065645

Title: Microrna-502-3p as a potential biomarker for alzheimer's disease

Authors: *D. DEVARA, B. SHARMA, M. TORRES, S. KUMAR;
Texas Tech. Univ. Hlth. Sci. Ctr. El Paso, El Paso, TX

Abstract: Alzheimer's disease (AD) is the leading cause of dementia and currently affects about 50 million people worldwide. Analysis of cerebrospinal fluid (CSF) for hyperphosphorylated tau (p-tau), total tau and Aβ42 protein content is one of the techniques currently used to diagnose AD; however, the disease would already have to be relatively advanced for successful diagnosis. Currently, there lacks minimally invasive peripheral biomarkers for early detection of AD. Therefore, it is important to develop new strategies to diagnose AD at an early stage. Recently, our lab discovered the presence of microRNA-502-3p (miR-502-3p) in the synapses of AD brains and characterized its potential role in disease pathology. In this study, we examined the biomarker potential of miR-502-3p for AD. The CSF samples from AD and unaffected healthy controls (HC) were obtained from NIH NeuroBioBank center. The CSF exosomes were extracted from AD and HC samples and characterized by electron microscopy and exosome marker analysis. Further, CSF miRNAs and CSF proteins were extracted from the AD and HC samples. MiR-502-3p expression was quantified by qRT-PCR analysis and the levels AD proteins- Aβ40, Aβ42, total tau and p-tau were quantified by ELISA analysis. Our results found that miR-502-3p is overexpressed in the CSF of AD samples compared to HC exosomes. The high levels of miR-502-3p were significantly correlated with AD proteins and neuropathology report of the CSF sample donors. Our studies found a strong correlation of miR-502-3p with Aβ levels, Aβ plaques, and neurofibrillary tangles of the patients. Therefore, miR-502-3p could be a promising synaptic and CSF biomarker for AD.

Disclosures: D. Devara: None. **B. Sharma:** None. **M. Torres:** None. **S. Kumar:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.01/R4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Alzheimer's Association (H.T.E)
Leon Levy foundation (H.T.E.)

The Rainwater foundation (H.T.E.)
NIH grant NS121786 (E.K.)

Title: Translational dysregulation in human iPSC-derived neuronal model of frontotemporal dementia

Authors: *S. VENKATESAN KALAVAI[1], H. EVANS[2], E. DENIL[3], E. KLANN[4];
[1]New York Univ. Neurosci. & Physiol., New York, NY; [2]New York Univ. Ctr. For Neural Sci., New York, NY; [3]New York Univ. Ctr. for Neural Sci., New York, NY; [4]New York Univ., New York, NY

Abstract: Protein synthesis is a vital biological process, important for many neuronal and cognitive functions, such as axon guidance and growth, synaptic plasticity, and long-term memory. Recently, studies have shown that dysregulated translation is a hallmark of many neurodegenerative diseases, such as frontotemporal dementia (FTD). Studies have shown that FTD-associated mutations to the neuronally enriched microtubule binding protein, tau, impair protein synthesis and ribosome formation (Evans et al., Acta Neuropathoc Comms, 2021; Koren et al., Acta Neuropathologica, 2019; Meier et al., J. Neuro, 2016). These studies however have relied upon overexpression models, such as transfected HEK cells or transgenic mice, presenting a clear confound. Furthermore, the effect of FTD-mutant tau on translation in human patients remains relatively unexplored. Here, we have investigated how FTD-mutant tau alters protein synthesis in human iPSC-derived neurons that have endogenous tau expression at normal physiological levels. We utilized various analyses, including puromycin labeling of de novo synthesized proteins, polysome profiling, and harringtonine run-off assays.
Using these techniques, we show that the FTD-tau mutant V337M results in reduced global translation by 4 weeks compared to isogenic controls, potentially due to a slowed rate of elongation. To explore the interaction between tau and ribosomes, we use polysome profiling. Our data suggests that V337M FTD-mutant tau decreases the quantity of the 60S ribosomal subunit. Together, our results demonstrate that FTD-mutant tau can impact protein synthesis and its machinery and that these impairments occur at physiological levels of tau.

Disclosures: S. Venkatesan Kalavai: None. **H. Evans:** 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.; Grants received from Alzheimer's Association, Leon Levy foundation, and the Rainwater foundation.. **E. Denil:** None. **E. Klann:** 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.; Grant funded by NIH(NS121786).

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.02/R5

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIA R21 (B.K)
NIA R01 (B.K)
Jeane.B. Kempner Fellowship (C.N)
The Don and Nancy Mafrige Professor in Neurodegenerative Disease Endowment (B.K)
Mitchell Center for Neurodegenerative Diseases (B.K)

Title: Phospholipase D1 signalosome disrupts glutamatergic transmission in behavioral variant Frontotemporal Dementia.

Authors: *C. NATARAJAN[1], S. GOPALKRISHNA SHETTY SREENIVASA MURTHY[2], B. THOMSON[3], P. PHAN[3], K. H. GARZA[2], B. KRISHNAN[4];
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Abstract: Background: Our lab showed the effect of elevated PLD1 driven by amyloidogenic insults involving Aß and tau in wild type mice and in 6-& 12 -months old 3xTg-AD mice model. PLD1 associated with protein-protein interaction is associated with the activation and cessation of activity of other key proteins associated that can affect several cellular activities. In this study, we report comprehensive protein profiling and evaluation of human bvFTD (behavioral variant fronto-temporal dementia) post-mortem brain tissues with primary focus on role of PLD1 in pathology. In this study, we are hypothesizing a role of elevated PLD1 in FTD patients relative to the control subjects and its relevance to pathology in tauopathy. **Methods:**We studied the association of PLD1 with tau, post synaptic receptors in human post-mortem frontal and temporal brain tissues using immunofluorescence. Synaptic perturbations of bvFTD patients in temporal and frontal regions were then investigated using an innovative approach called as Fluorescence assisted single synaptosome-long term potentiation. Further, to explain synaptic dysfunction in frontal and temporal lobe regions, we profiled protein expression levels of AMPA receptors. To elaborate the association between PLD1 and its signalosome partners we used immunoprecipitation to understand the interaction between pathological levels of PLD1 and m TOR, p-cofilin and PKCα. **Result:**We report elevated levels of PLD1 in temporal homogenates but not on that of the frontal lobes of bvFTD patients relative to control subjects. We then observed significant differences in association of PLD1 with full length tau and phosphorylated tau in bvFTD patients in tissue sections of human post-mortem brains. Interestingly, we were able to confirm the association of PLD1 with PSD-95 and thus pathological role of PLD1 driven synaptic transmission. Our FASS-LTP study findings revealed presence of synaptic deficits found in temporal and frontal lobe of bvFTD patients as compared to the control subjects. There was also a significant association between elevated PLD1 and Glial fibrillary acidic protein in temporal lobe of bvFTD patients relative to control non-demented subjects. **Conclusion:** We report elevated PLD1 expression drives cognitive decline via synaptic dysfunction in bvFTD specific to temporal pathology. Significant reduction in levels of glutamate receptors further explains the pathology driven by PLD1 and its regulators within the cytoplasm and also in the synaptic clefts— m TOR,PKC α and p-cofilin. The outcomes here, in turn, demonstrates a potential therapeutic target that can be combined with tau-targeting regimens in treatment of bvFTD.

Disclosures: **C. Natarajan:** None. **S. Gopalkrishna Shetty Sreenivasa Murthy:** None. **B. Thomson:** None. **P. Phan:** None. **K.H. Garza:** None. **B. Krishnan:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.03/R6

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH Grant U19NS110456
NIH Grant R01NS114656
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Supported by the Michael J. Fox Foundation

Title: Noninvasive detection of oxidative stress in preclinical models of synucleinopathy via positron emission tomography with [^{18}F]ROStrace

Authors: *E. GALLAGHER[1], C. HOU[2], C.-J. HSIEH[2], Y. ZHU[3], P. HENDERSON[3], R. CHRONEOS[3], M. SHELDON[3], S. RILEY[3], H. LEE[2], S. LI[2], K. XU[2], N. KOHLI[3], R. H. MACH[2], M. J. MCMANUS[3];
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Abstract: The synucleinopathies are a diverse group of neurodegenerative disorders characterized by aberrant aggregation of alpha-synuclein (aSyn) in neurons and/or glial cells. Oxidative stress—i.e. intracellular damage resulting from overproduction of reactive oxygen species (ROS)—is thought to be both a mediator and a consequence of aSyn aggregation in the synucleinopathies. Here, we sought to characterize the relationship between aSyn aggregation and ROS production in living brain via positron emission tomography (PET) with the recently-developed radiotracer [^{18}F]ROStrace. A53T mice (line M83) were used as a preclinical model of aSyn aggregation, and dynamic [^{18}F]ROStrace PET imaging was performed in male and female A53T and control mice at early (6-8mo) and middle (12mo) stages of disease progression. Additional cohorts of A53T and control mice were imaged 24h, 1m, or 5m following intraperitoneal lipopolysaccharide (LPS) injection, and all PET results were correlated with histological measures of aSyn aggregation and neuroinflammation at endpoint. As expected, A53T mice showed significantly higher whole-brain average [^{18}F]ROStrace signal than age- and sex-matched control mice at both the 6-8mo timepoint (n=27-28 animals per genotype; $p<.001$) and the 12mo timepoint (n=23-26 per genotype; $p<.001$). Significant elevations in PET signal were more widespread in 12mo animals compared to 6-8mo animals, consistent with a progressive accumulation of aSyn pathology in A53T brain over time. At both timepoints, elevations in PET signal were associated with increased histological evidence of oxidative stress and neuroinflammation, as well as significant disruptions to normal nesting and locomotor

behavior. Similar patterns were observed in LPS-injected animals, with LPS-injected A53T mice showing higher [^{18}F]ROStrace signal than LPS-injected control mice at 24h (n=8 per genotype; p=.014), 1m (n=5 per genotype; p=.09), and 5m (n=6 per genotype; p=.002) post-injection. Moreover, average brain [^{18}F]ROStrace signal remained elevated in LPS-injected A53T mice (1.12 at 24h post-injection vs 1.12 at 5m post-injection) but decreased over time in LPS-injected control mice (1.05 at 24h vs 1.02 at 5m). These data suggest that LPS treatment results in a persistent state of oxidative stress in A53T mice relative to similarly aged control mice. Collectively, our results indicate that aSyn aggregation in A53T mouse brain is consistently associated with increased ROS production as measured by [^{18}F]ROStrace, and they also demonstrate that A53T mice are more susceptible to—and less able to recover from—LPS-induced neurotoxicity.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.04/R7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Pathological Alpha Synuclein Causes Molecular Deficits in the PFF Model of Synucleinopathies.

Authors: *M. MILLETT, Jr.[1], N. E. CHAMBERS[1], A. HEUBERGER[1], A. M. COMITE[1], E. M. CASTOSA[2], P. WAGNER[1], I. GALLARDO[1], D. HALL[1], D. NABERT[1], M. MOEHLE[1]; [1]Univ. of Florida, Gainesville, FL; [2]Univ. of Florida, Gainesvillle, FL

Abstract: Synucleinopathies, a group of neurodegenerative disorders, are characterized by the aggregation of alpha synuclein (aSyn) into large insoluble inclusions termed Lewy bodies. Current treatment options for synucleinopathies are primarily symptomatic due to lack of mechanistic knowledge of how the aggregates affect cell function. To investigate the impact of Lewy pathology on molecular mechanisms, we employ the aSyn pre-formed fibril (PFF) model that causes the corruption of endogenous aSyn into pathological inclusions. PFF injected mouse brain tissue was then assayed using immunohistochemical, biochemical, expansion microscopy, and spatial transcriptomic techniques. Notably, Immunohistochemistry (IHC) revealed that that the majority of pathological aSyn aggregate subtypes localize within the nuclear envelope, and some penetrate inside nuclear compartments of the cell. These findings were confirmed in human tissue samples from patients with Lewy Body Dementia. Fluorescent in situ hybridization (FISH) analysis of PFF injected mouse brain tissue shows differential localization of RNA in cells with Lewy like pathology, suggesting altered RNA transport between nucleus and cytoplasm. Further IHC staining demonstrated the outer portion of aSyn aggregates localize within the endoplasmic

reticulum (ER), as the ER is continuous with the nuclear envelope. Cells with aSyn aggregates display abnormal levels of ER pathway markers, suggesting altered molecular signaling of the ER organelle as well. Lastly, neuronal cell models cultured in media supplemented with PFFs also displayed nuclear localization of aSyn aggregates and are currently being subjected to live cell assays to rigorously assess relevant organelle function. Cumulatively, our data defines the localization of aSyn aggregates in the nuclear envelope, nucleus, and the ER. Furthermore, we show that the homeostatic function of these cellular organelles is altered. This data begins to identify intracellular pathways that could potentially serve as etiological therapeutic targets and warrants further investigation.

Disclosures: **M. Millett:** None. **N.E. Chambers:** None. **A. Heuberger:** None. **A.M. Comite:** None. **E.M. Castosa:** None. **P. Wagner:** None. **I. Gallardo:** None. **D. Hall:** None. **D. Nabert:** None. **M. Moehle:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.05/R8

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH Grant R01NS132293-01

Title: Pathological α-synuclein dysregulates cholinergic signaling within the PL-mPFC in the PFF model of Lewy Body Dementias

Authors: *I. A. GALLARDO[1], N. E. CHAMBERS[2], M. F. MILLETT, Jr.[2], D. NABERT[2], D. HALL[1], M. S. MOEHLE[2];
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Abstract: The abnormal accumulation of α-synuclein (α-syn) into insoluble intraneural inclusions, known as Lewy Bodies, is a characteristic pathological feature shared by various neurodegenerative disorders, including Parkinson's Disease-Dementia (PDD) and Dementia with Lewy Bodies (DLB), collectively known as Lewy Body Dementias (LBD). Although the mechanisms by which pathological α-syn induces these cognitive dysfunctions are not fully understood, several clinical and pathological findings point to a unique role for altered cholinergic signaling to underlie cognitive disturbances in LBD. Progressive loss of cholinergic projection neurons of the basal forebrain, which project to critical cortical regions known to influence cognition, is predictive of PDD development in Parkinson's Disease (PD) patients and correlates with cognitive symptoms in DLB. Key among these cortical regions receiving cholinergic input is the prelimbic medial prefrontal cortex (PL-mPFC), a significant area linked to cognition and executive function. However, how aggregated α-syn impacts these circuits is not fully understood. To investigate this, we utilized a combination of pharmacological, electrophysiological, and fiber photometric approaches in the pre-formed fibril (PFF) mouse

model of LBD to understand the impacts of aggregated α-syn on cholinergic signaling in the PL-mPFC. We found that α-syn dysregulates both ACh release and ACh signaling in the PL-mPFC, leading to altered long-term plasticity in layer 5 of the PL-mPFC. Altogether, these results provide a potential mechanistic pathway for the α-syn-driven corruption of PL-mPFC circuits that lead to cognitive dysfunction in LBD

Disclosures: I.A. Gallardo: None. **N.E. Chambers:** None. **M.F. Millett:** None. **D. Nabert:** None. **D. Hall:** None. **M.S. Moehle:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.06/S1

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NINDS 1R01NS117968

Title: Dark-fret biosensors for real-time monitoring of alpha-synuclein oligomerization in live cells

Authors: *A. R. BRAUN, E. E. LIAO, N. NATHAN KOCHEN, J. N. SACHS;
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Abstract: Pathological misfolding and oligomerization of alpha-synuclein (aSyn) plays a crucial role in the pathogenesis of alpha-synucleinopathies. Monitoring aSyn conformation in disease relevant cell types is essential to unravel the underlying disease mechanisms and identify potential therapeutic targets. However, existing techniques, such as immunofluorescent cytochemistry and proximity ligation assays, require fixed samples, limiting real-time observation. Building upon our previous work with live-cell fluorescence lifetime based FRET (FLT-FRET) biosensors, we have developed a series of genetically encoded Dark-FRET biosensors capable of simultaneous monitoring of aSyn's oligomerization in two distinct cell populations. Through coupling bright-monomeric donor XFP with spectrally matched quenching XFPs we are able to monitor both biosensor donor lifetimes with minimal spectral crosstalk. This technology will allow for future co-culture FLT-FRET studies where it can provide a more comprehensive understanding of aSyn oligomerization dynamics in a more complex cellular model.

Disclosures: A.R. Braun: None. **E.E. Liao:** None. **N. Nathan Kochen:** None. **J.N. Sachs:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.07/S2

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH R01NS088485
VA Merit I01RX002340

Title: Genetic and Functional Analysis Reveals a Link between PERK Signaling and Tauopathy in Progressive Supranuclear Palsy

Authors: *A. GALDAMEZ[1], G. PARK[2], Y. YOO[2], W. YAN[2], K. KIM[2], L. CHEA[2], J. LIN[2]; [1]VA Palo Alto Healthcare Syst., Palo Alto, CA; [2]Stanford Univ., Palo Alto, CA

Abstract: This abstract presents a comprehensive study investigating the relationship between PERK (PKR-like Endoplasmic Reticulum Kinase) and Progressive Supranuclear Palsy (PSP), a neurodegenerative disease characterized by tau protein aggregation. Our research aimed to elucidate the mechanisms underlying the genetic association between tauopathy-associated PERK variants and neurodegeneration risk using a combination of genetic analysis, functional assays, and analysis of single-cell RNA sequencing (scRNA-seq) data. Firstly, we identified the pathogenic PERK Haplotype B variant as a functional hypomorph through cell culture studies, suggesting its potential involvement in tau aggregation. Building upon this finding, we employed bioinformatic tools to analyze pathogenic PERK variants and evaluate their impact on protein structure and pathogenicity. We specifically focused on missense changes introduced by tauopathy-associated PERK variants in the luminal domain, which disrupt hydrogen bonding and impair signal transduction. To further investigate the functional consequences, we utilized in vitro cell culture models to study the effect of PERK pathway modulation on tau aggregation. Our results demonstrated that both chemical and genetic inhibition of the PERK pathway led to increased tau aggregation, while its activation showed the opposite effect. These findings provide direct evidence of the functional impact of PERK dysregulation on tau pathology. Last, by analyzing bulk RNA-seq and scRNA-seq data from Braak tau pathology staged AD brains, we observed a significant reduction in the expression of the damaged PERK gene set specifically in AD brains with advanced tau pathology and especially in neurons, supporting the involvement of PERK dysregulation in tauopathy. In contrast, no such reduction was observed in glial cells. Collectively, our findings suggest that tauopathy-associated PERK variants contribute to PSP risk by impairing PERK signaling and promoting tau aggregation. This integrated approach combining genetic analysis, functional assays, and bulk- and scRNA-seq data analysis provides valuable insights into the molecular mechanisms underlying PSP pathogenesis. Further research is warranted to fully understand these effects and explore potential therapeutic strategies targeting the PERK pathway.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

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Program #/Poster #: PSTR199.08/S3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: LF Experiment - Lundbeckfonden

Title: Dating tau aggregates to reveal the spatial and chronological tauopathy progression in progressive supranuclear palsy

Authors: *M. RASMUSSEN[1], M. SCHWEIGHAUSER[2], M. HUANG[3], M. BACIOGLU[4], W. MCEWAN[3], M. SPILLANTINI[4], S. AZNAR[1], E. GARDE[5], K. PENKMAN[6], J. B. ROWE[7], S. KAALUND[1];
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Abstract: Progressive supranuclear palsy (PSP) is a severe neurodegenerative disorder characterized by impairments in movement, cognition, and behavior. The neuropathological processes begin many years before the onset of clinical symptoms. This creates a window for preventive strategies, but also uncertainties over the origins and mechanisms of progression of the pathology. PSP is a recognized tauopathy, with abundant aggregates of the four-repeat microtube-binding-protein tau (4R) in glial and neuronal cells. While neuropathology diagnostic stages suggest a typical pattern of spread of tau aggregates throughout the brain the rate and dynamics of this process are still unclear. Our aim is to date the tau aggregates and elucidate the spatial and chronological progression of tauopathy in PSP. In order to date tau aggregates we measure the spontaneous posttranslational process racemization, where the native and common L-form amino acids convert to the mirror-image, D-form. The rate of racemization is associated with age and is routinely used for dating fossils. Preliminary data strongly suggest that we can determine %D-amino acids from isolated tau fibrils and thus estimate the age of tau fibrils in the brain tissue from donors with PSP. This dating of tau aggregates is used to establish a better understanding of the chronological relationship between tau aggregation and clinical manifestations of PSP.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR199.09/S4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Movement Disorders Division/Postma Family Research Pilot Grant
NIH Grant R01 NS128467

Title: Comparing Lewy pathology interactomes between Parkinson's disease and multiple system atrophy

Authors: *S. CHOI[1], D. BETTS[2], J. GALLAGHER[1], R. BAROT[3], T. TITTLE[1], Y. CHU[4], J. KORDOWER[4], B. KILLINGER[1];
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Abstract: Synucleinopathies, such as Parkinson's disease (PD), Dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), are neurodegenerative disorders with shared clinical and pathological features. The hallmark of synucleinopathies is Lewy pathology (LP), consisting of aggregated alpha-synuclein (asyn) predominately phosphorylated at serine 129 (PSER129). For PD/DLB, LP is mainly observed in neurons (NCIs), but for MSA, LP is mostly found in oligodendroglia (GCIs). Extensive research has focused on characterizing NCIs, but there is less understanding of the GCIs in the MSA brain. We hypothesize that the distinct synucleinopathies MSA and PD/DLB share common pathological cellular pathways. To test this, we used an in-situ labeling technique called biotinylation by antibody recognition (BAR) to compare LP-interactomes (BAR-PSER129) and asyn-interactomes (BAR-MJFR1) between MSA (n=5) and PD/DLB (n=10) in the striatum and midbrain. Results showed that in both BAR-PSER129 and BAR-MJFR1, asyn was the most significantly enriched protein in PD/DLB and MSA. In PD/DLB, BAR-PSER129 identified 28 LP-interacting proteins, while BAR-MJFR1 identified 47 total-asyn interacting proteins. In contrast, MSA had only 7 and 46 proteins identified for each capture, respectively. When comparing MSA and PD/DLB, high overlap (53.3%) was observed between BAR-MJFR1 captured proteins, whereas very low overlap (6.7%) was observed for BAR-PSER129. Proteins including CRYAB, FTH1, FTL, CBR1, HAPLN2, PGAM1, and LDHA were distinguishing features of MSA LP, and their functional pathway enrichment analysis showed significant enrichment (padj. <0.05) for pathways including ferrous iron binding and extracellular exosome. In contrast, LP interactions in the PD/DLB were enriched for vesicle-mediated processes, lysosomes, and neurotransmitter release cycle. Surprisingly, only four proteins, including asyn, CRYAB, HAPLN2, and GDI2 were BAR-PSER129 enriched for both MSA and PD/DLB, and they were involved in amyloid fibril formation, axons, and lytic vacuoles (padj. <0.05). To sum up, synucleinopathies show divergent LP interactions indicative of distinct pathological mechanisms. MSA involves iron-mediated processes in glial cells, while vesicular processes in neurons dominate PD/DLB. The few shared LP interactions in MSA and PD/DLB suggest protein aggregation in neuronal axons in both diseases. CRYAB can prevent aberrant protein aggregation, and we speculate it acts as a neuroprotective mechanism for both

diseases. Future studies should target the identified overlapping proteins between the two diseases to prevent/inhibit asyn aggregation therapeutically.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.10/S5

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Parekh Center for Interdisciplinary Neurology
NINDS Grant 1R56NS131658
Parkinson's Foundation (Columbia University)
P30 AG066462 (Columbia University)

Title: Striatal cell type specific transcriptional dysregulation in multiple system atrophy

Authors: *T. C. MA[1], J. G. VONSATTEL[3], R. N. ALCALAY[4], S. LIDDELOW[2], U. J. KANG[1];
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Abstract: Multiple system atrophy (MSA) is a sporadic progressive neurodegenerative disease clinically defined by autonomic dysfunction with any combination of parkinsonism and cerebellar features. Along these lines, MSA patients are stratified into two main subtypes based on motor phenotype: *MSA-C for cerebellar* and *MSA-P for parkinsonian* predominant, which correlate with olivopontocerebellar atrophy and striatonigral degeneration, respectively. However, pathology is evident in both brain regions for most patients, indicating common pathogenic processes. Despite widespread neurodegeneration, the pathological hallmark of MSA is the accumulation of fibrillar forms of α-synuclein in glial cytoplasmic inclusions (GCIs) within oligodendrocytes. As overt loss of oligodendrocytes is variably reported, the nature of oligodendrocyte dysfunction has been a main focus of MSA pathogenesis research. α-synuclein is expressed at low levels by oligodendrocytes and whether the accumulation of α-synuclein within oligodendrocyte is cell autonomous remains debated. Additionally, the factors leading to α-synuclein accumulation (early events) and the subsequent consequences cellular physiology (late events) remain obscure. We hypothesize that the less affected tissue of each MSA subtype represents earlier stages in the progression of MSA: the striatum for MSA-C and cerebellum for MSA-P. Here we use single nucleus RNA sequencing (snRNAseq) of postmortem striatal tissue (putamen) from MSA-P, MSA-C, and healthy control donors to capture the transcriptional profile of nearly all striatal cell types to uncover the cell type-specific pathological alterations and their progression in MSA. We present striatal single cell transcriptomes for 6 control, 5

MSA-P, and 7 MSA-C cases. As with other studies, the transcriptional heterogeneity of recovered oligodendrocytes allowed identification of subtypes. The distribution of oligodendrocyte subtypes was altered in MSA with a progressive increase in the proportion of putatively pathological cells with the highest number in MSA-P. We will add profiling data from matching cerebellar tissue from these same subjects to determine whether this gradient of transcriptional dysregulation shifts towards MSA-C cases in the cerebellum.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.11/S7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE U01 NS110453
Mitsubishi Tanabe Pharma America

Title: Single-nucleus multi-region transcriptional characterization of sporadic and familial 4R-taupathic neurodegenerative disorders CBD, PSP, MAPT-N279K, and MAPT-P301L

Authors: *A. BERG[1], S. PINEDA[1], C. BOIX[2], M. TAMURA[3], S. MURATANI[4], S. TSURU[5], M. KELLIS[1];
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Abstract: Neurodegenerative diseases affect one in nine individuals above the age of 65 in the US. A subset of these diseases, called four-repeat (4R) tauopathies, are characterized by abnormal 4R-tau deposits within neurons, glia, and extracellular space. Both sporadic and familial 4R forms exist, with sporadic distinguished by their histopathological presentations, and familial by the specific mutations of the MAPT or PGRN gene. However, clear molecular signatures for 4R-tauopathies are still lacking, and little is understood about the drivers of their heterogeneity.

To address this gap, we generate 711k single-nucleus transcriptomic maps from 120 samples across 67 patients and two brain regions (prefrontal cortex, PFC, 303k nuclei; substantia nigra, SN, 408k nuclei), and across two sporadic and two familiar 4R-tauopathies: corticobasal degeneration (CBD, 20 patients), progressive supranuclear palsy (PSP, 18 patients), MAPT-N279K (N279K, 7 patients), and MAPT-P301L (P301L, 6 patients), the latter two (familial) resulting from the specified mutations to the tau-producing MAPT gene. We annotated 56 transcriptionally distinct cell subtypes, revealing previously-unappreciated differences between glial subtypes in the PFC and SN, which may underlie regional differences in cell type vulnerability. We found selective depletion of ventrally-biased dopaminergic neurons across all four 4R-tauopathies, and similar astrocytic and oligodendroglial subtype gliosis patterns in CBD and P301L. We also found downregulation of transcriptional regulators E2F4 and EGR1 for both sporadic 4R-tauopathies across nearly all PFC-based neuronal cell subtypes. Finally, we found striking cell-cell communication differences, with the PGRN-SORT1 signaling pathway in glial cells showing downregulation in PSP, but upregulation in the three other 4R-tauopathies. Our resource sheds important light on these devastating tauopathies, and our initial results already reveal highly specific hypotheses that can help guide therapeutic developments.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR199.12/S8

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: State of Arizona, DHS

Title: Proteolysis of Tau protein in neurodegenerative disease

Authors: N. M. FERNANDEZ[1], A. E. DALE[1], J. M. KANNER[1], N. S. REDDY[1], C. S. HOM[2], ***C. HUSEBY**[2];
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Abstract: Pathological Tau protein is found in a collective group of neurodegenerative diseases referred to as tauopathies. Tau has emerged as a surrogate marker of neurodegeneration, a source of neurotoxicity and a candidate vector for disease propagation. Identification of the molecular events associated with the initiation of Tau aggregation can lead to a new understanding toward etiology of disease. Tau is subject to a variety of post-translational modifications including proteolytic cleavage to smaller peptides. Research has revealed that proteolysis of Tau can

change normal neuronal interactions and promote aggregation propensity. Identification of active Tau peptides opens new disease pathways for future research and possible therapeutic targets. We mapped proteolytic cleavage sites of nineteen known and other yet to be discovered proteases along the longest human brain Tau isoform. Tau-derived peptides were ordered and received from commercial peptide synthesis services with greater than 95% purity. Thioflavin dye fluorescent assays were used to test for aggregate formation alone as well as ability to induce and incorporate with full-length human Tau isoforms in aggregation. Peptide aggregation inhibitory effects were also monitored screening for possible therapeutic pathways.
Our preliminary analysis of eighty Tau derived peptides show that truncated Tau protein species can form aggregate structures in vitro while others do not. This suggests that some proteolytic cleavage sites may be toxic while others are protective preventing aggregate formation. Toxic and protective peptides derived from Tau protein provide evidence for implicating specific proteases and their associated pathways in disease.

Disclosures: **N.M. Fernandez:** None. **A.E. Dale:** None. **J.M. Kanner:** None. **N.S. Reddy:** None. **C.S. Hom:** None. **C. Huseby:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.13/S9

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NRF-2020R1A5A1019023
NRF-2022R1A2C1004913
KHIDI-HU21C0071
BK21 Four Biomedical Science Program

Title: Self-aggregating tau fragment disrupts axon initial segment plasticity and displays an abnormal polarized distribution

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Abstract: Neurons possess highly compartmentalized structures with distinct axonal or somatodendritic domains. The axon initial segment (AIS) has a unique cytoskeletal organization that serves as a diffusion barrier, facilitating the segregation and polarized distribution of compartment-specific cargos. Dysregulation of polarized trafficking is associated with neuronal dysfunction and neurodegenerative disorders. In particular, the AIS exhibits activity-dependent structural plasticity, which plays a role in modulating neuronal excitability. Tau is a microtubule-associated protein that binds to and stabilizes microtubule filaments. While tau is highly abundant in the axons of brain neurons, the pathological accumulation of tau, which is a

hallmark of Alzheimer's disease (AD) and other tauopathies, is primarily observed in the somatodendritic region. Recent cryoelectron microscopy studies have identified a tau filament core (tau-AC) that drives the formation of tau aggregates in the AD brain. Based on this, we hypothesized that the structural integrity and plasticity of the AIS may contribute to the mislocalization and aggregation of tau-AC in the somatodendrites. Using confocal imaging technology in rat primary cultured neurons, we find that wild-type tau promotes AIS lengthening, whereas tau-AC disrupts AIS plasticity in response to chronic depolarization. Furthermore, the polarized trafficking of tau-AC was dysregulated, resulting in an increased distribution within the dendritic compartment and mislocalization onto dendritic spines. Our findings suggest that AIS plasticity plays a crucial role in maintaining the polarized distribution of tau in neurons. Further studies are necessary to elucidate the mechanisms underlying the polarized trafficking of tau that will contribute to a broader understanding of tauopathies.

Disclosures: S. Park: None. **J. Lee:** None. **M. Lee:** None. **Y. Suh:** None.

Poster

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Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: KBRI basic research program 23-BR-02-04
KHIDI R&D project HU21C0027

Title: Cyclodextrin exacerbates tau pathology in a mouse model of tauopathy: implication of the pathological role of cholesteryl ester

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Abstract: Abnormal accumulation of tau aggregates is a pathological hallmark of Alzheimer’s disease (AD) and other tauopathies. In AD, the sequential spreading of tau aggregation throughout the brain is strongly correlated with disease progression. Accumulating evidence suggests that intracellular aggregation and trans-cellular propagation of pathological tau are key pathological events underlying the spreading of tau pathology. Accumulating evidence strongly suggest that the disruption of lipid homeostasis is closely linked to AD pathologies, in particular dysregulation of cholesterol. Several evidence reported that cyclodextrin (CD), a cyclic oligosaccharide compounds widely used cholesterol depleting agent, reduces amyloid beta plaque in AD mouse model and insoluble alpha synuclein in cell model. Although increasing evidence suggests the pathological link between cholesterol metabolism and tauopathy, the detail mechanisms remain elusive. In the current study, we investigated the effect of cholesterol on tauopathy in PS19 transgenic mice expressing P301S mutant human tau by injecting 2-Hydroxypropyl-beta-cyclodextrin (HP-CD), one of the CD modified forms identified to have

good inclusion complexation and maximum in vivo safety for various of biomedical applications. HP-CD dramatically promoted pathological tau accumulation and neurodegeneration in the hippocampus. We further showed that the changes in cholesterol level in the various organs including blood plasma, liver, and brain. Interestingly, cholesteryl ester (CE) was increased by HP-CD in liver and brain cytosol. Recently, it has been reported that CE is accumulated in the brains of AD patients and AD mouse models, and CE increases tau phosphorylation. Based on these results, we hypothesized that increased cytoplasmic CE might exacerbate tau pathology. To confirm the hypothesis, we tested the effect of HP-CD in a seed-dependent tau aggregation cell model and found that HP-CD increased Triton X insoluble tau aggregation. In the future, we plan to prove that the increase in tau aggregation by HP-CD is reversed by using a compound that inhibits CE in cell model. Our data suggest that modulation of cholesterol ester may represent a practical strategy for AD therapy.

Disclosures: **E. Bok:** None. **J. Kim:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.15/T1

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: R01 AG075092 (HF)
R56 AG066782 (HF)
K01 AG056673 (HF)
AARF-17-505009 (HF)
BrightFocus foundation A2021027S (HF)

Title: Human neural organoids with the MAPT R406W mutation exhibit altered cholesterol metabolism, increased tau phosphorylation, and altered functional activity.

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Abstract: Tauopathies are a group of neurodegenerative diseases that are classified together by the significant role of aggregated tau in their pathogenesis. Mutations in the Microtubule

Associated Protein Tau (*MAPT)* gene, which encodes for the tau protein, can cause Frontotemporal Dementia (FTD) and several other tauopathies. Several *MAPT* gene mutations, such as *MAPT* R406W, have been used to model FTD and Alzheimer's disease (AD) in transgenic mice, cell culture, and more recently in human induced pluripotent stem cells (hiPSC) and organoid culture. Neural organoids provide a complex model with a diverse population of cell types, cortical areas with deep-layer and upper-layer organization of neurons, and functional neuronal and network activity. All of which can be essential in studying and understanding disease onset and pathology of FTD, AD, and other Tauopathies. We found that neural organoids generated from patient-derived hiPSCs with a heterozygous *MAPT* R406W mutation exhibited increased phosphorylated tau based on immunostaining and Western blot assay. The functional activity of the neural organoids was assessed by microelectrode array recordings and displayed altered activity compared to controls. The secretome lipidomics of mutant organoid culture medium also exhibited altered lipid profiles when compared to controls. Interestingly, the transcriptomic profile of neural organoids by single cell RNA-seq revealed differentially expressed genes that are associated with lipid metabolism including (VEGFB, GRAMD1B, HMGCS1, and LDLR) in excitatory neurons of mutants compared to control. Gene sets of pathways including chondroitin-sulfate binding, flotillin complex, and endoplasmic reticulum and plasma membrane contact sites were also enriched in mutants. Our data suggest the *MAPT* R406W mutation may have a significant impact on cholesterol storage, lipid synthesis, membrane transport, and signal transduction. Importantly, we investigate cholesterol transport in neurons and whether altered cholesterol and lipid metabolism may influence tau pathology. Our findings provide new insights into the relationship between cholesterol changes and tau pathology.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.16/T2

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Institut Curie
French National Research Agency ANR-20-CE13-0011
Fondation pour la Recherche Medicale (FRM) grant MND202003011485
France Alzheimer grant 2023
PSL-Biogen fellowship

Title: Lack of posttranslational polyglutamylation of microtubules impacts neuronal functions and can cause late-onset neurodegeneration

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Abstract: Microtubules (MTs) - a key component of the neuronal cytoskeleton- exert a multitude of essential functions in many aspects of neuronal cyto-architecture, function, and longevity. How these multiple roles are orchestrated still remains only partially understood. One underexplored mechanism is the regulation of MTs by posttranslational modifications (PTMs) of their basic building blocks - the tubulin proteins. One of these PTMs, polyglutamylation, is catalyzed by enzymes of the TTLL (glutamylases) and CCP (deglutamylases) protein families. We have previously demonstrated that excessive polyglutamylation due to mutations in the key deglutamylase CCP1 lead to early-onset neurodegeneration in mice[1] and humans[2]. We further showed that polyglutamylation acts as a rheostatic controller of axonal transport, suggesting that strictly controlled levels of this PTM are essential for neuronal homeostasis[3].
To assess the role of polyglutamylation as a physiological modulator of MT functions in neurons, we generated novel mouse models with reduced polyglutamylation, lacking main neuronal glutamylases. In contrast to mice with excessive polyglutamylation that develop early neurodegeneration, ageing brains of mice with reduced polyglutamylation revealed signs of late-onset neurodegeneration. Magnetic resonance imaging (MRI) revealed a progressive reduction in the size of the olfactory bulb and the prefrontal cortex. Immunohistochemistry on brain sections further showed loss of the mature neurons in the glomerular layer of the olfactory bulb and a reduced thickness of the prefrontal cortex. To investigate whether these anomalies translate into perturbations of neuronal functions, we performed manganese enhanced MRI, which indeed uncovered signs of altered neuronal activity in mice with reduced polyglutamylation. We thus aimed at determining the effect of reduced polyglutamylation on cognitive parameters of our mouse models. Using an automated ‘Intellicage’ system, we found that mice with reduced polyglutamylation show signs of better long-term memory, a result we are further investigating. By determining the role of tubulin polyglutamylation and its regulation in the physiology of neuronal cells, we aim at gaining insights into its potential role in the pathogenesis of neurodegenerative disorders. Our data suggest that regulating polyglutamylation might open promising new prospects for the development of therapies tackling neurodegenerative diseases in which tubulin PTMs are deregulated.
[1]Magiera et al, EMBO J, 2018
[2]Shashi et al., EMBO J, 2018
[3]Bodakuntla et al., EMBO J, 2021

Disclosures: S. Chakraborty: None. **S. Poggini:** None. **N. Ciano Albanese:** None. **D. Boido:** None. **L. Ciobanu:** None. **I. Branchi:** None. **C. Janke:** None. **M. M. Magiera:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.17/T3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Inclusion of a cryptic exon in ELAVL3 results in RNA misprocessing and proteome alterations across TDP-43 proteinopathies

Authors: *M. CHUNG[1,2], E. MISHKOVSKY[1,2], N. RAJ[1,2,3], *M. CHUNG[4], D. DUONG[5], E. B. DAMMER[5], A. N. TRAUTWIG[5], A. VEIRE[8], T. F. GENDRON[8], M. E. MURRAY[8], M. G. TANSEY[9], T. L. KUKAR[6], N. T. SEYFRIED[5], J. D. GLASS[7], G. J. BASSELL[1,2], Z. T. MCEACHIN[1,2,3];
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Abstract: The mislocalization, phosphorylation, and aggregation of the nuclear RNA binding protein, TAR DNA-binding protein 43 (TDP-43), is a neuropathological hallmark of several neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's Disease (AD), and Limbic Age-related TDP-43 encephalopathy (LATE-NC). Notably, TDP-43 pathology is observed in disease-relevant regions of the CNS and correlates with neurodegeneration. Previous studies have suggested that toxic gain-of-function mechanisms due to the deposition and aggregation of phosphorylated TDP-43 (pTDP-43) underlie the associated toxicity. However, recently, several lines of evidence suggest that the nuclear depletion of TDP-43 drives neurotoxicity through the loss of nuclear TDP-43 function. In addition to the well-established roles of TDP-43 in alternative splicing and mRNA stability, TDP-43 has been shown to repress the inclusion of cryptic exons in several transcripts. We have identified a cryptic exon regulated by TDP-43 in the ELAV-like 3 (ELAVL3) gene that results in reduction of ELAVL3 expression. ELAVL3 is a neural-specific RNA binding protein important for alternative splicing (AS) and alternative polyadenylation (APA). We observe inclusion of a cryptic exon in ELAVL3 across TDP-43 proteinopathies. RNA sequencing of iPS derived motor neurons with ELAVL3 knocked down results in significant alternative splicing and polyadenylation events. Additionally, we observe significant changes in the proteome in iPS-MNs with reduced ELAVL3. Together, our data suggest that TDP-43-dependent loss of ELAVL3 results in global changes in the transcriptome and proteome and may contribute to disease pathogenesis. Understanding these disease pathways is critical for identifying novel therapeutic targets for TDP-43 proteinopathies.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.18/T4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: COBRE Grant 1P20GM148326-01
NIA-ADRC Grant P30 AG072946 "University of Kentucky Alzheimer's Disease Research Center"

Title: The role of Spermidine/spermine N1-acetyltransferase 1 and the polyamine stress response in TDP-43 proteinopathies

Authors: *C. R. SAUNDERS[1], P. ROCHA-RANGEL[1], J. A. GRIFFITH[1], J. B. HUNT, Jr.[1], H. ZHU[3], P. M. THOMPSON[4], D. C. LEE[2], M.-L. B. SELENICA[1];
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Abstract: TAR DNA-binding protein 43 kDa (TDP-43) is a nuclear DNA/RNA binding protein linked to the neuropathology of a spectrum of disease. TDP-43 undergoes several post-translational modifications (PTM), proposed to alter its structure and cellular function. Our laboratory is focused on molecular mechanisms underlying TDP-43 pathology in AD Related Dementias (ADRD) and hence investigating the effects of Spermidine/spermine N1-acetyltransferase 1 (SSAT1), a major acetyltransferase in the catabolic polyamine pathway, on TDP-43 pathology. This study aims to investigate the impact of SSAT1 activity and polyamine metabolism on TDP-43 structure and cellular function, as crucial pathways implicated in protein aggregation and accumulation in neurodegenerative diseases.We performed LC MS/MS analysis in a disease-state mouse model (TAR) overexpressing human wtTDP-43. While aged TAR4 mice (heterozygous, mild pathology) displayed significant levels of putrescine, arginine, and glutamic acid, the young homozygous TAR4/4 mice (progressive pathology) showed a 3-fold increase in putrescine levels followed by significant reduction in spermine and glutamic acid levels in the cortex. Similarly, acetyl-putrescine (3-fold) and acetyl-spermidine (1-fold) were significantly increased in TAR4/4 mice, suggesting that progressive TDP-43 pathology drives a classical polyamine stress response (PSR) in these models. To investigate the contributing effects of the PSR on TDP-43 pathology and aggregation, we performed thioflavin-T kinetics with recombinant TDP-43 low-complexity domain (LCD) with putrescine, spermidine, spermine, and their respective acetyl forms. We found a decrease in t1/2 (elongation phase) following incubation with acetyl polyamine, suggesting that increased catabolic polyamine pathway accelerates TDP-43 nucleation phase. As the TAR model demonstrated increased SSAT1 levels in a gene-dependent fashion, we demonstrated the effects of SSAT1 activity on acetylation of eIF5A, a direct target of SSAT1 recently identified in our laboratory. Additionally, SSAT1 gene deletion significantly reduced acetylated eIF5a levels, validating acetylation of EIF5A as a direct target of SSAT1 activity in mice. Utilizing recombinant protein systems and mass spectrometry analysis we discovered that SSAT1 is the main acetyltransferase responsible for TDP-43 acetylation, acetylating 5 out of 20 lysine epitopes. From this data we generated 3 specific antibodies for K79, K136, and K160, two of which are found to contribute to TDP-43

aggregation in literature. This work will aid in unraveling the role of SSAT1 and PSR-dependent TDP-43 pathology in disease.

Disclosures: C.R. Saunders: None. **P. Rocha-Rangel:** None. **J.A. Griffith:** None. **J.B. Hunt:** None. **H. Zhu:** None. **P.M. Thompson:** None. **D.C. Lee:** None. **M.B. Selenica:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.19/T5

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: ioGlutamatergic Neurons CRISPR Ready cells: a functional genomics tool to easily generate disease-specific models for drug discovery and development

Authors: *J. CONDE VANCELLS[1], A. GRANDCOLAS[2], C. TALBOT-COOPER[1], E. SPENCER[1], G. BELLI VALLETTA[1], G. SHIPLEY[1], B. KLAPHOLZ[1], S. SALIC[2], A. BYRNE[2], K. FIRTH[1], F. PATELL-SOCHA[1], M. METZAKOPIAN[1], T. BURCKSTUMMER[2], M. KOTTER[1];
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Abstract: Human induced pluripotent stem cells (hiPSC) provide an unlimited source of any human cell due to their unique capability to self-renew and differentiate into any cell type, allowing for disease specific models for drug testing. This is particularly important for human cells otherwise difficult to obtain such as neurons. Traditional animal models or cell lines might not fully replicate human diseases. hiPSC-derived cells can more accurately mimic human physiology, therefore providing a more reliable prediction of drug efficacy and toxicity.
Use of hiPSC-derived cells is restricted by complex and lengthy directed differentiation protocols or inconsistent cellular reprogramming methods. Our proprietary opti-ox™ (optimised inducible overexpression) technology overcomes these restrictions by enabling the precisely controlled expression of transcription factors to provide a rapid, consistent, and reliable source of any specific cell type of interest. ioGlutamatergic Neurons have been precision reprogrammed from hiPSC using opti-ox technology. Within days, cells convert consistently to mature, functional glutamatergic neurons characterised by >80% expression of glutamate transporter genes VGLUT1 and VGLUT2 and the formation of electrically active neuronal networks at 17 days.
We have engineered ioGlutamatergic Neurons with a transgene that constitutively expresses the Cas9 nuclease, termed ioGlutamatergic Neurons CRISPR Ready Cells, to allow for easy gene knockout generation. ioGlutamatergic Neurons CRISPR Ready Cells express pan-neuronal markers (MAP2, TUBB3) and glutamatergic neuron specific markers (VGLUT1 and VGLUT2) by RT-qPCR and ICC. Bulk RNA sequencing shows comparable gene expression profiles to parent ioGlutamatergic Neurons. Cas9 is expressed and functional from day 1 post-thaw. Using single guide RNAs delivered by either lentiviral transduction or lipid-based transfection, we

show successful knockout of *SOX11,* evidenced by more than 80% indel formation. ioGlutamatergic Neurons CRISPR Ready Cells provide an easy-to-use functional genomics tool to allow researchers the study of any genes, diseases, or signaling pathways in a physiologically relevant hiPSC-derived cellular model. These cells offer easy disease-specific model generation for drug development.

Disclosures: **J. Conde Vancells:** A. Employment/Salary (full or part-time):; bit.bio. **A. Grandcolas:** A. Employment/Salary (full or part-time):; bit.bio discovery. **C. Talbot-Cooper:** A. Employment/Salary (full or part-time):; bit.bio. **E. Spencer:** A. Employment/Salary (full or part-time):; bit.bio. **G. Belli Valletta:** A. Employment/Salary (full or part-time):; bit.bio. **G. Shipley:** A. Employment/Salary (full or part-time):; bit.bio. **B. Klapholz:** A. Employment/Salary (full or part-time):; bit.bio. **S. Salic:** A. Employment/Salary (full or part-time):; bit.bio discovery. **A. Byrne:** A. Employment/Salary (full or part-time):; bit.bio discovery. **K. Firth:** A. Employment/Salary (full or part-time):; bit.bio. **F. Patell-Socha:** A. Employment/Salary (full or part-time):; bit.bio. **M. Metzakopian:** A. Employment/Salary (full or part-time):; bit.bio. **T. Burckstummer:** A. Employment/Salary (full or part-time):; bit.bio discovery. **M. Kotter:** A. Employment/Salary (full or part-time):; bit.bio.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.20/T6

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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National Research Foundation (NRF) funded by the Korean government (MSIT) (2022M3E5E8017395)
KIST Program (2E32211)

Title: Investigation of methods for the study of the neuronal mitochondrial morphology

Authors: *S. KIM[1,2], K. STRUCINSKA[3], B. OSEI[3], J. CHOI[4], W.-K. JEONG[4], T. LEWIS[3,5], K. HAN[2], S.-K. KWON[1,6];
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Abstract: Mitochondria morphology is distinct in neuronal dendrites and axons with dendritic mitochondria being long and tubular, while axonal mitochondria are short and punctate. In the neuron, mitochondria directly impact neuronal function via ATP generation and calcium clearance, and these actions are closely associated with their specific morphology. Mitochondria are also highly sensitive to surrounding environment altering their morphology in response to cues such as oxidative stress, aging, or disease. Therefore, it is essential to optimize the methods for analyzing neuronal mitochondrial shape to obtain a comprehensive understanding of the functional properties of neurons. In this study, we first investigated whether the fixation condition is a determinant for mitochondrial morphology. Surprisingly, fixative solutions containing only paraformaldehyde (PFA) or hypoxic conditions during intracardiac perfusion induced fragmentation of mitochondria. However, this disruption was not observed with glutaraldehyde addition or oxygen-supplemented direct perfusion of fixative without PBS pre-flushing, respectively. Furthermore, under suboptimal fixation conditions, it became challenging to distinguish the effects of neurodegeneration from fixation on mitochondria. After establishing the optimal condition for preserving mitochondrial morphology, we employed MitoVis, a deep-learning based program, to improve the rigor and robustness of the analysis of neuronal mitochondrial structures in a compartment-specific manner. MitoVis allowed the examination of mitochondrial length in synucleinopathy model neurons in an unbiased yet 10 times faster speed than manual methods. Using MitoVis, we find that the average length of dendritic mitochondria is significantly reduced in this model (3.57±0.05μm, 9,657 mitochondria for control, 3.066±0.03μm, 11,434 mitochondria for α-Syn, $p<0.0001$). Additionally, the average length of axonal mitochondria was decreased from 1.354±0.02μm to 1.197±0.02 μm (2,711 mitochondria for control, 2,579 mitochondria for α-Syn, $p<0.0001$). Taken together, investigating compartment-specific neuronal mitochondrial morphology with an appropriate method is crucial for understanding the neuronal function and related diseases.

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Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.21/T7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Sanofi

Title: Aav-arsa mediated gene replacement for the treatment of metachromatic leukodystrophy

Authors: S. RAMACHANDRAN[1], J. ARDINGER[1], J. BU[1], M. RAMOS[1], E. WISCHHOF[1], L. GUO[2], M. GONCALVES[2], D. GHOSH[2], M. HOSSAIN[2], S. AYLOO[1], J. ADAMS[1], D. DUBREUIL[1], Y. LUO[1], ***E. J. W. CROSIER**[1], A. RICHARDS[1], R. JACKSON[1], J.

HOGESTYN[1], S. NASS[1], J. SULLIVAN[1], C. O'RIORDIAN[1], M. GOULET[1], C. MUELLER[1]; [1]Genomic Med. Unit, [2]Precision Med. and Computat. Biol., Sanofi, Waltham, MA

Abstract: Metachromatic leukodystrophy (MLD) is an autosomal recessive neurodegenerative disorder caused by mutations in the arylsulfatase A (*ARSA*) gene, resulting in lower sulfatase activity and the toxic accumulation of sulfatide in the central and peripheral nervous system. In MLD patients, this leads to progressive demyelination, cerebral atrophy, peripheral neuropathy, and shortened life expectancy. Therapeutic benefit of ARSA replacement has been demonstrated in a clinical setting and the sustained expression of ARSA by gene therapy is a promising strategy with potential to restore myelinogenesis, prevent additional atrophy and provide long lasting therapeutic benefit from a one-time treatment. We present an AAV-mediated gene replacement strategy to treat MLD and report therapeutic benefit in a MLD mouse model, and meaningful ARSA expression and biodistribution in non-human primates (NHPs). We report the nomination of a novel capsid AAV.GMU1, demonstrating better biodistribution and higher transgene expression in the CNS of NHPs, compared to a gold-standard CNS-tropic capsid. We demonstrate that AAV.GMU1-h*ARSA* mediated gene replacement in MLD mice (*Arsa*$^{-/-}$) resulted in the reversal of MLD-associated pathology. AAV.GMU1-h*ARSA* treated mice show increased sulfatase activity in the brain and spinal cord and a concomitant reduction in sulfatide levels (LC-MS) in the brain, spinal cord, CSF, and plasma. Treated MLD mice exhibit prominent and persistent h*ARSA* expression (measured up to 13 months post-dosing), secretion, and cross-correction. Furthermore, *Arsa*$^{-/-}$ mice treated with AAV.GMU1-h*ARSA* showed near complete absence of MLD-associated histopathology and absence of MLD-associated phenotypes. Further, a dose-range finding pharmacology and safety study was carried out in juvenile cynomolgus monkeys, that received four increasing doses of AAV.GMU1-h*ARSA.* We report widespread and dose-dependent increase in AAV.GMU1-h*ARSA* vector biodistribution in NHP brain, with clinically meaningful h*ARSA* expression in >90% of the brain. NHPs showed uniform h*ARSA* expression in the spinal cord and DRGs along the spinal rostral-caudal axis, and presented no clinical signs (functional or behavioral) for the duration of the study. In summary, we propose AAV.GMU1-h*ARSA* mediated gene replacement as a clinically viable approach to achieve broad and therapeutic levels of ARSA in the CNS and PNS.

Disclosures: S. Ramachandran: A. Employment/Salary (full or part-time):; Sanofi. **J. Ardinger:** A. Employment/Salary (full or part-time):; Sanofi. **J. Bu:** A. Employment/Salary (full or part-time):; Sanofi. **M. Ramos:** A. Employment/Salary (full or part-time):; Sanofi. **E. Wischhof:** A. Employment/Salary (full or part-time):; Sanofi. **L. Guo:** A. Employment/Salary (full or part-time):; Sanofi. **M. Goncalves:** A. Employment/Salary (full or part-time):; Sanofi. **D. Ghosh:** A. Employment/Salary (full or part-time):; Sanofi. **M. Hossain:** A. Employment/Salary (full or part-time):; Sanofi. **S. Ayloo:** A. Employment/Salary (full or part-time):; Sanofi. **J. Adams:** A. Employment/Salary (full or part-time):; Sanofi. **D. Dubreuil:** A. Employment/Salary (full or part-time):; Sanofi. **Y. Luo:** A. Employment/Salary (full or part-time):; Sanofi. **E.J.W. Crosier:** A. Employment/Salary (full or part-time):; Sanofi. **A. Richards:** A. Employment/Salary (full or part-time):; Sanofi. **R. Jackson:** A. Employment/Salary (full or part-time):; Sanofi. **J. Hogestyn:** A. Employment/Salary (full or part-time):; Sanofi. **S. Nass:** A. Employment/Salary (full or part-time):; Sanofi. **J. Sullivan:** A. Employment/Salary (full or part-time):; Sanofi. **C. O'Riordian:** A. Employment/Salary (full or part-time):; Sanofi. **M. Goulet:** A. Employment/Salary (full or part-time):; Sanofi. **C. Mueller:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.22/T8

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Cln3 ko as a model of lysosomal dysfunction and neurodegenerative disease

Authors: *H. S. WALD, S. JINN, D. TOOLAN, L. YAO, N. HATCHER, S. SMITH, J. MARCUS;
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Abstract: Lysosomal dysfunction is thought to underlie the pathophysiology of neurodegenerative disease (NDD). This is supported by evidence that NDD shares many pathological features with lysosomal storage diseases (LSD), and that mutations in lysosomal pathway genes confer disease risk. In both, deficits in lysosomal functions, including degradation, trafficking and clearance, lead to accumulation of undegraded cellular material, contributing to neurodegeneration. As such, models of LSDs are useful to understand these pathways and to develop neurodegenerative therapeutics that enhance lysosomal function. While these treatments are developing, biomarkers of lysosomal dysfunction that inform on lysosomal changes and efficacy of pharmacological treatment are still not validated or well-understood. This project investigates lipid biomarkers of lysosomal dysfunction through the characterization of CLN3 loss-of-function mutation, which causes the juvenile LSD, Batten disease, in a human iPSC-derived neuronal model. This includes comparison with iPSC models of two other LSDs, Niemann-Pick Type C and Krabbe disease. In characterizing the phenotypic consequence of CLN3 loss, this project explores assays to measure lysosomal exocytosis as a potential biomarker to measure lysosomal changes and to investigate cellular clearance under lysosomal stress and in response to pharmacological agents that stimulate lysosomal function. Assays also include the assessment of cerebrospinal fluid (CSF) from aged non-human primates to complement *in vitro* work by linking age-associated lysosomal dysfunction to lysosomal endpoints validated in cellular models. This project aims to contribute to the understanding of lysosomal function in the context of cellular models of LSD, and to translate the findings into establishing biomarkers of lysosomal dysfunction.

Disclosures: H.S. Wald: None. **S. Jinn:** None. **D. Toolan:** None. **L. Yao:** None. **N. Hatcher:** None. **S. Smith:** None. **J. Marcus:** None.

Poster

PSTR199. Proteinopathies: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.23/T9

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Lisa Dean Foundation

Title: Role of metabolic defects in axonal degeneration of WIPI4 deficient iPSC induced-neurons

Authors: *L. RAMOS-RODRIGUEZ[1,2], X. ORTIZ-GONZALEZ[1,2];
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Abstract: Beta-Propeller Associated Neurodegeneration (BPAN) is a genetic disease uniquely characterized by a progression from infantile encephalopathy into a Parkinson-like movement disorder. While BPAN patients typically present thinning of the corpus callosum, and human neuropathological studies show axonal swellings with protein aggregation, it is currently unknown why axonal integrity is uniquely affected during BPAN progression. BPAN is caused by mutations in the gene encoding the protein WIPI4, involved in autophagic initiation. Macro-autophagy (herein autophagy) is the mechanism by which cells eliminate dysfunctional organelles and proteins. When this pathway is activated, a target is isolated by a double membrane (autophagosome biogenesis) and sent to fuse with lysosomes for degradation. WIPI4 deficient cells have decreased in response to autophagy inducers. Demonstrating that cells with a WIPI4 deficiency are less responsive to autophagy-initiating factors, prompting the question of whether specific organelles have decreased quality control. Indeed, mouse models of BPAN have shown swollen axonal mitochondria, suggesting that WIPI4 deficiency impairs mitochondrial quality control in axons. While fibroblast studies show that WIPI4 mutations lead to decrease ATP production. While poor mitochondrial quality control on its own can trigger caspase 3-related apoptosis, recent findings have shed light on axon-specific degeneration that involves NADH depletion. This process works by localizing the protein SARM1 to mitochondria in the axon where it degrades NADH precursor NAD+. **Given these findings, our hypothesis was that WIPI4 deficiency decreases axonal mitochondrial quality control leads to NADH depletion and neurodegeneration.** To examine axonal mitochondria function in WIPI4 deficient iPSC-derived neurons (iNeu) we used mitochondrial membrane potential-dependent dyes in microfluidic chambers to evaluate compartment-specific (axonal and soma). To assess neuronal mitochondrial oxygen consumption and ATP production we used Seahorse Xfe96. To measure NADH availability and the fraction of NAD(P)H bound to proteins, we used NADH NAD+ quantification assay and fluorescence lifetime imaging microscopy (FLIM). Our results show that mitochondrial membrane potential decreases farther away from soma, while oxygen consumption and ATP production were significantly decreased in WIPI4 mutants compared to control iNeu. While NADH concentrations and the fraction of NADH bound to proteins do not show a clear trend in the preliminary experiments. Suggesting that mitochondrial quality control in axons is limited due to WIPI4 deficiencies.

Disclosures: L. Ramos-Rodriguez: None. **X. Ortiz-Gonzalez:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.01/T10

Topic: C.06. Neuromuscular Diseases

Support: NINDS NS125490

Title: Sex-biased role of myeloid receptor Clec7a in regulating motor phenotypes and disease progression of SODIG93A mouse models

Authors: *X. CHEN[1], H. YAN[2], J. HU[2], Y. YANG[3];
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Abstract: Clec7a (also named Dectin-1), is a mammalian C-type lectin receptor (CLR) expressed on the surface of various myeloid cells. Previous transcriptomic analysis of post-mortem cortex of amyotrophic lateral sclerosis (ALS) patients and spinal cords of SOD1G93A ALS mouse models found selective up-regulation of Clec7a mRNA in disease microglia. However, currently there is no knowledge whether and how Clec7a is involved in ALS pathogenesis. Here we aim to investigate the potential pathogenic role of Clec7a in ALS by generating SOD1G93A^{+}Clec7a$^{-/-}$ mice and examining ALS-related motor phenotypes, pathology, and overall survival of SOD1G93A^{+}Clec7a$^{-/-}$ mice. We first showed that Clec7a protein is selectively up-regulated in (60%) Iba1^{+} sciatic nerve (SN) macrophages of SOD1G93A mice at pre-symptomatic disease stage (P80) and then (80%) Iba1^{+} spinal cord (SC) microglia of SOD1G93A mice at disease progression stage (P110-115). By performing hanging wire and rotarod tests, we observed a significantly reduced latency in both tests in SOD1G93A^{+}Clec7a$^{-/-}$ mice compared to SOD1G93A^{+} mice as early as P70, especially in males. In parallel to the reduced latency, significantly higher number of fully denervated neuromuscular junctions (NMJs) in gastrocnemius muscles was observed in SOD1G93A^{+}Clec7a$^{-/-}$ than SOD1G93A^{+} male mice at P80 following immunostaining of NMJs, while the Clec7a deficiency alone has no effect in NMJ denervation. In addition, we found that the proportion of activated SN macrophages indicated by increased CD68 immunoreactivity was significantly increased as a result of the Clec7a deficiency in SOD1G93A^{+}Clec7a$^{-/-}$ male mice at P80, suggesting that the loss of Clec7a promotes the early activation of SN macrophages, which may potentially contribute to increased NMJ denervation observed in SOD1G93A^{+}Clec7a$^{-/-}$ male mice. By monitoring weekly body weight and righting reflex-based end-stage, our results further revealed a significantly delayed disease progression period, defined by the duration from 10% weight loss to the end-stage, in female (but not male) SOD1G93A^{+}Clec7a$^{-/-}$ mice compared to SOD1G93A^{+} mice. However, the overall survival showed no difference between male and female SOD1G93A^{+}Clec7a$^{-/-}$ and SOD1G93A^{+} mice. In summary, Clec7a deficiency preferentially results in early motor deficits in SOD1G93A male mice but leads to delayed disease progression in female SOD1G93A^{+}Clec7a$^{-/-}$ mice, suggesting a sex-biased and distinct role of Clec7a in regulating motor phenotypes and disease progression in the SOD1G93A ALS mouse model.

Disclosures: X. Chen: None. **H. Yan:** None. **J. Hu:** None. **Y. Yang:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.02/U1

Topic: C.06. Neuromuscular Diseases

Support: Independent Research Fund Denmark (8020-00330A)
Læge Sofus Carl Emil Friis og Hustru Olga Doris Friis Legat

Title: Spinal motoneurones from symptomatic mice with C9orf72 repeat expansions show increased persistent inward currents and disrupted nodes of Ranvier.

Authors: V. REHÁKOVÁ[1], S. DRÆBY[1], M. KADLECOVÁ[1], D. B. JENSEN[2], *C. F. MEEHAN[1];
[2]Dept. of Neurosci., [1]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: An increased excitability of the motor system has consistently been observed in Amyotrophic Lateral Sclerosis (ALS) at the cortical, spinal and peripheral level. Research from our laboratory and others has shown that the core pathological feature of this disease - nuclear depletion and cytoplasmic aggregation of TDP-43 protein - is sufficient to drive such changes. However, the majority of research investigating hyperexcitability in ALS uses mouse models with an atypical form of the disease not expressing TDP-43 pathology. In our current experiments, we have investigated excitability in a relatively new ALS mouse model expressing TDP-43 pathology, based on the most common mutations found in both familial and sporadic ALS: C9orf72 repeat expansions (C9orf72RE). As excitability changes in peripheral axons are more pronounced in C9orf72RE carriers with ALS than C9orf72RE carriers without, we focused our experiments on C9orf72RE mice showing a slowly progressing motor phenotype. These mice all showed a clasping behaviour when suspended by the tail. In vivo intracellular recordings from spinal motoneurones were performed at around 250 and 400 days of age in C9Orf72RE mice and WT littermates. Most basic excitability parameters were unchanged, except motoneurones from C9Orf72RE mice (both ages) showed signs of increased persistent inward currents. Axon initial segments of the motoneurones appeared structurally relatively normal, however, the nodes of Ranvier showed many structural abnormalities. The changes seen in this model are therefore less extreme than those seen in SOD1 models at more advanced disease stages and more consistent with our observations in SOD1 mice at earlier stages.

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Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.03/U2

Topic: C.06. Neuromuscular Diseases

Support: Lazio Innova -Bandi per Gruppi di Ricerca 2020- AMETISTA to S.M. (Prot. GeCoWEB n. A0375-2020- 36668; CUP: F85F21003700009)
MUR Grant of Excellence Department 2023-27 to Department of Science, University Roma Tre

Title: Altered expression of peroxisomal proteins and antioxidant enzymes in amyotrophic lateral sclerosis

Authors: M. TERRICOLA[1,2], C. FIORUCCI[1], R. PRICE[1,2], S. SCARICAMAZZA[2], M. MUZZI[1], M. CERVELLI[1,2], F. BERARDINELLI[1,2], C. VALLE[2], A. FERRI[3], *S. MORENO[1,2]; [1]Sci., Univ. Roma Tre, Rome, Italy; [2]IRCCS Santa Lucia Fndn., Rome, Italy; [3]CNR, Rome, Italy

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, affecting both upper and lower motor neurons, and leading to paralysis. Energy dysmetabolism and oxidative stress, related to mitochondrial impairment, have been recognized as major contributors to ALS pathogenesis. Besides nervous tissue, skeletal muscle is also primarily involved, switching its metabolism towards an oxidative phenotype and preferentially using fatty acids as fuel[1]. Based on the role of peroxisomes in energy metabolism and on their crosstalk with mitochondria, the aim of this study is to address their involvement in ALS. Indeed, these two classes of organelles cooperate in multiple metabolic and signaling networks, such as lipid metabolism (*e.g.*, fatty acids β-oxidation) and reactive oxygen species (ROS) detoxification[2]. Peroxisomes are dynamic organelles whose anabolic and catabolic functions can adjust to a changing environment, under control of peroxisome proliferator activated receptors (PPARs), a subfamily of transcription factors. PPAR action depends upon binding to their ligands and is regulated by coactivators as PGC1α[3]. In this study we used *in vivo* and *in vitro* models of ALS, specifically skeletal muscle and neural tissues from *SOD1*^{G93A} transgenic mice at 120 days of age, and neural cell line NSC34 overexpressing *SOD1*^{G93A} mutation. We performed immunoblotting and qRT-PCR analyses to assess the expression of peroxisomal markers in the gastrocnemius muscle and spinal cord of transgenic mouse and NSC34 cells. The results obtained show significant differences between SOD1^{G93A} and WT control groups of peroxisomal fatty acyl β-oxidation enzymes (thiolase and acyl-CoA oxidase 1), peroxisome membrane protein of 70kDa (PMP70, a fatty acid transporter) and antioxidant enzymes (catalase and superoxide dismutase 2). Such up-regulation is accompanied by PPARα induction, emphasizing the crosstalk between mitochondria and peroxisomes in the pathological condition. These data support an involvement of peroxisomes in ALS, possibly coping with redox imbalance and mitochondrial dysfunction. *[1.] Scaricamazza et al. 2021 Cells 10:525. [2.] Wahli & Michalik 2012 TEM 23:351.[3.] Islinger et al. 2018 Histochem Cell Biol 150:443*

Disclosures: M. Terricola: None. **C. Fiorucci:** None. **R. Price:** None. **S. Scaricamazza:** None. **M. Muzzi:** None. **M. Cervelli:** None. **F. Berardinelli:** None. **C. Valle:** None. **A. Ferri:** None. **S. Moreno:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.04/U3

Topic: C.06. Neuromuscular Diseases

Support: NIH IRP ZIA-HD008966

Title: Investigating alterations preceding neurodegeneration in TDP43 deficient motor neurons

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and ultimately fatal neurodegenerative disease that affects thousands of patients and their families. 97% of ALS patients display a common pathology where the essential nucleic acid binding protein, TDP-43, is mislocalized outside of neuronal nuclei. Due to its role in RNA splicing, mislocalization of TDP43, out of the nucleus results in the aberrant splicing of numerous transcripts, including the inclusion of normally repressed cryptic exons. To understand the early changes associated with nuclear loss of TDP-43, we examined the functional and transcriptomic alterations in a mouse model where TDP-43 is conditionally deleted from motor neurons. These animals develop progressive motor symptoms due to motor neuron loss over the course of 2-3 months of age. However, the exact mechanisms by which loss of TDP-43 results in motor neuron death are not well understood. By compound muscle action potential recordings from the tibialis anterior muscle, we detect a significant reduction in motor neuron function preceding motor neuron loss and muscle denervation. We hypothesize changes in motor neuron gene expression may account for this functional deficit. Therefore, we performed single nucleus RNA sequencing of lumbar motor neurons to examine broad changes in gene expression as well as RNA sequencing of the lumbar spinal cord to assess potential mis-splicing of transcripts in this model. We will assess expression of genes involved in establishing motor neuron identity and function, stress response genes, and misregulated pathways to better understand pathological progression associated with loss of TDP-43. Ultimately these findings could provide potential therapeutic approaches for ALS patients.

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Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.05/U4

Topic: C.06. Neuromuscular Diseases

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ALS Association Grant 1114-471-454
Kavli Neural Systems Institute Pilot Award

Title: Mitochondrial Profiling of ALS-Vulnerable and Resilient Cortical Neurons Using SOD1^{G93A}Mouse Model

Authors: *B. A. COTTO[1], N. HEINTZ[3], E. F. SCHMIDT[2];
[2]Lab. Mol Biol, [1]Rockefeller Univ., New York, NY; [3]Rockefeller Univ, Howard Hughes Med. Inst., New York, NY

Abstract: Neurodegenerative diseases are often defined by a marked anatomical and cellular specificity. Among them, Amyotrophic Lateral Sclerosis (ALS) results from the selective loss of corticospinal motor neurons in the motor cortex and lower motor neurons of the spinal cord eventually leading to paralysis and death. Despite significant progress in identifying the genetic and molecular factors of ALS, little is known about the exact mechanisms that contribute to the selective vulnerability of motor neurons. A major anomaly that remains is many ALS-causing gene mutations are ubiquitously expressed throughout the body, yet only a subset of neurons is vulnerable. Mitochondrial dysfunction has emerged as a common and early phenomenon in both familial and sporadic ALS suggesting an important role for loss of mitochondrial integrity in the etiology of ALS. To date, studies of ALS-associated mitochondria dysfunction have relied on whole brain or regions of brain tissue for analysis. The usefulness of this information is limited given the cellular heterogeneity in the cortex which diminishes the ability to detect and distinguish meaningful changes in the relatively rare, corticospinal neurons. Here, we developed and applied a strategy to facilitate the isolation of mitochondria in a cell type specific manner. The use of our novel retroAAV-mediated approach, term TOM-TAG, for the cell type specific immunopurification of mitochondria by magnetic beads from whole tissue is a powerful tool for the rapid assessment of mitochondria across cell populations in complex tissues like the cortex. Using this strategy in combination with proteomic profiling and real-time metabolic analysis, we have identified stark differences in isolated mitochondria from ALS “resilient” versus “vulnerable” populations at baseline and in a relevant animal model. Taken together, our findings indicate intrinsic differences in mitochondrial physiology of these distinct neuronal populations that may contribute to disease susceptibility.

Disclosures: B.A. Cotto: None. **N. Heintz:** None. **E.F. Schmidt:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.06/U5

Topic: C.06. Neuromuscular Diseases

Support: NIH NINDS R01 NS110953
Startup funds from the College of Pharmacy, the Provost Office and The George and Anne Ryan Institute for Neuroscience

Title: Dysregulation of homeostatic plasticity in ALS: investigating the homeostatic response of mutant SOD1 mouse spinal motoneurons to chronic diazepam treatment

Authors: E. J. REEDICH[1,2], R. D. IMHOFF-MANUEL[1,2], ***M. MANUEL**[1,2];
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Abstract: Dysregulation of homeostatic plasticity has recently emerged as a potential overarching mechanism underlying motoneuron disease pathophysiology. In this context, we are investigating the dysregulation of homeostatic plasticity using mutant SOD1 mice as a model system for neurodegenerative motoneuron diseases. We propose that mutant SOD1 motoneurons exhibit dysregulated homeostatic plasticity, characterized by overcompensation and subsequent oscillations, resulting in increased motoneuron morbidity.
We are rigorously testing this hypothesis by investigating the homeostatic response of mutant SOD1 motoneurons to chronic diazepam treatment, a benzodiazepine that exerts its effects through enhancing the inhibitory neurotransmission mediated by gamma-aminobutyric acid (GABA) receptors.
This double-blind study includes four experimental groups: SOD1-G93A mice treated with diazepam, SOD1-G93A mice treated with vehicle only, wild-type (wt) littermates treated with diazepam, and wt littermates treated with vehicle only. Mice are treated with daily injections of diazepam (15 mg/kg, SC) or the vehicle alone for 10 days, with the last treatment administered on the day of the recording.
Intracellular recordings of motoneurons are performed at age 45-55 days old, encompassing a critical period of disease progression. We are assessing changes in intrinsic excitability and firing properties of motoneurons, including measurements of input resistance, rheobase, and firing rates. Furthermore, synaptic inputs, both excitatory and inhibitory, are investigated to evaluate alterations in the balance of synaptic transmission.
Based on the hypothesis of dysregulation of homeostatic plasticity, we predict that mutant SOD1 motoneurons will exhibit exaggerated homeostatic responses following chronic diazepam treatment compared to wt littermates. Specifically, we expect that the chronic treatment will lead to alterations in the intrinsic electrical properties, resulting in increased firing rates and enhanced excitatory synaptic transmission, in both mutant SOD1 and wt control motoneurons, but that these alterations will be more pronounced in the mutant group.

Disclosures: E.J. Reedich: None. **R.D. Imhoff-Manuel:** None. **M. Manuel:** None.

Poster

PSTR200. ALS: Non-Human Models

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Program #/Poster #: PSTR200.07/U6

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant AR077191
NIH Grant NS091540
University of Wisconsin Foundation
UW Stem Cell & Regenerative Medicine Center

Title: Muscle-specific Bet1L knockdown induces neuromuscular denervation, motor neuron degeneration, and motor dysfunction in ALS model rats

Authors: A. ECKARDT[1,2], S. ROBERTSON[1], C. MARBLE[1], B. FERN[1], H. MORITZ[1], C. REBANCOS[1], *M. SUZUKI[1,2];
[1]Comparative Biosci., [2]Stem Cell & Regenerative Med. Ctr., Univ. of Wisconsin Madison, Madison, WI

Abstract: Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease characterized by specific loss of motor neurons. Recent observations support the 'dying back' hypothesis, which states that the degeneration of these motor neurons initially occurs at peripheral sites such as skeletal muscle and neuromuscular junctions (NMJs). Although accumulated results support this hypothesis, the specific processes involved in skeletal muscle and at the NMJs are still largely unknown. We recently reported that a vesicle trafficking protein Bet1L (Bet1 Golgi Vesicular Membrane Trafficking Protein Like) would be a new molecule possibly linked to NMJ degeneration in ALS (Lynch et al., Experimental Neurology, 2021). Through RNA sequencing, Bet1L was initially identified as one of four genes commonly downregulated in skeletal myocytes differentiated from ALS patient-derived induced pluripotent stem cells. Focal expression of Bet1L protein was detected in the basal lamina of the NMJ with decreased expression over time in ALS model rats (SOD1-G93A transgenic). Importantly, the expression levels began to decrease early in the disease process. Based on these findings, we next hypothesized that Bet1L knockdown in skeletal muscle could influence NMJ integrity, motor neuron function and survival. To test this hypothesis, small interference RNA (siRNA) targeting BET1L was injected on a weekly basis into the hindlimb muscle of pre-symptomatic ALS rats. Sham, vehicle and scramble siRNA-injected rats were prepared as controls. First, immunohistochemistry and blinded image quantification revealed that 1 week of Bet1L siRNA intramuscular injections significantly increased the number of denervated NMJs in the siRNA-injected muscles of both wildtype and ALS model rats when compared to four control groups ($p<0.05$, $n=4$). After 3 weeks of siRNA injections, BET1L knockdown significantly decreased motor neuron size in the lumbar spinal cord where siRNA-injected hindlimb muscles had been innervated ($p<0.05$, $n=4$). Lastly, the Basso Beattie and Bresnahan (BBB) locomotor rating scale indicated that there was a trend of impaired motor function in the hindlimbs of Bet1L siRNA-injected rats ($n=3$). These results not only demonstrate that BET1L knockdown induces denervation of NMJs, but also that this knockdown induces early signs of motor neuron

degeneration and motor dysfunction similar to that observed in ALS. Our results provide new evidence to support potential roles of Bet1L as a key molecule in ALS pathogenesis.

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Poster

PSTR200. ALS: Non-Human Models

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Program #/Poster #: PSTR200.08/U7

Topic: C.06. Neuromuscular Diseases

Support: NIH T32 Grant 5T32AG071444-02
NINDS NIH R01 NS089585

Title: Ribosomal profiling of motor neurons degenerating in ALS suggests a neuroprotective role for FGF21

Authors: *W. STANSBERRY[1], E. NEWELL[2], J. SHADRACH[3], B. PIERCHALA[2]; [2]Indiana Univ. Sch. of Med., [1]Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Univ. of Mchigan, Univ. of Mchigan, Ann Arbor, MI

Abstract: The neuromuscular junction (NMJ) is a chemical synapse that is the site of skeletal muscle innervation by spinal motor neurons. This connection between the nervous system and muscle allows for coordinated motor function, and the maintenance of the NMJ is critical for maintaining musculoskeletal homeostasis. Under normal physiological conditions, spinal motor neurons have significant regenerative potential and can regrow axons in response to peripheral nerve injury. In diseases such as amyotrophic lateral sclerosis (ALS), the NMJ is dismantled and motor neurons selectively degenerate resulting in progressive muscle wasting and eventual fatal paralysis. We adapted the RiboTag methodology developed by Sanz *et al*. to perform ribosomal profiling of motor neurons in mice to assess how nerve injury and ALS affect motor neurons *in vivo*. We purified motor neuron-specific transcripts after the sciatic nerve crush model of acute injury, and in the *Sod1*^{G93A} ALS model. We identified 267 transcripts that were upregulated following sciatic nerve crush, and of those transcripts, 38% were also upregulated in 4-month-old *Sod1*^{G93A} ALS mice, demonstrating some translational overlap between regenerative and degenerative processes. However, the majority of upregulated genes in injured (58%) and ALS (78%) conditions were specific for either injury or neurodegeneration, respectively. One of the most highly upregulated transcripts was *Fgf21,* which was only induced in *Sod1*^{G93A} mice. *Fgf21* is a stress-inducible hormone that is critically involved in glucose turnover, and its expression may cause metabolic disturbances in ALS. Immunolabeling experiments in *Sod1*^{G93A} and *Tdp43*^{A315T} mice revealed that FGF21 protein is increased both in motor neuron cell bodies and in the periphery in motor axons and muscle. Transgenic mice in which *Fgf21* was selectively deleted in motor neurons in the *Sod1*^{G93A} model showed reduced motor neuron survival and

increased NMJ dennervation. Survival trials with *Sod1*^{G93A} mice showed a dramatic reduction in lifespan when *Fgf21* was conditionally deleted in motor neurons compared to controls. These data suggest that FGF21 functions in a neuroprotective capacity in ALS pathology. We are evaluating the mechanisms in whch FGF21 promotes motor neuron survival with the ultimate goal of identifying new therapeutic strategies for ALS.

Disclosures: W. Stansberry: None. **E. Newell:** None. **J. Shadrach:** None. **B. Pierchala:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.09/U8

Topic: C.06. Neuromuscular Diseases

Support: ALS Ride for Life
NIH R01AG079898

Title: Tdp-43 pathology causes differential expression of retrotransposons in a tdp-43-q331k mouse model

Authors: *S. KORADA[1], O. TAM[3], D. WILLBOLD[4], M. HAMMELL[3], J. DUBNAU[5,2], R. SHER[1];
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Abstract: Pathological aggregates of TDP43 are seen in patients with ALS, FTD and AD. TDP43 is a nucleic acid binding protein with important functions such as translational regulation, stress granule formation and retrotransposon repression. Retrotransposable elements (RTE) are mobile elements capable of inserting copies into different genomic locations. Studies in flies have established the causal role of RTEs in mediating both the intracellular toxic effects of TDP43, and the intercellular spread of that toxicity from glia to neurons. The role of TDP43 in RTE regulation has also been replicated in postmortem human tissue. Here, we establish the first rodent model to examine the effects of TDP43 pathology on RTEs. We look at TDP43 proteinopathy in a mouse model where the human TDP43 transgene, with or without the Q331K familial ALS mutation, is overexpressed 1.5 times. We used hindlimb clasping and rotarod to quantify motor deficits in 1.5, 3, 6, 10 and 15 mo mice. The TDP43-Q331K Tg animal shows hindlimb clasping and lower latency to fall on the rotarod starting at 3 months, while the TDP43-WT Tg animal shows a delayed onset of motor deficits starting at 15 months. To investigate the RTE transcript levels, we sequenced total RNA from the motor cortex (MC) of TDP43-Q331K Tg, TDP43-WT Tg and nTg littermates. We see a significant upregulation of RTE at 3 months in

the TDP43-Q331K Tg animals and at 15 months in the TDP43-WT Tg animals. This upregulation of RTEs coincides with the onset of motor defects in each of these transgenic lines. To explore the number, location and cell types in which retrotransposition events occur, we used the L1-EGFP reporter mouse that expresses EGFP after a retrotransposition event occurs. We crossed the two transgenic lines with L1- EGFP animals and imaged GFP positive cells in multiple brain regions at different ages. The TDP43-Q331K Tg animals show significantly higher GFP positive glia and neurons, which occur in large clusters in the striatum(Str) and nucleus accumbens(NA) at 3 months and in the MC at 6 months. The TDP43-WT Tg animals show similar clusters in the NA and Str at 10 months. Thus, both the transgenes drive retrotransposition of L1, that first appear in NA and Str, and later appear in MC. Interestingly, both the RTE transcript levels in the MC and the clusters of GFP positive cells within each brain region appear transiently, becoming undetectable at later time points, consistent with the hypothesis that the cells that contain these de novo transposition events are not viable. In conclusion, we show evidence of RTE expression and retrotransposition events in a cell type, brain region specific, and age- dependent manner in TDP43 pathology mice.

Disclosures: S. Korada: None. **O. Tam:** None. **D. Willbold:** None. **M. Hammell:** None. **J. Dubnau:** None. **R. Sher:** None.

Poster

PSTR200. ALS: Non-Human Models

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Program #/Poster #: PSTR200.10/U9

Topic: C.06. Neuromuscular Diseases

Support: Lundbeckfoundation - Treating ALS disease by rescuing interneuron-motor neuron connections (IA)
DSfN Scholarstipend (AS)
University of St Andrews
University of Copenhagen

Title: Rescue of ALS phenotype by Esyt1 systemic overexpression in the SOD1^{G93A} mouse model

Authors: *A. STUCKERT[1,2], S. MORA[2], K. PIETERSZ[4], R. SELVAN[2,3], J. VERHAAGEN[4], I. ALLODI[1,2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive paralysis and loss of motor neurons (MN). Our previous work showed loss of synapses from spinal V1 inhibitory interneurons (IN) onto MNs prior to MN

degeneration. These interneurons control locomotor speed and express Engrailed-1 (En1). Loss of inhibitory synapses may contribute to imbalanced MN excitability and death. By overexpressing the presynaptic protein Extended synaptotagmin 1 (Esyt1), which promotes synaptic growth and stabilization, we aim to stabilize the V1 IN-MN connectivity. Previous results from our laboratory demonstrated increased MN survival and amelioration of locomotor phenotypes upon V1-restricted Esyt1 overexpression in the lumbar spinal cord via intraspinal injections. It remains unknown if similar improvements can be obtained using non-invasive, systemic administration of Esyt1. The present study investigates Esyt1 systemic overexpression in V1 INs using an AAV-PHP.eB viral vector, which passes the blood-brain-barrier upon intravenous delivery and transduces neurons. $En1^{cre}$ restricted targeting was achieved utilizing a *cre*-dependent strategy. First, an AAV-PHP.eB-DIO-mCherry virus was used to assess spread, efficiency and V1-specific transduction. Upon systemic administration at postnatal day 30 (P30), immunohistochemistry showed preferential targeting of En1+ neurons within the spinal cord, with ~2.3 times more positive neurons when compared to the midbrain, and ~8.5 times more when compared to the somatosensory cortex. Validation of En1 specificity was performed by RNAscope. Secondly, double transgenic $SOD1^{G93A}$;$En1^{cre}$ mice were used for *cre*-dependent Esyt1 overexpression to assess potential side effects due to off-target Esyt1 expression. Exploration, memory, anxiety, and social behavior were assessed 3 weeks post-injection. Motor functions were evaluated weekly by gait and kinematic analysis. $SOD1^{G93A}$ mice injected with a control virus overexpressing GFP and wild type mice injected with saline were used as controls. Results showed no significant differences in the cognitive paradigms for the $SOD1^{G93A}$;$En1^{cre}$ mice overexpressing Esyt1, compared to $SOD1^{G93A}$ mice. Moreover, $SOD1^{G93A}$;$En1^{cre}$ mice exhibited improvement in locomotor parameters including speed, step frequency, and acceleration when compared to $SOD1^{G93A}$ mice. Thus, the study indicates that systemic administration of Esyt1 is safe, efficiently targets V1 INs, and ameliorates the locomotor phenotype in ALS. Altogether, our approach is noninvasive and holds translational potential for future investigations.

Disclosures: **A. Stuckert:** None. **S. Mora:** None. **K. Pietersz:** None. **R. Selvan:** None. **J. Verhaagen:** None. **I. Allodi:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR200.11/U10

Topic: C.06. Neuromuscular Diseases

Title: Phenotypic evaluation of SOD1-G93A transgenic mice as a model of amyotrophic lateral sclerosis and the potential of CMAP (compound muscle action potential) as an early biomarker

Authors: *K. PARK, J. LEE, T. KIM, J. KIM, T. KIM, Y. YOON, H. PARK, S. NA, L. PARK; Naason Sci., Cheongju-si, Korea, Republic of

Abstract: SOD1-G93A transgenic mice, expressing human SOD1 with the G93A mutation under the control of the cistronic human SOD1 promoter, have been widely utilized as a valuable preclinical model to investigate neuromuscular disorders, specifically familial amyotrophic lateral sclerosis (ALS). ALS, also known as Lou Gehrig's disease, is a devastating neurodegenerative condition primarily associated with mutations in the SOD1 gene. In this study, we characterized the phenotypic similarities between SOD1-G93A mice and human ALS, while exploring the potential therapeutic effects of two compounds. The SOD1-G93A mice displayed a progressive phenotype closely resembling ALS in humans, with the onset of limb paralysis observed within a few weeks after birth. Additionally, these mice exhibited age-dependent body weight loss, and disease scores (DS) increased significantly after 15 weeks of age. Notably, alterations in rotarod performance (RR) were evident from 14 weeks of age in the transgenic (TG) mice. To assess the functional status of the motor unit pool, we employed electrophysiological techniques, specifically compound muscle action potential (CMAP) measurements. Remarkably, CMAP analysis revealed a significant difference between wild-type (WT) and TG mice as early as 10 weeks of age, suggesting CMAP's potential as an early-stage biomarker for ALS. Furthermore, we evaluated the therapeutic efficacy of two compounds, Riluzole and Donepezil. Riluzole is a well-known ALS therapeutic, whereas Donepezil is utilized in the treatment of neurodegenerative diseases. However, neither compound exhibited a curative effect on CMAP, RR, or DS in our experimental model. Collectively, our findings highlight CMAP as a potential early-stage biomarker for ALS, surpassing other functional assessments. Moreover, we demonstrate that Riluzole and Donepezil did not exhibit consistent efficacious effects in behavioral tests. These observations underscore the necessity for alternative therapeutic strategies in the treatment of ALS. The SOD1-G93A mouse model serves as a valuable tool for studying the influence of novel drugs on neuromuscular disorders, including ALS.

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Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.12/V1

Topic: C.06. Neuromuscular Diseases

Support: ANR grant

Title: Neuronal and microglialP2X4 receptors contribute differently to the progression of ALS

Authors: *S. CARRACEDO VICENTE[1], E. BERTIN[1], A. FAYOUX[2], C. RIFFAULT[1], S. BERTRAND[2], E. BOUE-GRABOT[1];
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Neurosciences Cognitives et Intégratives d'Aquitaine (INCIA), Univ. of Bordeaux, Bordeaux, France

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a selective loss of motor neurons (MNs), leading to muscular weakness and death within 3-5 years after diagnosis. The aggregation of misfolded proteins such as SOD1 or TDP-43 is the pathological hallmark of ALS and has been associated with neuroinflammation and cellular degeneration. Growing evidence point ALS to be the result of a complex interplay between the nervous and the immune system. ATP released by neurons and glial cells modulates the neuroglial communication via activation of P2X receptors. Among these, P2X4 receptor, which is a non-selective cationic channel has been recently involved in ALS pathogenesis using SOD1G93A ALS mouse model (SOD1). In our previous research, we have demonstrated that misfolded SOD1 proteins interfere with the endocytosis machinery of P2X4 receptors resulting in a notable increase in the density of P2X4 receptors on the cell surface. To evaluate the impact of P2X4 in ALS pathogenesis, we generated double transgenic SOD1 mice expressing either P2X4 internalization-defective knocking gene (SOD1:P2X4KI) or lacking the P2X4 gene (SOD1:P2X4KO). Surprisingly, both genotypes exhibited improved motor performance and survival of SOD1 mice pointing out a complex cell-specific function of P2X4. In order to address the respective role of P2X4 in neurons and glial cells in ALS, we have developed and characterized novel SOD1 mice, expressing either P2X4KI or P2X4KO, selectively in macrophage/microglia or neurons. Using behavioral, immunohistological and cellular approaches, we observed that both the neuronal absence of P2X4 (Synapsin Cre:P2X4KO:SOD1 mice) or the surface increase of P2X4 within microglia cells (CD11BCre:P2X4KI:SOD1 mice) have a beneficial effect on motor performance and survival of SOD1 mice. This effect seems to be associated in both mouse lines with a higher survival of MNs and a decrease on microglia reactivity during later diseases stages. Altogether, our findings suggest that P2X4 receptors in both neurons and microglia contribute to the complex neuroimmune interactions that occur during ALS. Further studies are warranted to precisely evaluate the role of P2X4 receptors in the interplay between MNs death and spinal microglia reactivity throughout the ALS progression. Investigating the cellular role of P2X4 receptors in this context can provide valuable insights into the underlying mechanisms of the disease and potential therapeutic targets.

Disclosures: **S. Carracedo Vicente:** None. **E. Bertin:** None. **A. Fayoux:** None. **C. Riffault:** None. **S. Bertrand:** None. **E. Boue-Grabot:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.13/V2

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: ALS Canada

Title: Cholinergic antagonists improve symptomatology and delays end stage in amyotrophic lateral sclerosis mouse model.

Authors: *R. POPOLI, T. L. WELLS, T. AKAY;
Med. Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: SfN Abstract
In amyotrophic lateral sclerosis, symptom onset does not occur until a significant number of motor neurons have died, suggesting the existence of a compensatory mechanism. Our previous research has shown that genetically silencing C-boutons in ALS mutant mSOD1G93A mice leads to earlier symptom onset and worsening of behavioural performance, indicating that these synapses are involved in delayed symptom onset relative to motor neuron death. We have shown that C-boutons are upregulated during intensive exercise routines, such as swimming. The literature suggests that this type of exercise is detrimental to disease progression in mSOD1G93A mice. However, when the C-boutons are genetically silenced and mice are exercised three times a week in the form of swimming, an activity where these synapses would typically be upregulated, behavioural performance is dramatically improved. Genetic manipulations, however, are not used in clinical settings, presenting a challenge to improving neurological care in patients with ALS. In our most recent study, we investigated a clinically relevant approach through the use of two cholinergic antagonists in two conditions: (i) frequent exercise under the influence of these pharmacological agents and (ii) resting conditions. These groups were compared among themselves, along with saline controls. Our preliminary results show that both pharmacological agents improve behavioural performance and increase lifespan by approximately 8%, suggesting it may improve the quality of life in patients with ALS. Based on these results, however, it is unclear whether these positive effects are C-bouton dependent or not since these drugs act on several cholinergic systems in the body. In order to reveal whether the mechanism behind these positive results are C-bouton dependent or not, we are currently working with mice that have genetically silenced C-boutons and the use of pharmacological antagonists.

Disclosures: R. Popoli: None. **T.L. Wells:** None. **T. Akay:** None.

Poster

PSTR200. ALS: Non-Human Models

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Program #/Poster #: PSTR200.14/V3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Ulla-Carin Lindquist´s Foundation for ALS Research
The Swedish Research Council
Västerbottens läns landsting

Title: Ultrastructural imaging of distinct SOD1 strains in ALS transgenic mouse models

Authors: *I. SIGFRIDSSON, T. BRÄNNSTRÖM;
Umeå Univ., Umeå, Sweden

Abstract: Abnormally assembled proteins play a central role in neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and amyotrophic lateral sclerosis (ALS). In these diseases, evolving evidence show the capability of misfolded proteins to assemble into different strains and acquire different biological activities. Moreover, several studies suggest that the large clinicopathological heterogeneity in these diseases may be explained by the existence of the different conformational strains. The presence of aggregates formed by misfolded superoxide dismutase-1 (SOD1) proteins are hallmarks of ALS caused by mutations in the *SOD1* gene. In our laboratory, two structurally distinct strains of human (h) SOD1 aggregates (denoted A and B) have previously been identified in transgenic (Tg) hSOD1 mice. It was shown that strain A and B affect the progression of the disease differently, suggesting a difference in pathogenesis provoked by the distinct strains. The specific pathological mechanisms of the structurally different hSOD1 strains have previously not been determined. In this study, we investigated the ultrastructural morphology of hSOD1 strain A and B in order to explore their pathological function in the cell. Antibodies targeting strain specific sequences were used to label hSOD1 aggregates in spinal cords of $hSOD1^{G85R}$ and $hSOD1^{D90A}$ Tg mice. The aggregates were visualized using correlative light-electron microscopy. Our results show a successfully developed protocol for ultrastructural visualization of strain A and B within their cellular environment. Both strains show randomly arranged fibrils in the cytoplasm although indicating a difference in deposit morphology. These findings form the basis to further investigate how conformational changes within SOD1 fibrils can alter the pathological function in the cell and be linked to specific variants of ALS.

Disclosures: I. Sigfridsson: None. **T. Brännström:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.15/V4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: ALS Association 22-PDF-601

Title: Dysfunction and Degeneration of Corticospinal Tract Neurons in ALS Mouse Models

Authors: *J. KALAMBOGIAS[1,2], N. A. SHNEIDER[1], Y. YOSHIDA[2], F. IMAI[2], A. AMER[2], S. LANG[2], S. LAURINO[2], M. BOIANO[2];
[1]Columbia Univ., New York, NY; [2]Burke Neurolog. Inst. | Weill Cornell Med., White Plains, NY

Abstract: In human patients with amyotrophic lateral sclerosis (ALS), deficits in motor control occur as a consequence of the degeneration of corticospinal tract (CST) neurons. In ALS mice

however, the dysfunction of the CST is surprisingly limited and is likely due to the different connections patterns between the CST and spinal motor neurons (SMNs) in humans versus mouse. In human, CST circuits includes corticomotoneuronal (CM) connections to SMNs, as well as to spinal interneurons (INs). In contrast, mice have few CM, only forming connections with SMNs, via INs, and therefore incompletely model the human CST in disease. We will therefore use a PlexinA1 conditional knockout mouse (KO) which display abundant human-like CM connections, together with $SOD1^{G93A}$ transgenic mice, to analyze CST circuits. Our central hypothesis is that progressive defects in CST motor circuitry will be exacerbated by the establishment of CM connections in $SOD1^{G93A}$ mice. Here we test the role of CM connections on CST neuropathology in $SOD1^{G93A}$ mouse models with and without CM circuits at symptomatic (P60) and endstage (P130) timepoints. We compared four groups of animals: the "humanized" mouse, PlexA1 KO; the "humanized" ALS mouse, $SOD1^{G93A}$; PlexA1 KO (SOD1;A1KO); the ALS mouse without CM connections, $SOD1^{G93A}$ (SOD1); and control animal. Using sensitive viral tracing techniques, we assayed for CST white matter axon projections of the dorsal column (DC), as well as gray matter terminations in the cervical enlargement (C7-C8) spinal cord. Analysis in SOD1;A1KO mice revealed a significant reduction in CST axon projections in the DC, as well as gray matter terminations, compared to SOD1, control and PlexA1 KO animals at P130, suggesting exacerbated degeneration of the CST. Furthermore, SOD1;A1KO mice displayed fewer CM connections (confirmed with vesicular glutamate transporter 1, VGluT1) onto ChAT+ SMNs compared to PlexA1 KO mice suggesting disruptions of CM circuitry at P130. Additionally, to assay for CM dysfunction in SOD1;A1KO mice, we used the electrophysiological technique, stimulus triggered averaging (StTA), of EMGs recorded from forelimb muscle in response to motor cortex stimulation. StTA has revealed the CM connection in primate and in the PlexA1 KO mouse. SOD1;A1KO mice showed long latencies at P60 and P130, compared to PlexA1 KO mice suggesting progressive dysfunction of CM circuits. Taken together, these data show defects along the CST circuitry in SOD1;A1KO mice. Future experiments will be critical in further verifying CST pathology and whether the presence of the CM connection results in more exacerbated deficits along the CST-MN axis in the "humanized" $SOD1^{G93A}$ mouse.

Disclosures: **J. Kalambogias:** None. **N.A. Shneider:** None. **Y. Yoshida:** None. **F. Imai:** None. **A. Amer:** None. **S. Lang:** None. **S. Laurino:** None. **M. Boiano:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.16/V5

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Annexon Biosciences

Title: C1q inhibition reduces neuronal damage, preserves neuromuscular junctions, and improves compound muscle action potential in the SOD1G93A mouse model

Authors: *C. HUYNH, A. TASSONI, V. MATHUR, J. VEREEN, L. KUHN, D. R. ARTIS, T. YEDNOCK, Y. ANDREWS-ZWILLING;
Annexon Biosci., Brisbane, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a sporadic or genetic disease associated with peripheral loss of synaptic connectivity at the neuromuscular junction (NMJ) and central loss of motor neurons. The SOD1G93A mouse model has been widely used to study a familial form of ALS. C1q, the initiating molecule of the classical complement cascade, marks synapses in the central nervous system for glial elimination during normal development, but it also triggers aberrant synapse loss in neurodegenerative disorders. We hypothesized that excessive synaptic pruning initiated by C1q contributes to motor deficits in ALS and that pharmacologically inhibiting this process would be beneficial. First, we confirmed deposition of C1q deposition at the NMJ in SOD1G93A mice. This was accompanied by correlation between NMJ and disease progression. Treatment of the mice for 9 weeks (age 7 to 16 weeks) with a C1q-blocking antibody reduced C1q levels in plasma, spinal cord, and muscle tissue, along with inhibition of downstream classical complement activation. Treated mice showed significant preservation of NMJ density, as measured by bungarotoxin labelling of the gastrocnemius muscle, and improvement in the amplitude of compound muscle action potential, potentially demonstrating that C1q inhibition leads to increased synaptic connectivity at the NMJ. Furthermore, anti-C1q treatment reduced NfL levels in the cerebrospinal fluid and plasma of SOD1G93A mice compared to NfL levels seen in untreated SOD1G93A mice, marking reduction in neuronal damage in this model. These findings indicate that inhibition of the classical complement pathway results in reduced neuronal damage, as measured by NMJ preservation; improved muscle nerve conduction; and reduced NfL levels following anti-C1q therapy treatment in the SOD1G93A mouse model of ALS. A Phase 2 study of ANX005, an anti-C1q therapy, in ALS patients is ongoing (ClinicalTrials.gov: NCT04569435).

Disclosures: C. Huynh: A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **A. Tassoni:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **V. Mathur:** A. Employment/Salary (full or part-time):; Acelot, formerly, Annexon Biosciences. **J. Vereen:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **L. Kuhn:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **D.R. Artis:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **T. Yednock:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **Y. Andrews-Zwilling:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.17/V6

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: JSPS KAKENHI 21K07270

Title: Analysis of molecular mechanisms underlying neuron-to-neuron transmission of FUS

Authors: *T. HASHIMOTO[1], N. WATANABE[2], Y. KISHINO[2], K. MATSUKAWA[2], D. DORMANN[3], T. IWATSUBO[2];
[1]Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [2]The Univ. of Tokyo, Tokyo, Japan; [3]Johannes Gutenberg-Universität, Inst. of Mol. Physiol., Mainz, Germany

Abstract: Neuron-to-neuron transmission of disease-related proteins is a key process in the progression of neurodegenerative disorders. Cytoplasmic accumulation of fused-in-sarcoma (FUS) is a pathological feature of a subset of patients with amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD); however, the neuron-to-neuron transmission of FUS has not been fully elucidated. To investigate whether FUS can be transferred intercellularly, we developed a novel cell-based transfer assay using NanoBiT bimolecular luminescence complementation. We generated HEK293 cells stably expressing LgBiT- or SmBiT-tagged FUS (Lg-FUS or Sm-FUS), to detect the luminescence induced by reconstitution of NanoLuc luciferase through an interaction of Lg-FUS and Sm-FUS. Co-culture of the Lg-FUS and Sm-FUS stable cells showed an increase in the luminescence, suggesting that FUS is transferred intercellularly. We found that substitution of Arg495, Arg498, and Arg503 with Ala (FUS RAmt) significantly reduced the intercellular transfer of FUS, suggesting that these Arg residues are involved in the intercellular transmission of FUS. We also found that treatment of the co-culture with a methyltransferase inhibitor, adenosine-2',3'-dialdehyde (AdOX), significantly increased luminescence, suggesting that hypomethylation of FUS enhances intercellular transmission of FUS. Next, to visualize FUS transmission between neurons, we constructed an adeno-associated virus-based expression vector encoding dTomato-P2A-FUS (AAV-FUS) driven by the human synapsin I promoter, which expresses both dTomato and FUS in neurons. AAV-FUS-infected "donor" neurons express both dTomato and FUS as individual proteins by self-cleavage of P2A. In contrast, FUS-receiving "recipient" neurons have FUS but no dTomato. After 4 weeks of infection with AAV-FUS, nuclei and cytoplasm of neurons in the neocortex were positive for FUS and dTomato; in particular, the cytoplasm of neurons close to the FUS/dTomato-double positive neurons was positive for FUS but not for dTomato, supporting neuron-to-neuron transmission of FUS. Furthermore, after 4 weeks of infection with AAV-FUS RAmt, FUS/dTomato-double positive neurons were also observed, but the number of FUS-positive dTomato-negative neurons was significantly lower compared to AAV-FUS. Taken together, we suggested that FUS is transmitted interneuronally and that hypomethylation of Arg495, Arg498, and Arg503 residues of FUS are involved in the transmission.

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Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR200.18/V7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: FightMND 2022 Drug Development Grant

Title: Evaluation of the phase-3 clinical candidate RRx-001 as a novel therapeutic agent for Amyotrophic Lateral Sclerosis

Authors: ***M. KUZNETSOVA**[1], N. BIRCH[2], M. YULE[2], R. HENDERSON[3], P. MCCOMBE[3], T. R. REID[4], S. CAROEN[4], B. ORONSKY[5], R. GORDON[2];
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Abstract: RRx-001 is a brain-penetrant direct inhibitor of the NLRP3 inflammasome currently in Phase 3 clinical trials as a radio and chemo-protective agent and anti-cancer therapeutic. We have previously demonstrated that NLRP3 inflammasome activation is a central driver of chronic immune activation and persistent neuroinflammation in the Central Nervous System (CNS). Pharmacological inhibition of NLRP3 in the CNS, is an effective disease-modifying therapeutic strategy for neuroprotection using the systemic NLRP3 inhibitor MCC950 (Gordon et al, Science Translational Medicine 2018). Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressing fatal neurodegenerative disorder which affects the brain and spinal cord. Currently, approved treatments only manage symptoms, or do not adequately slow or halt disease progression. Therefore, there is an urgent need to develop new and more effective treatments. In this study, we evaluated if RRx-001 could be be an effective disease-modifying therapy for ALS using a combination of in vitro studies and preclinical models of neuropathology. We confirmed that nanomolar doses of RRx001 were effective at reducing NLRP3-activation and inflammatory neuropathology triggered by neurotoxic C9ORF72 dipeptide aggregates (poly-GA) in immune cells. Our studies using neuronal cells also demonstrate that RRx-001 improves mitochondrial function and NRF2 activation which we confirmed in vivo. Once weekly dosing with RRx-001 at 2 to 5 mg/kg also improved exercise intolerance and markers of muscle injury such as malonaldehyde. Our ongoing studies are also evaluating the therapeutic potential of RRx-001 to prevent and rescue neuropathology in two experimental models of ALS, the SOD1 G93A mouse and the TDP-43 (Wt-TAR6/6) and in patient-derived iPSCs. Our early suggest that RRx-001, could have therapeutic potential for ALS by targeting multiple pathological mechanisms linked to disease progression, including NLRP3 activation, motor neuron death, mitochondrial dysfunction, and muscle degeneration. Given that RRx-001 has been tested in over 200 patients

to date, with no dose limiting toxicity, our early results highlight the prospect of RRx-001 as a new disease-modifying therapeutic for ALS with the potential for rapid clinical translation.

Disclosures: **M. Kuznetsova:** None. **N. Birch:** None. **M. Yule:** None. **R. Henderson:** None. **P. McCombe:** None. **T.R. Reid:** None. **S. Caroen:** None. **B. Oronsky:** None. **R. Gordon:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.19/V8

Topic: C.06. Neuromuscular Diseases

Support: Muscular Dystrophy Association Grant #865871
R35 Grant NS122140

Title: Modifying the RNA Exosome Complex as a Potential Treatment in Amyotrophic Lateral Sclerosis

Authors: *K. C. K. EHSANI, F. ARNOLD, S. MICHELS, M. COLWIN, W. G. SITU, A. R. LA SPADA;
Univ. of California Irvine, Irvine, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of motor neurons in the brain and spinal cord for which there is currently no cure. Previous research has indicated that dysregulation of RNA metabolism appears to be a key cellular mechanism in ALS. Several of the RNA-binding proteins implicated in ALS are associated with a central regulator of RNA metabolism, the RNA exosome complex. The intent of this study is to investigate the RNA exosome complex as a potential therapy target for C9orf72 Amyotrophic Lateral Sclerosis (ALS), the most common genetic form of the disease. A hexanucleotide repeat expansion in the C9orf72 gene accounts for the highest proportion of all familial ALS cases. The exosome complex has previously been shown to degrade the C9orf72 associated GGGGCC (G4C2) repeat RNA, specifically the catalytically active subunit EXOSC10. By modulating the protein expression of RNA exosome subunits, we were able to confirm that knockdown of EXOSC10 and other core exosome subunits increases the levels of the G4C2 repeat RNA, while overexpression decreases the G4C2 repeat RNA. Following these results, we were also able to conclude that of the RNA exosome complex's three primary cofactor complexes (TRAMP, NEXT, and PAXT) that the PAXT complex is the one that is most likely recruiting G4C2 repeat RNA to the exosome complex. Based on these data in cell models of ALS, we generated new mouse models conditionally overexpressing EXOSC9 or EXOSC10. We have confirmed that the health of mice overexpressing EXOSC9 or EXOSC10 is generally unaffected and that we achieve approximately 2-fold increased protein in all tissues. We are currently conducting a large experiment to determine if EXOSC9 or EXOSC10 overexpression improves the phenotype of a well characterized mouse model of C9orf72 ALS, in

which AAVs encoding 149-repeat G4C2 repeat RNA is delivered via intracerebroventricular injection at postnatal day 0.

Disclosures: K.C.K. Ehsani: None. **F. Arnold:** None. **S. Michels:** None. **M. Colwin:** None. **W.G. Situ:** None. **A.R. La Spada:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.20/V9

Topic: C.06. Neuromuscular Diseases

Support: ERC starter grant 805426 - FutureTrophicFactors
ERC POC 101069256 - REGENERA

Title: Novel CDNF and MANF variants delay disease onset and preserve motor function in a SOD1-G93A mouse model of ALS

Authors: *L. BECKETT, K. VALKONEN, T. KOPPINEN, M. H. VOUTILAINEN;
Univ. of Helsinki, Helsinki, Finland

Abstract: Amyotrophic Lateral sclerosis (ALS) is a progressive neurodegenerative disease characterised by the selective death of upper and lower motor neurons (MNs) in the brain and spinal cord respectively, leading to patient death within 3-5 years. Superoxide dismutase 1 (SOD1) gene mutation is causative in 12% of familial and 2% of sporadic cases of ALS and is the most studied animal model of ALS. One key component of ALS and the SOD1 gene mutation is the pathological induction of endoplasmic reticulum (ER) stress. Cerebral dopamine neurotrophic factor (CDNF) and Mesencephalic astrocyte-derived neurotrophic factor (MANF) attenuate the unfolded protein response to promote the survival of ER stressed neurons. One key drawback of MANF and CDNF is that they cannot pass the blood-brain barrier (BBB). Here we have developed a MANF variant (vMANF) and CDNF variant (vCDNF) that are capable of passing the BBB and are protective both *in vivo* and *in vitro*.
SOD1-G93A transgenic mice were grouped in sex and motor behaviour matched groups. Mice were injected S.C with vMANF (4mg/kg), vCDNF (4mg/kg) or PBS (5ml/kg) weekly. Mice were sacrificed at week 16 or end-point, for further histological analysis. To assess the *in vitro* effect of vMANF and vCDNF, NSC-34 cell line was treated with was used in combination with vMANF and vCDNF in a cell survival assay.
vMANF and vCDNF treated transgenic mice showed a delay in symptom onset (vMANF: 17.5 days, vCDNF: 7 days), protection of clinical score at weeks 14 to 16 (vMANF) and weeks 14 and 15 (vCDNF). vMANF group had a median survival increase of 11 days. Both vCDNF and vMANF groups showed significant improvement in motor ability assessed by rotarod. Histological analysis showed both vCDNF and vMANF treated mice had more lumbar MNs remaining at week 16, and a reduced microglial activation, while vMANF also showed a

reduction in activated astrocytes. *In vitro,* vMANF and vCDNF provides dose dependant protection against toxin-induced apoptosis from 5ng/ml to 5µg/ml.
Here we show that both vMANF and vCDNF are neuroprotective compounds, capable of passing the BBB. Further to this, we show that weekly subcutaneous injection of vMANF or vCDNF delays symptom onset, preserves motor ability and for vMANF, prolongs life in a mouse model of ALS. We show that vMANF and vCDNF protects LMN from SOD1-linked degeneration, and has no effect on naïve cells. vMANF and vCDNF show potential as a promising treatment for ALS, a disease critically lacking in therapeutic options.

Disclosures: L. Beckett: None. **K. Valkonen:** None. **T. Koppinen:** None. **M.H. Voutilainen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.21/V10

Topic: C.06. Neuromuscular Diseases

Support: Funded in part by a gift from the Ralph L. Smith Foundation (to DAL)

Title: Preclinical effects of red dragon fruit betacyanins in the G93A hSOD1 mouse model of ALS

Authors: *C. PENA, L. KOZA, M. SINAR, E. BUECHLER, A. BAYBAYON-GRANDGEORGE, A. SMITH, C. SARANGI, T. SAVOLT, D. A. LINSEMAN;
Biol. Sci., Univ. of Denver, Denver, CO

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disorder that affects nerve cells in the brain and spinal cord. It is characterized by the loss of cortical and spinal motor neurons and by the presence of oxidative and nitrosative stress, inflammation, and mitochondrial dysfunction. Research on nutraceuticals known as betacyanins, water-soluble pigments found in red dragon fruit, have demonstrated the powerful antioxidant, anti-inflammatory, and free-radical scavenging properties of these compounds; activities which may be beneficial for ameliorating underlying disease pathology and slowing disease advancement in ALS. The present study aimed to characterize the therapeutic effects of a betacyanin-rich red dragon fruit extract (DFE) in the G93A hSOD1 transgenic mouse model of ALS. In this animal model, disease onset typically occurs at 90 days old with the development of skeletal muscle atrophy, hind limb weakness (and eventual paralysis), and weight loss; disease end-stage is reached at approximately 120 days old. G93A hSOD1 mutant mice were treated orally with 5% (v/v) DFE in drinking water ad libitum, from disease onset until end-stage. Each group of mice (n=20; equal number of males and females) were sex-matched littermates, consisting of two mutants (one treated with DFE, one untreated) and a wildtype (WT) control. Body weight was

monitored weekly throughout the treatment, and grip strength and rotarod behavioral tests were conducted to assess muscle strength and endurance. The DFE treated G93A hSOD1 mice had a significant increased median lifespan as well as a significant preservation of muscle strength when compared to their untreated littermates. Additionally, histopathological analyses show reduced astrogliosis and microgliosis, increased motor neuron survival, and improved neuromuscular junction complexity and innervation in the treated G93A hSOD1 mice compared to their untreated littermates. These findings indicate that DFE, or purified betacyanin compounds, could potentially be used as a therapeutic intervention for patients with ALS.

Disclosures: **C. Pena:** None. **L. Koza:** None. **M. Sinar:** None. **E. Buechler:** None. **A. Baybayon-Grandgeorge:** None. **A. Smith:** None. **C. Sarangi:** None. **T. Savolt:** None. **D.A. Linseman:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.22/V12

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant 1R03NS130425
The University of Pittsburgh Momentum Funds
UPMC Competitive Medical Research Fund

Title: A novel calcium channel gating modifier that enhances synaptic function and maintains innervation in a mouse model of Amyotrophic Lateral Sclerosis

Authors: *Y. BADAWI[1], Y. LI[1], A. SABER[1], K. FETZER[1], P. WIPF[2], S. D. MERINEY[1]; [1]Neurosci., [2]Chem., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Amyotrophic Lateral Sclerosis (ALS) in a neurodegenerative disease that results in the progressive deterioration and loss of function of the motor neurons leading to paralysis. Studies indicated that synaptic transmission at neuromuscular junctions (NMJs) is reduced in early stages of the disease - before denervation and motor neuron death. To date, there are no treatments for ALS that target improving neuromuscular transmission, which could improve quality of life for ALS patients both by enhancing neuromuscular strength and triggering trophic support for motoneurons. Here, we tested the effect of GV-58, a novel Cav2-specific voltage-gated calcium channel gating modifier that we have developed, on neuromuscular function in $SOD1^{G93A}$ ALS model mice. First, we show significant denervation in the epitrochleoanconeus (ETA) muscle of $SOD1^{G93A}$ mice (which has not previously been documented as vulnerable) at the early symptomatic stage (P90). Our results also demonstrate impaired magnitude of transmitter release in the ETA muscle of $SOD1^{G93A}$ mice at the early symptomatic stage (P90), and that treating *ex vivo* nerve muscle preparations from $SOD1^{G93A}$ mice with GV-58 significantly increased quantal content (by ~40%). We hypothesized that a GV-58-mediated increase in synaptic activity may

reduce denervation and provide better support for the motoneurons. Interestingly, a chronic treatment of GV58 starting at P90 delayed disease progression, significantly enhanced end plate potential amplitude, and maintained synapse innervation for the length of the treatment (20 days post-injection), compared to vehicle-treated mice. Therefore, we hypothesize that GV-58 could prove to be a new intervention approach to strengthen synaptic transmission, improve neuromuscular function, delay the loss of motor skills, increase the quality of life, and potentially prolong the life-span of ALS patients.

Disclosures: Y. Badawi: None. **Y. Li:** None. **A. Saber:** None. **K. Fetzer:** None. **P. Wipf:** None. **S.D. Meriney:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.23/V13

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: JST ERATO (JPMJER1801)
Institute for AI and Beyond of the University of Tokyo

Title: Als linked mutant sod1-flot2 interaction causes zinc toxicity

Authors: *S. TANAKA[1,2], Y. IKEGAYA[1,3], T. FUJISAWA[2], H. ICHIJO[2];
[1]Univ. of Tokyo, Tokyo, Japan; [2]Pharma Sci, Univ. Tokyo, Tokyo, Japan; [3]Inst. for AI and Beyond, Univ. Tokyo, Tokyo, Japan

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by selective loss of motor neurons. Despite its severity, there are no effective treatments. *Copper-zinc superoxide dismutase (SOD1)* gene has been identified as the most common causative gene for familial ALS. While SOD1 is primarily known as an antioxidant enzyme, it is widely accepted that ALS-linked mutant SOD1 ($SOD1^{mut}$) is recognized for its acquisition of toxic functions that contribute to disease progression. Although various mechanisms, including endoplasmic reticulum stress, zinc toxicity, and disrupted axonal transport, have been proposed, no precise mechanisms have been elucidated. We previously uncovered a novel function of SOD1. We demonstrated that wild-type SOD1 ($SOD1^{WT}$) changes its conformation under zinc deficiency. The conformationally altered $SOD1^{WT}$ interacts with FLOT2, a regulator responsible for endocytosis of diverse proteins on the plasma membrane. We showed that the SOD1-FLOT2 interaction inhibits the endocytosis of some zinc transporters, thereby increasing the amount of the transporters on the plasma membrane for promoting zinc uptake.Given that SOD1 is a causative gene for ALS and that zinc excess is one of the toxic mechanisms in ALS, we explored the impact of the SOD1-FLOT2 interaction in the case of $SOD1^{mut}$. We found that mutant SOD1 interacts with FLOT2 and the $SOD1^{mut}$-FLOT2

interaction increases the amount of zinc transporters on the plasma membrane. These results suggest that the SOD1-FLOT2 interaction induces ALS by promoting the excess uptake of zinc.

Disclosures: S. Tanaka: None. **Y. Ikegaya:** None. **T. Fujisawa:** None. **H. Ichijo:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.24/V14

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH 5R01 NS081303-09

Title: Loss of NEK1 aggravates C9orf72 repeat expansions-mediated toxicity

Authors: *D. MAWRIE[1], E. N. ANDERSON[1], A. GLEIXNER[2,3], C. WARD[4], O. CHAUHAN[5], C. ZAMMERILLA[4], R. ROY[6], C. J. DONNELLY[2,3], E. KISKINIS[7], U. B. PANDEY[1,4];
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Abstract: An expanded GGGGCC (G4C2) repeat in chromosome 9 open reading frame 72 (C9orf72) is the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). Heterozygous loss-of-function (LOF) mutations in NEK1 (NIMA-related kinase 1) was shown to be a causative gene in ALS with evidence of reduced penetrance. Reports suggest the co-occurrence of heterozygous LOF mutations of NEK1 in C9orf72 carriers. However, the functional implications of NEK1 in C9 ALS have not been explored. Here, we develop an *in vivo* model to assess the impact of ALS risk factor gene NEK1 on C9orf72-mediated neurodegeneration. We found that reduced levels of NEK1 protein in a *Drosophila* model of C9orf72 ALS deteriorates eye phenotype, cause neuromuscular junction defects, impairs motor function, and decreases lifespan. Furthermore, *in vitro* expression of G4C2 repeats decreases NEK1 protein and mRNA, and C9 iMNs showed reduction in NEK1 mRNA. Strikingly, reduce levels of NEK1 *in vivo* and *in vitro* elevated toxic G4C2 RNA suggesting that NEK1 may modify C9orf72 toxicity via G4C2 RNA. Interestingly, NEK1 reduction in C9orf72 alters the percent of motile mitochondria and transport. Our data provide evidence that the reduce levels of NEK1 is a potential causative factor of C9-ALS toxicity.

Disclosures: D. Mawrie: None. **E.N. Anderson:** None. **A. Gleixner:** None. **C. Ward:** None. **O. Chauhan:** None. **C. Zammerilla:** None. **R. Roy:** None. **C.J. Donnelly:** None. **E. Kiskinis:** None. **U.B. Pandey:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.25/V15

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH R01 AG066729-01A1
VA Merit I01BX005762
BrightFocus Foundation A2022041S

Title: Hsp90 loss of function increases TDP-43 protein accumulation but protects against disease phenotypes in a C. elegans model of amyotrophic lateral sclerosis (ALS)

Authors: L. GARCIA TOSCANO[1,2], J. C. HINCKS[1], H. N. CURREY[1], ***N. LIACHKO**[1,2]; [1]VA Puget Sound Hlth. Care Syst., Seattle, WA; [2]Univ. of Washington, Seattle, WA

Abstract: Neuronal inclusions of hyperphosphorylated TDP-43 are hallmarks of disease for most patients with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD-TDP). Mutations in *TARDBP*, the gene coding for TDP-43, can cause familial inherited ALS (fALS), indicating dysfunction of TDP-43 drives disease. To study the cellular, molecular, and genetic underpinnings of TDP-43 mediated neurotoxicity in a tractable model system, we have developed *C. elegans* models of TDP-43 proteinopathy. Expression of fALS-causing mutant TDP-43 in all *C. elegans* neurons causes severe progressive motor dysfunction, and recapitulates some characteristic features of ALS and FTLD-TDP including decreased lifespan, neuronal degeneration, and accumulation of detergent insoluble aggregates containing hyperphosphorylated, ubiquitinated and C-terminal truncated TDP-43. Aggregated, phosphorylated TDP-43 may contribute to disease phenotypes; alternatively, TDP-43 aggregation may be a protective cellular response sequestering toxic protein away from the rest of the cell. The chaperone Hsp90 has been previously shown to interact with TDP-43, stabilize its normal conformation, and modify toxicity of wild-type TDP-43 in yeast and cultured cell models. However, there may be biological differences between wild-type and fALS mutant TDP-43 that alter the consequences of Hsp90 chaperone activity. To test whether Hsp90 loss of function modulates mutant TDP-43 protein accumulation and neurotoxicity in a whole animal, we used a well characterized loss-of-function mutation in the *C. elegans* Hsp90 homolog *hsp-90/ daf-21*. We find that loss of function of the heat shock protein HSP-90 protects against mutant TDP-43 neurotoxicity and subsequent neurodegeneration in *C. elegans*. Interestingly this protection is accompanied by an increase in both total and phosphorylated TDP-43 protein. We also find a selective increase in *hsp-70* but not other chaperones in *hsp-90(-);TDP-43 Tg* animals, indicating changes within the chaperone network may be underlying TDP-43 protein accumulation. Our results indicate that Hsp90 chaperone activity can contribute to adverse outcomes in fALS driven by TDP-43 mutations, and that TDP-43 aggregation may protect against worsened disease phenotypes.

Disclosures: **L. Garcia Toscano:** None. **J.C. Hincks:** None. **H.N. Currey:** None. **N. Liachko:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.26/V16

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Development of a Novel, Orally Active, Brain Penetrable HDAC6 Inhibitors Shows a Therapeutic Potential for Amyotrophic Lateral Sclerosis (ALS)

Authors: *H. VANKAYALAPATI, Z. LI, C. LIN, K. MEDLEY, D. BEARSS;
Biolexis Therapeutics, Inc., American Fork, UT

Abstract: Objective: An *in vivo* evaluation of HDAC6-specific small molecule leads BLX-0270 and BLX-0279 in ALS pre-clinical SOD1-G93A transgenic B6SJL.SOD1-G93A, in rNLS8 model of TDP-43 mouse models with PK/PD, biomarkers, safety, and familial SOD1, FUS, TDP-43, and C9orf72 genes from ALS patient's samples ex-vivo.
Background: ALS is a progressive neurodegenerative disease that gradually and irreversibly affects motor movement due to the death of motor neurons in the brain and spinal cord. Mutations in over 20 genes have been associated with familial ALS, with mutations in 4 genes accounting for most ALS familial cases. ALS disease ultimately leads to paralysis and eventually death, usually because of respiratory failure within 3 to 5 years after symptom onset, typically in spinal, including limb weakness. HDAC6 is an enzyme that regulates the acetylation of proteins, including those involved in transporting important cellular components along the axons of neurons. Studies have shown that HDAC6 plays a role in the clearance of abnormal protein aggregates, such as SOD1 and TDP-43, which are implicated in ALS pathogenesis. Evidence suggests that HDAC6 inhibitors could have potential therapeutic effects in ALS. We have discovered a series of new HDAC6 inhibitors to develop as a treatment for ALS.
Methods: Fluorescence-based quantification, NanoBRET using HEK293 cells transiently expressing NanoLuc-HDAC6 CD2 cell-free and High Content IF/ICC and WB cellular efficacy, PK, ADME-Tox, and B6SJL.SOD1-G93A experiments were conducted.
Results: Utilizing an HDAC6-refined crystal structure and our MolecuLern^{TM} RO3 fragment-based technology, we designed, synthesized a series of >40 novel, reversible HDAC6i, and BLX-0270 and BLX-0279 selected as leads. The HDACs selectivity, target engagement assays confirm the on-target potency of BLX-0270 and BLX-0279 with IC_{50s} of 8.13 and 3.63 nM. Our lead BLX-0279 demonstrated its potent activity with an IC_{50} of 3.40 nM in the NanoBRET assay. BLX-0270 and BLX-0279 exhibited potent cellular efficacy in both High Content IF/ICC and WB experiments in a dose-response manner against HDAC6 expressed acetylated α-tubulin in SH-SY5Y neuroblastoma cells with an IC_{50} of 1.33 and 0.82 nM. BLX-0270, and BLX-0279 had excellent PK profile in mouse species with oral bioavailability of 50%, 53% including brain uptake, and 6.0 hrs of half-life.

Conclusions: Our preliminary results support the inhibition of HDAC6, rescue of ALS phenotypes through our ALS-patient-derived iPSC, ICC, and WB motor neurons model experiments. These two compounds were nominated as candidates for additional safety, toxicokinetic, and other investigational studies.

Disclosures: H. Vankayalapati: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Arrien Pharmaceuticals. **Z. Li:** A. Employment/Salary (full or part-time):; Biolexis Therapeutics, Inc. **C. Lin:** A. Employment/Salary (full or part-time):; Biolexis Therapeutics, Inc. **K. Medley:** A. Employment/Salary (full or part-time):; Biolexis Therapeutics, Inc. **D. Bearss:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biolexis Therapeutics, Inc., Halia Therapeutics, Inc., University of Utah.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.27/V17

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NES22001-001-00000

Title: Bioenergetic stress triggers Amyotrophic Lateral Sclerosis-like symptoms in mice

Authors: *S. SAHA, A. PAPANERI, G. CUI;
Natl. Inst. of Hlth., Durham, NC

Abstract: Optimum “bioenergetic balance” in the mitochondria, the powerhouse of neurons, is a key factor for proper brain functioning. The perturbance of this balance over time leads to a wide array of neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS). ALS is a progressive, lethal neuromuscular disease characterized by degeneration of the cholinergic motor neurons (CMN), resulting in loss of voluntary movements, paralysis, and eventually death. Mitochondrial transcription factor A (Tfam) plays indispensable roles in regulating cellular bioenergetics by reducing oxidative stress and maintaining mitochondrial DNA and health. Here, we seek to elucidate the cause of neurodegeneration in ALS, which remains unknown, by studying the direct role of bioenergetic stress in its etiopathogenesis. For this, a new cholinergic Tfam-deletion mouse strain was developed (ChAT-cre;$Tfam^{-/-}$, abbreviated as ChTf) with progressive loss of mitochondrial function in cholinergic neurons. In these ChTf mice, compared to their littermate controls, we observed a rapid decrease in body weights following 13.5 weeks of age. As they approach the 16th week of age, they show stooped posture and limited mobility in their home cages, potentially due to weakened muscle activity, and they resemble the predominant form of ALS (i.e. limb onset ALS). Behaviorally, this observation was reinforced in the rotarod test, when we found that ChTf mice showed persistent reduced motor performance

and coordination age-dependently, exhibiting motor deficits with rotarod acceleration. In open field and Y-maze test, the ChTf mice did not show changes in exploratory behavior and memory. Further, the histological analysis of ChAT neurons revealed no damage to the basal forebrain and striatal cholinergic neurons. Together, the findings suggest the causality of bioenergetically stressed CMN in movement dysfunctions, implicating the potential damage of the CMNs in the brain stem and spinal cord. Most interestingly, the selective vulnerability of lower motor neurons revealed by equally applied bioenergetic stress bestows a newer angle to the susceptibility and treatment of CMN neurons in ALS. This indicates that differential bioenergetics is an important mechanism underlying CMN degeneration in ALS. Further, histopathology of the spinal cord, along with fiber photometry using ATP and cholinergic sensors, will validate the perturbed bioenergetic dynamics in multiple brain regions of this ALS model.

Disclosures: S. Saha: None. **A. Papaneri:** None. **G. Cui:** None.

Poster

PSTR200. ALS: Non-Human Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR200.28/V18

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant NS078375
NIH Grant AA027079-05

Title: Mechanisms of Motor Unit Dysfunction at onset of disease in a mouse model of ALS

Authors: *J. PARK[1], N. DELESTRÉE[2], V. MENON[3], G. Z. MENTIS[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive fatal neurodegenerative disease affecting mostly spinal motor neurons. Its hallmarks include motor neuron loss, muscle atrophy, paralysis and death. Studies in ALS using genetic mutants in mouse models have identified numerous changes in motor neurons at early stages, including excitotoxicity, oxidative stress, denervation at neuromuscular junction (NMJ), mitochondrial abnormalities and motor neuron excitability. Such changes are accompanied by abnormal motor unit (MU) function and motor output. Despite attempts to identify early changes in ALS, the causal relationship between abnormal motor output - broadly defined as MU dysfunction - and the reported pathological changes have not been understood. Here, we used male mice of C57BL6/J-SOD1 G93A mouse model of ALS together with behavioral, physiological and morphological assays to identify transcriptional changes in vulnerable motor neurons involved in motor dysfunction during ALS onset. We found that P50 was an early age in which SOD1-G93A mice exhibited ~40% reduction in the distance travelled/day/mouse. Additionally, there was a significant ~50% loss of functional Tibialis Anterior (TA) muscle motor units, determined by *in vivo* experiments in

which individual MUs were counted following incremental stimulation intensities and quantifying the elicited muscle force. To this end, there was also a significant overall decrease in the twitch force in the TA muscle. Furthermore, we observed an ~40% NMJ denervation in the TA muscle. Yet, SOD1-G93A male mice revealed resistance to fatigue compared to controls at onset of disease. At earlier ages than P50, SOD1-G93A male mice revealed no overt pathological changes. Thus, to identify transcriptional changes at P50 that may be involved in the pathogenesis of ALS, large TA motor neurons (presumed to be Fast MUs) from control and SOD1 males were subjected to RNAseq following collection through Laser Capture Microdissection following labelling with a fluorescent tracer through intramuscular injection. The resultant differentially expressed genes (max expression ≥ 100 counts/million, FDR-adjusted $p < 0.05$) included 6 upregulated and 10 downregulated transcripts. These results identify potential novel transcriptional targets in vulnerable motor units early in the course of disease. Future experiments will determine whether any of these targets contribute to the motor neuron phenotype, or represent a protective/compensatory response.

Disclosures: **J. Park:** None. **N. Delestrée:** None. **V. Menon:** None. **G.Z. Mentis:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.01/V19

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

Support: Canadian Institutes of Health Research
Parkinson Canada
Michael Smith Foundation for Health Research

Title: Brain pericytes are highly vulnerable to thiol oxidation

Authors: *C. GROTEN[1], S. WENDT[4], N. WEILINGER[5], L.-P. BERNIER[2], S. EBERT[6], M. TOWRISS[1], B. A. MACVICAR[3];
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Abstract: Brain dysfunction in various disorders such as stroke, Parkinson's, and Alzheimer's disease is promoted by oxidative stress, a pathological state resulting from an excess of reactive oxygen and nitrogen species. These molecules impede brain function by oxidizing various proteins and lipids which impact essential cellular processes and can ultimately lead to neuronal dysfunction and death. Hence, it is crucial to identify the primary cellular and molecular targets that are impacted by oxidative stress and contribute to brain dysfunction. To address this, we assessed cell death evoked by oxidative stress in acute cortical brain slices from rodents by

measuring propidium iodide (PI) uptake with two-photon microscopy. Oxidation of thiols on proteins and glutathione is a prominent downstream impact of reactive oxygen and nitrogen species. Therefore, we triggered oxidative stress in acute brain slices using thiol oxidation. We observed that exposure of brain tissue to a thiol oxidizing agent was sufficient to induce PI loading, indicating cell death. Interestingly, this cell death largely occurred in cells associated with the vasculature rather than non-vascular cells. Moreover, the uptake of PI by these vessel-associated cells occurred rapidly, within 10 minutes after thiol oxidation. Given their distinct morphology and association with blood vessels, we explored the possibility that these vulnerable cells were pericytes. Utilizing live imaging of pericytes labeled with the fluorescent marker NeuroTrace (NT), we discovered that thiol oxidation led to concurrent PI uptake and NT loss in a significant portion of pericytes-indicating robust cell death. Our findings demonstrate that pericytes possess high sensitivity to thiol oxidation, which is a prominent impact of reactive oxygen species and oxidative stress. Pericytes are implicated in the regulation of blood vessel stability, the integrity of the blood-brain barrier, and cerebral blood flow. Consequently, our data suggests that dysfunction of pericytes might significantly contribute to brain pathologies triggered by oxidative stress. Future investigations aimed at unraveling the mechanisms underlying the susceptibility of pericytes to cell death during oxidative stress will be essential to test this hypothesis.

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Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.02/Web Only

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

Title: Progressing neurodegeneration induced by morphine in mouse brain.

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Abstract: Opioids are the most effective drugs commonly prescribed to treat pain. Despite widespread abuse of opioids, we know little about the long-term consequences of chronic use. Toxic effects of opioids have been documented not only for heroin abusers, but also for patients with a history of long-term prescription opioid use. Recently, our lab demonstrated that chronic oxycodone exposure induces oxidative and nitrosidative stresses in the rat's brain, as well as the expression of the stress-related proteins in the nucleus accumbens, cortex, and cerebellum. In addition, we demonstrated that animals chronically treated with oxycodone developed damage in subcortical white matter. To investigate the effect of chronic morphine use, we treated female mice with either water or morphine (15 mg/kg/day) intraperitoneally. We observed the development of tolerance to morphine after 7 days of treatment, measured by the Hot Plate and

the Open Field tests. To investigate the dynamics of neuronal degeneration in mice brains, we monitored expression levels of pro-apoptotic Bax, Brain-derived neurotrophic factor (BDNF), and Platelet-derived growth factor receptor A (PDGFR A) in brains of mice treated with morphine for 12 or 30 days. Bax is a protein involved in the Bcl-2 pathway that functions as an apoptotic activator. We observed an increase in the level of Bax expression in mouse striatum at 12 and 30 days, which suggests that chronic morphine administration may induce pro-apoptotic signaling in brain. We also observed an increase in the level of BDNF, a protein that is involved in the growth and survival of neurons and synapses. Levels of BDNF increase during periods of inflammation and oxidative stress. Our result suggests that chronic morphine administration induces survival mechanisms in neurons through increased expression of BDNF. We also observed an increase in PDGFR A, a receptor tyrosine kinase involved in the regulation and maintenance of oligodendrocyte differentiation and maturation. Oligodendrocytes produce myelin in the central nervous system that is crucial for the propagation of nerve signals. Our results suggest that chronic morphine administration may affect the rate of maturation of oligodendrocytes, leading to lower expression levels of myelin basic protein (MBP) and demyelination of axons. The findings of this project support the hypothesis that chronic opioid use causes a negative impact on neuronal health.

Disclosures: **C. Brazile:** None. **N. Korneeva:** None. **R. Fan:** None. **T. Arnold:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.03/V20

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

Support: R01MH119000
2R01NS065957
2R01DA032444

Title: The intraluminal chemistry of endolysosome iron oxidation induced by acidification or iron chelation prevents nicotine and ethyl alcohol cytotoxicity

Authors: *J. D. GEIGER, P. HALCROW, R. SOLLOWAY, D. QUANSAH, K. TIEGEN; Biomed. Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: Nicotine and ethyl alcohol are commonly consumed psychoactive drugs. As legal but regulated drugs, nicotine and ethyl alcohol adversely affect the health of people consuming them. Nicotine and ethyl alcohol increase levels of reactive oxygen species (ROS) and can cause neurotoxicity; however, by unknown mechanisms. Ferrous iron (Fe^{2+}) is well known to increase levels of ROS via Fenton-like chemistry, and endosomes and lysosomes (endolysosomes) contain high levels of readily releasable [Fe^{2+}] that when released are sufficient to affect cytosolic and mitochondrial Fe^{2+} levels. Correspondingly, endolysosome acidity maintains iron

homeostasis and endolysosome de-acidification triggers iron release from endolysosomes and iron accumulation in the cytosol and mitochondria; nicotine and ethyl alcohol de-acidify endolysosomes. Although well-known as an antioxidant, deferoxamine (DFO) is an endolysosome-specific iron chelator that binds ferric iron (Fe^{3+}), induces Fe^{2+} to Fe^{3+} oxidation, and increases [H^{+}]. In addition, endolysosome acidification via mucolipin synthetic agonist 1 (ML-SA1) inhibits endolysosome de-acidification-induced neurotoxicity; however, by unknown mechanisms. Thus, it was important to determine how DFO and ML-SA1 affect the intraluminal chemistry of endolysosome iron and, accordingly, identify mechanisms by which nicotine and ethyl alcohol induce neurotoxicity. Using SH-SY5Y neuroblastoma cells and U87MG astrocytoma cells, we showed that nicotine and ethyl alcohol (1) de-acidified endolysosomes, (2) decreased endolysosome Fe^{2+} levels, (3) increased cytosolic and mitochondrial Fe^{2+} and ROS levels, (4) depolarized mitochondrial potentials, and (5) induced cell death; effects all blocked by DFO and ML-SA1. Understanding the role of endolysosome iron in nicotine and ethyl alcohol-induced neurotoxicity may provide new insight into the pathological implications of these commonly used psychoactive drugs.

Disclosures: **J.D. Geiger:** None. **P. Halcrow:** None. **R. Solloway:** None. **D. Quansah:** None. **K. Tiegen:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.04/V21

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

Support: 1RF1AG068292
AFAR BIG21042

Title: Pirh2-dependent DNA damage in neurons induced by the G-quadruplex ligand pyridostatin

Authors: *R. DIAZ ESCARCEGA[1], A. A. PATIL[1], J. F. MORUNO-MANCHON[1], A. URAYAMA[1], S. P. MARRELLI[1], N. KIM[2], D. MONCHAUD[3], L. D. MCCULLOUGH[1,4], A. S. TSVETKOV[1,5];
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Abstract: Non-canonical base pairing among four guanines (G) within single-stranded G-rich sequences leads to the formation of a G-quartet. Self-stacking of G-quartets results in the formation of a columnar four-stranded DNA structure known as the G-quadruplex (G4 or G4-DNA). In cancer cells, G4-DNA regulates a broad variety of DNA-dependent processes

including transcription, replication, and telomere function. How G4s function in neurons, however, is poorly understood. Here, we performed a genome-wide gene expression analysis (RNAseq) to identify genes modulated by a G4-DNA ligand, pyridostatin (PDS), in primary cultured neurons. PDS promotes the stabilization of G4 structures, thus allowing us to define genes directly or indirectly responsive to G4 regulation. Our results demonstrate that 901 genes are differentially expressed in neurons treated with PDS out of a total of 18,745 genes with measured expression. 505 genes are downregulated and 396 genes are upregulated, involving networks of genes regulating p53 signaling, immune response, learning and memory, and cellular senescence. Within the p53 network, we discovered that the E3 ubiquitin ligase Pirh2 (Rchy1), a modulator of DNA damage responses, is upregulated by PDS. Ectopically overexpressing Pirh2 promotes the formation of DNA double-stand breaks, suggesting a new DNA damage mechanism in neurons that depends on G4 stabilization. Intriguingly, Pirh2 downregulates DDX21, an RNA helicase that unfolds G4-RNA and R-loops, suggesting that accumulation of R-loops may contribute to PDS-mediated DNA damage. We also demonstrate that Pirh2 increases G4-DNA levels in the neuronal nucleoli. Overall, our data reveal the genes that are responsive to PDS treatment and suggest similar transcriptional regulation by endogenous G4-DNA ligands and identify how G4-dependent regulation of transcription is connected to the DNA damage mechanisms in neuronal cells.

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Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR201.05/V22

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

Support: NIH Grant R01 NS110754

Title: Heat stress response mechanisms regulating neuronal recovery in the mammalian central nervous system

Authors: *C. SELUZICKI[1], S. S. MARGOLIS[2];
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Abstract: Elevated brain temperature is one of the most poorly understood environmental stressors impacting the central nervous system (CNS). Brain temperature fluctuates 2-3°C in a healthy human, but elevated temperature beyond this point, resulting from brain injury, illicit drug usage, or heat stroke, is detrimental to CNS function and survival. These data suggest intrinsic molecular mechanisms for heat stress response reside in the brain for neuronal function.

To study this, we heat stressed primary mouse neuronal cultures at 42°C and found that during heat stress, neurons failed to upregulate canonical heat shock protein, Hsp70, and downregulated global translation. We observed that neurons have a time-sensitive window to adapt to heat stress, beyond which, they failed to recover translation and die. Surprisingly, deep-sequencing analysis revealed that *Hspa1a,* which codes for Hsp70 protein, is upregulated nearly 350-fold during heat stress, without detectable protein. We found that *Hspa1a* was preferentially occupied by monosomes during heat stress, and exclusively accumulated polysomes when neurons were returned to 37°C to recover from heat stress, which eventually yielded detectable Hsp70 protein after two hours. We hypothesized that neurons accumulate monosomes on *Hspa1a* to poise the system for recovery if it is an option, and that without Hsp70 protein, neurons fail to recover and die. Hsp70 inhibition and genetic mouse Hsp70 knockouts demonstrated that Hsp70 is important during recovery for neuronal viability. Our study characterizes molecular events modulating neuronal proteostasis during heat stress and Hsp70 regulation, which has broader relevance in the context of environmental stress and neurological disease.

Disclosures: C. Seluzicki: None. **S.S. Margolis:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.06/V23

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

Support: CONACyT fellowship 373878 tp MGS

Title: Effect of DHEAS on chemical hypoxia-induced damage on egg-laying in the model organism Caenorhabditis elegans.

Authors: *L. HERNANDEZ, M. D. J. GALLEGOS SAUCEDO, D. W. AGUILAR OCAMPO, A. CASTILLO-ROMERO, R. CORTÉS-ZÁRATE, S. A. GUTIERREZ-RUBIO, A. HERNÁNDEZ-CHÁVEZ, G. CAMARGO-HERNÁNDEZ;
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Abstract: Too low oxygen levels to sustain normal functions, namely Hypoxia, occur in many physiological and pathophysiological processes. It can cause severe central nervous system (CNS) dysfunction because of neuronal death. At the cellular level, the mechanism of action by which neurosteroids such as Dehydroepiandrosterone (DHEA) can confer protection against hypoxia-induced damage is not entirely elucidated, since the cellular response to hypoxia has not been fully clarified. Neuroprotection research has been endorsed by animal model works, including Caenorhabditis elegans (C. elegans). C. elegans is among the simplest organisms with a nervous system performing similar functions to those in more complex organisms' nervous systems. Particularly, it includes the components of the serotonergic and dopaminergic systems present in vertebrates and its activity can be studied through stereotyped behaviors such as egg-

laying. Furthermore, C. elegans responds to hypoxia with cellular pathways and processes conserved in mammals. Here, we implemented a chemical hypoxia model with Sodium Sulfite (SS, Na2SO3), which mimics the pathophysiological processes in physical hypoxia. This work aimed to investigate the effect of DHEAS on the damage induced by sodium sulfite on egg-laying in the model organism Caenorhabditis elegans. In this study, age-synchronized N2 (Wild Type) worms strain cultured on NGM-agar plates at 19°C was employed. We established an untreated group (CTL), a hypoxic group (SS), a hypoxic group with DHEAS (SS+DHEAS), a hypoxic group treated with Dopamine (SS+DOP), a hypoxic group treated with Serotonin (SS+ SER) and a group treated only with DHEAS (DHEAS). Hypoxia was induced by exposing the worms to SS for 16 h at 20ºC, followed by a rest for 24 h at 20ºC in NGM-OP50. To observe the egg-laying behavior, a defined number of worms were transferred to NMG Agar boxes with food (E. coli OP50 strain), and the eggs laid on the surface of the NGM-Agar plate, were counted by one hour. We found that DHEAS completely reversed the 50% reduction in egg-laying induced by SS and that exogenous dopamine did not show significant differences with CTL, and exogenous serotonin did cause a significant increase in egg-laying with CTL. The observed results suggest that DHEAS confers protection against oxidative damage to the systems involved in the C. elegans egg-laying, mostly the dopaminergic pathway, probably due to its antioxidant activity, neurotrophic action, or a combined effect.

Disclosures: L. Hernandez: None. **M.D.J. Gallegos Saucedo:** None. **D.W. Aguilar Ocampo:** None. **A. Castillo-Romero:** None. **R. Cortés-Zárate:** None. **S.A. Gutierrez-Rubio:** None. **A. Hernández-Chávez:** None. **G. Camargo-Hernández:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.07/V24

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

Support: CIHR PJT-162103

Title: Modulation of mitochondrial dynamics by the delta-opioid receptor

Authors: *L. CÔTÉ, J. DEGRANDMAISON, P. LABRECQUE, L. GENDRON, J.-L. PARENT;
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Abstract: Mitochondria are highly dynamic organelles that fuse and divide constantly to maintain their size, shape and distribution. Lately, a few G protein-coupled receptors such as the cannabinoid receptor 1 were shown to modulate this dynamic. Interestingly, many studies revealed the mito- and neuroprotective roles of the delta-opioid receptor (DOP). Despite all the effects on mitochondria that were elucidated, little is known about how the DOP contributes to mitochondrial dynamics. Here, studies performed by LC-MS/MS analyses on

immunoprecipitated FLAG-tagged DOP from brain homogenates of knock-in mice identified numerous endogenous mitochondrial proteins as DOP interactors in its native environment. Since several members of the mitochondrial dynamics' proteins were identified in our LC-MS/MS analyses, we investigated the contribution of DOP in mitochondrial dynamics. We first confirmed the interaction between DOP and mitofusin-1 (Mfn1), mitofusin-2 (Mfn2), dynamin-related protein 1 (Drp1) and optic atrophy 1 (Opa1) by co-immunoprecipitation studies. Confocal microscopy analyses show that the stimulation of the DOP modulates the size and the shape of mitochondria. Our results also suggest that the observe effects are dependent of the mitochondrial localized DOP.

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Poster

PSTR201. Cellular Stress and Death Mechanisms

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Program #/Poster #: PSTR201.08/V25

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

Support: NCI/NIH R01 CA208623
NCI/NIH R01 CA208623-S2
NCI/NIH R21 CA149814
NHLBI/NIH HHSN268201000046C

Title: Role of oxidative stress and TXNIP pathway in chemotherapy-induced peripheral neuropathy

Authors: *J. DU[1,2], K. SHAHVERDI[2], L. C. SUDLOW[2], H. ZHOU[2], M. D. WOOD[4], M. SHAMIM[3], H. HU[5], M. Y. BEREZIN[2];
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Abstract: Oxaliplatin, a third generation of platinum-based chemotherapy drug, remains one of the lead treatment for colorectal cancers. Despite its high efficacy, oxaliplatin frequently induces debilitating side effects, with chemotherapy induced peripheral neuropathy (CIPN) being most prevalent among patients. Almost 90% of patients receiving oxaliplatin experience acute neuropathy, and upon repetitive chemotherapy sessions, 70% of them develop chronic complications. To improve the life quality of patients and ensure the chemotherapy efficacy, a better management of the neuropathy is in demand. However, the underlying mechanisms of CIPN has not been fully elucidated. Reducing the effect of the neuropathy is an unmet challenge.

To understand the mechanism of CIPN, we first established a mouse model that mimics the CIPN in human. The animal model was validated by behavioral, biochemical, and electrophysiological changes. Nerve histology with TEM shows abnormal mitochondria in the sciatic nerve suggesting the mitochondrial damage through role of oxidative stress in the development of CIPN. Meanwhile, the burst of reactive oxygen species (ROS) was observed in rat sciatic nerve in vivo after oxaliplatin injection. We further validated the involvement of ROS pathway through bulk RNA-seq on mice dorsal root ganglia (DRG). KEGG analysis of differentially expressed genes identified oxidative stress-associated pathways as one of oxaliplatin's top-regulated metabolic pathways. A subsequent correlation network analysis of transcriptome in these pathways identified thioredoxin interacting protein (TXNIP) as a key gene in the network with a large fold change and high degree of connections to other genes. In vitro CIPN experiments with DRG explants indicated that the inhibition of the oxidative stress by TXNIP suppressor verapamil can protect DRGs from oxaliplatin and preserve neurite outgrowth. Similarly, animals treated with verapamil alongside oxaliplatin demonstrated a decrease in TXNIP expression in DRG and partial improvement of nerve conduction velocity (NCV). Our findings indicate that TXNIP, with its central role as a regulator of the oxidative stress, is a promising therapeutic target to treat chronic CIPN.

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Poster

PSTR201. Cellular Stress and Death Mechanisms

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.09/W1

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

Title: Deciphering predominant ROS producers in epilepsy

Authors: *P. K. SINGH, A. SAADI, T. SHEKH-AHMAD;
Sch. of Pharm., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Epilepsy affects ~1% of the global population, with over 30% of epileptic patients resistant to currently available anti-seizure medications. Burgeoning evidence suggests that reactive oxygen species (ROS) generation contribute to neuronal damage and cell death in seizures and epilepsy. Nevertheless, the primary source of ROS in epilepsy remains obscure. Traditionally, mitochondria have been proposed to be the main ROS producer in seizures and epilepsy. More recently, we have demonstrated that NADPH oxidase 2 (NOX2) expression has a transient response to seizure which temporally vary depending on seizure duration and brain region (cortex or hippocampus). In this study, we aim to investigate the contribution of these two ROS sources, i.e., mitochondria and NOX2, to the ROS burden in epilepsy. We used genetically encoded redox-sensitive probes (Mito-roGFP2-Orp1 and p47-roGFP) to monitor the ROS generation during in vitro seizures in neuronal cultures, as well as in in-vivo seizure models

using in vivo fiber photometry along with ECoG recordings in freely behaving rats. Our *in-vitro* results demonstrated that following low Mg^{2+} induced seizure activity in mixed cortical culture, mitochondrial ROS generation decreased by 30%, while NOX2 derived ROS generation was increased by 60% within 1 hour of seizure activity. When tested *in vivo*, there was no significant change in mitochondrial ROS following acute seizure induced by pentylenetetrazole. Interestingly, we demonstrated a ~25% increase in NOX2-derived ROS (normalized p47-roGFP fluorescence) which started 5-10 minutes prior to seizure and lasted for ~20 minutes. When monitored following KA-induced seizure, p47-roGFP fluorescence was increased by 30-40% for the whole 2 hours of SE activity. On the other hand, a progressive decrease in mitochondrial ROS was observed throughout the whole SE. These findings provide valuable insights into the dynamics of ROS generation in epilepsy and shed light on the potential contributions of mitochondria and NOX2 to the pathophysiology of epilepsy. Further research in this area may pave the way for novel therapeutic strategies targeting ROS-related mechanisms in epilepsy.

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Poster

PSTR201. Cellular Stress and Death Mechanisms

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Program #/Poster #: PSTR201.10/W2

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

Support: NIH NS045734
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GM10350

Title: Deletion of the integrated stress response kinase HRI promotes oligodendrocyte survival, attenuates inflammation and improves functional recovery after thoracic spinal cord injury

Authors: *S. SARASWAT[1], S. A. MYERS[3], B. L. BROWN[2], L. SLOMNICKI[3], G. Z. WEI[3], K. ANDRES[3], R. HOWARD[4], M. D. FORSTON[5], S. R. WHITTEMORE[6], M. HETMAN[6]; [1]Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY; [2]Kentucky Spinal Cord Injury Res. Ctr., University of Louisville, KY; [3]Kentucky Spinal Cord Injury Res. Ctr., [5]Anatom. Sci. and Neurobio., [6]Neurolog. Surgery, [4]Univ. of Louisville, Louisville, KY

Abstract: The integrated stress response (ISR) pathway plays a critical role in maintaining cellular homeostasis under physiological and pathological conditions. The mammalian ISR is mediated by several kinases that phosphorylate eIF2α (eukaryotic initiation factor 2α) and inhibit general protein synthesis. Conversely, stress response genes and/or proteins are upregulated via both transcriptional and translational mechanisms. After acute injuries, ISR may promote survival and/or initiate death of damaged cells, and also regulate injury-associated inflammation. Therefore, we took advantage of mouse genetics to determine the pathogenic contributions by

three ISR kinases, PKR/EIF2AK2, GCN2/EIF2AK4, and HRI/EIF2AK1 after moderate contusive spinal cord injury (SCI) at the T9 level. All those ISR kinases were activated 24 h after SCI. No significant differences in locomotor recovery were observed between *Pkr*$^{-/-}$ and wt mice. Moderately improved recovery was found in *Gcn2*$^{-/-}$ mice. In contrast, *Hri*$^{-/-}$ mice showed strongly improved locomotor recovery. In addition, those effects were associated with reduced ISR signaling acutely after SCI and chronic increases in white matter sparing and oligodendrocyte survival at the injury epicenter. Moreover, a remarkable reduction in neuroinflammation was observed together with decreased accumulation of lipid-loaded macrophages/microglia. In cell culture, *Hri*$^{-/-}$ microglia and/or macrophages showed impaired ISR response, reduced inflammatory potential and enhanced phagocytosis of myelin. Therefore, the HRI-mediated ISR is a major positive regulator of SCI-associated secondary injury. Enhancement of cytotoxic neuroinflammation is at least one mechanism that underlies such deleterious activity.

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Poster

PSTR201. Cellular Stress and Death Mechanisms

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Program #/Poster #: PSTR201.11/W3

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

Support: NIH Grant 5P20MD006988

Title: Docosahexaenoic Acid Inhibition of Lipotoxicity in Schwann Cells Involves Regulation of Autophagy and Fatty Acid Binding Protein 5

Authors: *F. ZAMORA, J.-W. LIU, M. L. MONTERO, M. DE LEON;
Basic Sci., Loma Linda Univ. Sch. of Med., Loma Linda, CA

Abstract: High levels of palmitic acid can elicit lipotoxicity (PA-LTx) in nerve cells, which may render the onset of chronic neuropathic pain (NP). Our previous reports have shown that PA-LTx in Schwann cells involves decreased calcium in the ER, followed by ER stress, mitochondrial depolarization, and apoptotic cell death. PA-LTx can be inhibited if immortalized Schwann cells (iSCs) are co-treated with calcium chelator BAPTA-AM, antioxidant MC-186, or docosahexaenoic acid (DHA). Further, an omega-3 fatty acid-enriched diet produces an anti-inflammatory metabolomic profile and significantly reduces NP. Because activation of autophagy has been observed during NP, the present study examines whether autophagy plays a significant role during PA-LTx in iSCs. Following our previous published methods, iSC cultures were treated with PA: BSA at 300 µM:150 µM (2:1 ratio) for 24 and 48 hours to induce LTx. The cultures were also co-treated with DHA to inhibit LTx or with Chloroquine (CQ) to inhibit

autophagic flux. Protein levels of autophagy marker LC3-II and stress-response marker fatty acid binding protein 5 (FABP5) were assessed using Western blot. Additionally, real-time qPCR measured the expression of autophagy-related genes (ATGs) and FABP5, and cell viability was measured at 48 hours using Crystal Violet. Like previous findings, PA treatment decreased cell viability, while DHA co-treatment fully protected against PA-LTx. Treatment with CQ alone did not affect cell viability, however exposure to PA+CQ exacerbated the effects of PA-LTx. Interestingly, adding CQ did not inhibit DHA's neuroprotection from PA-LTx. Assessment of autophagy revealed that PA increased LC3-II and ATG 12 expression, suggesting activation of autophagy. In addition, iSCs exposed to PA or CQ showed increased FABP5 mRNA and protein levels that were suppressed with DHA co-treatment. These findings suggest that PA-LTx in iSCs increases autophagy activity as an initial attempt to counteract and survive the injury, and its inhibition may exacerbate apoptotic cell death. Treatment with DHA inhibits PA-LTx, at least in part, by restoring a healthy autophagic flux, which is consistent with DHA's neuroprotective actions and ability to reduce neuropathic pain.

Disclosures: **F. Zamora:** None. **J. Liu:** None. **M.L. Montero:** None. **M. De Leon:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.12/W4

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

Support: Dr. Miriam and Sheldon G. Adelson Foundation

Title: Neuroprotection via HDAC inhibition: Following the FerroMap to decipher a long sought treasure

Authors: *I. CHAMBERS[1], S. S. KARUPPAGOUNDER[2], C. CORONA[2], E. RAMIREZ[3], A. SILVA[3], R. R. RATAN[2];
[1]Burke Med. Res. Inst., White Plains, NY; [2]Burke Neurolog. Inst., White Plains, NY; [3]UCLA, Los Angeles, CA

Abstract: Ferroptosis is an iron-dependent and programmed form of necrosis that is distinct from apoptosis or parthanatos implicated in myriads of neurological conditions. HDAC inhibitors have been demonstrated by our laboratory and others to protect against ferroptosis as well as enhance regeneration, inhibit tumor growth, and improve learning and memory. There robust and broad effects have led to many efforts trying to identify isoform selective inhibitors that can individually protect or repair the brain. Despite decades of intense study in our lab we have not been able to identify an HDAC inhibitor that had broad salutary effects without obvious toxicity at protective concentrations *in vitro*. This led us to embark on a novel, unbiased approach to discover interdictors of ferroptosis by producing an unbiased ResearchMap of this pathway. Our “FerroMap” therefore incorporates causal molecular data on ferroptosis to build an

integrated view of validated pathways supported by molecular manipulation in available publications *in silico*. Once this map of ferroptosis was complete it was then processed using a connectivity map (CMap) pipeline, which has generated more than 1.5 million genetic profiles of greater than 5,000 compounds and 3,000 genetic manipulations from the Broad Institute, to identify small molecule drugs whose genetic profiles suggested that they would inhibit ferroptosis. The top six hits from our analysis to interdict ferroptosis were all the same drug, Belinostat, a pan-HDAC inhibitor. Our studies to date have shown that Belinostat, which is already FDA approved and used in humans for cancer, is more potent and effective than isoform selective inhibitors in preventing ferroptotic death *in vitro.* Here we demonstrate that: Belinostat is protective against both Erastin and Hemin-induced ferroptosis in primary cortical neurons without evidence of the toxicity observed with past HDAC inhibitors; Belinostat treatment upregulates several genes including the master regulator GPX4, whose overexpression has been shown by our lab to protect neurons from ferroptosis *in vitro* and both major stroke types *in vivo;* Belinostat's effect is dependent on GPX4, with both pharmacological and molecular reduction of GPX4 abrogating Belinostat protection of neurons; Belinostat treatment *in vitro* results in a dose responsive acetylation of cytosolic and nuclear targets; finally, Belinostat *in vivo* results in enhanced objection placement recognition, indicative of improved memory.

Disclosures: I. Chambers: None. **S.S. Karuppagounder:** None. **C. Corona:** None. **E. Ramirez:** None. **A. Silva:** None. **R.R. Ratan:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.13/W5

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

Support: K08NS119882

Title: Transcriptomic meta-analysis identifies zika's oncolytic impact in glioblastoma multiforme

Authors: *S. SINGH[1], A. HORVATH[1], F. TOMASSO[1], Y. A. KOUSA[1], T. A. MANSOUR[2]; [1]Children's Natl. Med. Ctr., Washington, DC; [2]Univ. of California, Davis, CA

Abstract: Glioblastoma multiforme (GBM) is an aggressive, lethal brain cancer with few effective treatments. Toward improving survival rates in treatment of GBM, oncolytic viruses are emerging as a potential adjuvant therapy. Among neurotropic viruses, Zika virus has emerged as a promising therapeutic agent due to its ability to selectively infect and kill GBM cells. Neuroblastomas (NBMs) have also shown vulnerability to Zika infection and oncolysis, despite having a markedly distinct profile. It remains unclear if shared or distinct mechanisms mediate the impact on different tumor types. Our goal is to elucidate Zika's neuro-oncolytic mechanisms toward expanding the landscape of oncolytic therapies and improve treatment and prognosis for

patients with GBM. We began work toward this by performing a transcriptomic meta-analysis comparing the molecular signatures of Zika infection in GBM and NBM. We performed a comprehensive search through Gene Expression Omnibus (GEO) Datasets and identified three RNA sequencing experiments characterizing the effect of Zika infection on GBM and NBM. We harmonized the four datasets from these three publications to systematically evaluate their transcriptional profiles. Sequencing reads were trimmed by Trimmomatic v.39 and aligned by bowtie2 using refSeq annotation. Quantification of gene expression was done by Salmon and differential analysis was done by DeSeq2. Subsequent functional over-representation analysis and visualization were done using WebGestalt. We confirmed gene expression changes with qPCR, using six biological replicates in each of two GBM cell lines. Using NBM as an outgroup, we evaluated shared genes and molecular networks that could be mediating Zika's role in oncolysis. We identified upregulation of genes driving canonical tumor pathways, including TNF, NF-kappa B, and p53 signaling pathways, and a refined list of consistently dysregulated long non-coding RNAs (lncRNAs) that could be driving oncolytic impact. Functional review of these candidates revealed their potential regulatory role in Zika-mediated oncolysis and posited further investigation of the less-researched targets. Validation of these lncRNAs in GBM identified several targets that matched expected dysregulation after Zika infection. Altogether, our analysis suggests specificity in Zika's neuro-oncolytic effect and provides novel insights into the molecular mechanisms underlying the effect of Zika on GBM. We highlight potential therapeutic targets that could be further interrogated to improve the efficacy of tumor cell death and the utility of Zika as an adjuvant virotherapy for GBM and other related cancers.

Disclosures: S. Singh: None. **A. Horvath:** None. **F. Tomasso:** None. **Y.A. Kousa:** None. **T.A. Mansour:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.14/W6

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

Support: The Research Institute for Veterinary Science, Seoul National University
NRF-RS-2023-00208475
2021R1A5A103315713
BK21 Four Future Program for Creative Veterinary Science Research

Title: High glucose-suppressed Parkin-mediated neuronal mitophagy is ameliorated by sodium butyrate, via inhibition of the HDAC8/NF-kB complex

Authors: *J. CHO, C. CHAE, J. YOON, H. HAN;
Seoul Natl. Univesity, Seoul, Korea, Republic of

Abstract: Damaged mitochondria accumulation in diabetes is one of the early features which contribute to increased incidence of neurological disorders by inducing apoptosis. Butyrate is the major metabolite of microbiota that has protective effects in neuronal apoptosis. But, the detailed mechanisms of how butyrate can enhance mitophagy remains unclear. Here, we examined the regulatory role of sodium butyrate (NaB) on high glucose induced mitophagy dysregulation, neuronal apoptosis, and cognitive impairment using human induced pluripotent stem cell-derived neurons, SH-SY5Ys, and streptozotocin (STZ)-induced diabetic mice. In our results, STZ mice showed that gut-microbiota dysbiosis, which especially decreased butyrate-producing bacteria, as well as reduced NaB plasma concentrations. Oral administration of NaB prevented hippocampal neuronal apoptosis and improved cognitive impairment in STZ mice. Furthermore, high glucose-induced neuronal mitochondrial dysfunction and dysregulation of Parkin-mediated mitophagy subsequently accumulated abnormal mitochondria that lead to apoptosis. Meanwhile, NaB ameliorated Parkin-mediated mitophagy and prevented apoptosis by high glucose. High glucose-stimulated NF-κB and increased HDAC8 translocation to the nucleus caused the HDAC8/NF-κB complexes, that bound to the PARK promoter region and induced epigenetic repression. NaB restored Parkin expression by inhibiting the HDAC8/NF-κB complex via reducing HDAC8 nuclear translocation through AMPK activation in the cytoplasm, and direct inhibition of HDAC8 in the nucleus. Moreover, HDAC8 overexpression blocked the effect of NaB in restoring mitophagy dysfunction and neuronal apoptosis induced by high glucose. In conclusion, NaB enhances neuronal mitophagy through Parkin upregulation via inhibiting HDAC8/ NF-κB complexes, suggesting that NaB is an important substance for protecting neuronal apoptosis in diabetes-associated neurogenerative disorders.

Disclosures: **J. cho:** None. **C. Chae:** None. **J. Yoon:** None. **H. Han:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.15/W7

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

Support: NIH/NINDS R01NS115800
Iowa Neuroscience Institute

Title: Aberrant neuronal calcium dynamics as a spatiotemporal determinant of cytotoxic injury in acute brain slice preparations

Authors: *P. SURYAVANSHI, S. BAULE, J. GLYKYS;
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Abstract: The acute brain slice preparation is a widely used experimental model in neuroscience to study neuronal function at the molecular, synaptic, and cellular levels. However, preparing acute brain slices can alter neuronal structure and function and cause tissue damage, including

cell death. Specifically, trauma during brain slice preparation causes Ca^{2+} loading, which can be deleterious to neuronal health. Injured neurons with high Ca^{2+} load often exhibit abnormal nuclear accumulation of the predominantly cytosolic Ca^{2+} biosensors (e.g., genetic-encoded Ca^{2+} indicators or GCaMPs), rendering them "filled" in appearance. Thus, to minimize data acquisition from injured tissue, it is critical to understand the spatiotemporal limits of cytotoxic injury in brain slices and to identify depths and incubation times that yield optimal neuronal function. Using multiphoton imaging, we examined the relationship between neuronal viability and Ca^{2+} load at various depths and incubation times. We prepared acute brain slices from neonatal mice (postnatal days 8-12) expressing neuronal GCaMP6s under the Thy1 promoter. We incubated them with a red-shifted cell-death indicator (propidium iodide or PI) to simultaneously observe neuronal Ca^{2+} dynamics and cell death at multiple tissue depths. We found that both PI-labeled cells and GCaMP-filled neurons were most abundant in the superficial tissue (at depths less than 90 microns), with an exponential decrease in their numbers in the deeper tissue. Additionally, PI-labeled cells significantly correlate with both GCaMP-filled neurons and the Ca^{2+}-loaded neuropil. The number of PI-labeled and GCaMP-filled cells in the deeper tissue significantly increased with prolonged incubation. These results show a functional relationship between high neuronal and neuropil Ca^{2+} load and cell viability in acute brain slices at different depths. As GCaMPs and other Ca^{2+} biosensors become popular tools for measuring neuronal activity, considering aberrant neuronal Ca^{2+} dynamics in acute brain slices will help minimize injury-related variability in results.

Disclosures: P. Suryavanshi: None. **S. Baule:** None. **J. Glykys:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.16/W8

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

Support: National Science Centre Sonata Bis grant no. 2018/30/E/NZ1/00144
ESF, POWR.03.02.00-00-I028/17-00

Title: Vulnerability of hippocampal pyramidal neurons to NMDA-induced damage is associated with subregion-specific direction of arginine metabolism pathways.

Authors: *A. KACZYNSKA[1], M. BERESEWICZ-HALLER[2], A. OWCZAREK[1], M. PODGAJNA[1], K. SKOWRONSKA[3], M. ZIELINSKA[3], B. ZABLOCKA[2], M. WEGRZYNOWICZ[1];
[1]Lab. of Mol. Basis of Neurodegeneration, [2]Mol. Biol. Unit, [3]Dept. of Neurotoxicology, Mossakowski Med. Res. Inst. Polish Acad. of Sci., Warsaw, Poland

Abstract: Polyamines (PAs) are polyvalent cations involved, among others, in neurotransmission and neuroprotection. Hippocampus contains high levels of polyamines, but

their distribution and roles in this structure are not well understood. The preliminary analysis carried out in our Laboratory indicates that CA2 is characterized by a specific expression of genes related to the metabolism of arginine (Arg). In the brain, Arg can be a substrate for two mutually competing pathways: synthesis of nitric oxide (NO) or production of polyamines (PAs) (putrescine (Put), spermidine (Spmd) and spermine (Spm)). The aim of the study was to verify the hypothesis that the hippocampus is characterized by regional differences in the direction of Arg metabolism and that differential vulnerability of hippocampal pyramidal neurons to NMDA-induced damage may be dependent on endogenous synthesis of PAs. Spatial distribution of mRNAs of PAs metabolism-related genes was studied with Allen Brain Atlas. The experimental work was performed using WT mice and mouse model of loss of arginase 2 (Arg2) (Arg2 KO line), a first enzyme in PAs synthesis pathway which is responsible for conversion of Arg to Ornithine (Orn). Analysis of Arg2 distribution within the hippocampus was done by immunostaining. Levels of Arg and Orn were measured by HPLC (n=10-13 per group). The role of PAs synthesis pathway for CA2 protection from neuronal injury was studied using organotypic cultures of rat hippocampal slices exposed to NMDA (n=4). The CA2 region, known for its resistance to damage, has been identified as having a unique expression of PAs metabolism-related genes. Arg2 protein was specifically expressed in pyramidal neurons of CA2, and absent in other regions (CA1, CA3). Loss of Arg2 resulted in accumulation of Arg (increase by 75%, $p<0.001$; t-test) and reduction of Orn (loss by 12%, $p=0.025$; t-test). Inhibition of PAs synthesis in organotypic slice model of excitotoxicity was found to induce injury of, otherwise protected, CA2 pyramidal neurons. My data suggest a distinct PAs metabolism in hippocampal subregions, and, in combination with a detailed analysis of available sources (in situ data from Allen Brain Atlas) clearly point at prominent enhancement of PAs synthesis in CA2. The results of my in vitro experiments suggest significant involvement of PAs synthesis pathway in local resistance to excitotoxicity, one of well established, but still unexplained, phenotypes of pyramidal neurons of CA2.

Disclosures: **A. Kaczynska:** None. **M. Beresewicz-Haller:** None. **A. Owczarek:** None. **M. Podgajna:** None. **K. Skowronska:** None. **M. Zielinska:** None. **B. Zablocka:** None. **M. Wegrzynowicz:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.17/W9

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

Support: R01 NS102196
NIH R56 NS124707-01A1
Kentucky Spinal Cord and Head Injury Trust grant 22-1A

Title: Rit2 is an important mediator of MAP kinase signaling in TLR7/9 induced cortical neurodegeneration

Authors: *M. PANDEY[1], C. L. MONCMAN[1], W. LEI[2], D. A. ANDRES[3];
[1]Mol. and Cell. Biochem., Univ. of Kentucky Col. of Med., Lexington, KY; [2]Dept. Biochem. and Mol. Biol., Univ. of Florida Col. of Med., Gainesville, FL; [3]Col. of Med., Lexington, KY

Abstract: Neuronal apoptosis is a critical aspect of many neurological diseases, and a deeper understanding the molecular mechanisms involved is key to developing potential therapeutic treatments. Recent studies have shown that stimulation of endosomal Toll-like receptors (TLRs) is novel innate immune-mediated pathway resulting in neuronal death. TLRs are stimulated both by pathogen-associated and damage-associated molecular patterns, including microRNAs and mitochondrial DNA released from damaged neurons. Here we demonstrate that endosomal TLR7/9 activation results in acute activation of both ERK and JNK MAPK cascades in cortical neurons. Importantly, only pharmacological JNK inhibition, but not ERK blockade, was found to protect cortical neurons from TLR7/9-dependent neurodegeneration. As Ras-like GTPases are critical upstream regulators of MAPK cascades, we reasoned that a small GTPase was involved in TLR7/9-mediated degenerative signaling. Using a RNAi-GTPase gene silencing screen in primary cortical neurons, we identified RIT2 (Rin, Ras-like in neurons), a small GTPase expressed in neural tissue, as crucial for TLR7/9-mediated JNK activation, axonal degeneration and neuronal death in vitro. To control for potential off-target RNAi effects, we generated a transgenic RIT2 knockout mouse model and demonstrate that RIT2 is required both for TLR7/9-dependent JNK activation and in vivo axonal degeneration. Genome wide association studies have identified the locus containing the RIT2 gene with an increased risk for Parkinson's disease (PD), as well as other psychiatric disorders, and additional in vitro studies have implicated RIT2 in control of neurite outgrowth, calcium signaling, and dopamine transporter trafficking. Our exciting data highlight the importance of RIT2 as a key mediator of TLR-dependent neuronal MAPK signaling and suggest that further investigation if RIT2-JNK signaling is warranted.

Disclosures: M. Pandey: None. **C.L. Moncman:** None. **W. Lei:** None. **D.A. Andres:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.18/W10

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

Support: UNAM-DGAPA-PAPIIT 208119
UNAM-DGAPA-PAPIIT 212522
CONACYT 251510

Title: Effects of chronic exposure to Atrazine in Female Rats: Exploring the Role of Estrogens Neuroprotection.

Authors: *V. PASTOR[1], G. L. SEVILLA[1], J. YÉPEZ[2], S. MENDOZA-TREJO[2], W. PORTILLO[3], R. CORONA[2], I. HERNÁNDEZ[4], V. RODRIGUEZ CORDOVA[5];

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Abstract: Chronic exposure to the herbicide atrazine (ATR) has been associated with alterations in locomotor activity and dopaminergic markers in male rats. However, recent studies have revealed a sex-dependent effect, with female rats exhibiting less impairment in the dopaminergic system. The principal aim of this study was to investigate the potential role of estrogens in the observed neuroprotection in female rats exposed to ATR. Rats were divided into four groups: ovariectomized and non-ovariectomized rats exposed to 10 mg ATR/kg body weight or control feeding. The rats were exposed daily to ATR for 13 months, during which spontaneous locomotor activity was assessed monthly for 12 months. At the end of the 13 months, anxiety levels, spatial memory, motor coordination, and olfactory discrimination were evaluated. The results revealed that control ovariectomized rats displayed motor coordination impairments and reduced locomotor activity. Notably, ovariectomized rats exposed to ATR also showed a decline in motor coordination, although to a lesser extent than the control ovariectomized group. In addition, ovariectomized groups showed variations in olfactory discrimination toward social stimuli. However, no significant changes were observed in anxiety levels or spatial memory among groups. These findings suggest that the observed sex-dependent effect cannot be only attributed to neuroprotection mediated by estrogens. However, further investigations are necessary to understand the underlying mechanisms involved in these observations fully.

Disclosures: V. Pastor: None. **G.L. Sevilla:** None. **J. Yépez:** None. **S. Mendoza-Trejo:** None. **W. Portillo:** None. **R. Corona:** None. **I. Hernández:** None. **V. Rodriguez Cordova:** None.

Poster

PSTR201. Cellular Stress and Death Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR201.19/W11

Topic: F.04. Neuroimmunology

Support: K00NS120365
R01NS119178
NMSS RFA-2203-39228
K22AI125566

Title: Novel alternative splicing in PSMB8 and its potential role in multiple sclerosis

Authors: *B. C. SHAW[1], J. L. WILLIAMS[2];
[1]Neurosciences, Cleveland Clin., Northfield, OH; [2]Neurosciences, Cleveland Clin., Cleveland, OH

Abstract: Multiple sclerosis (MS) is a chronic demyelinating autoimmune disorder of the central nervous system (CNS). In its initial stages, MS is characterized by peripheral immune cell invasion of the CNS resulting in lesions of degraded myelin and neuroaxonal loss. As patients age, however, these peripheral immune cells are no longer present in the CNS yet lesions continue to grow indicating a role for the resident CNS cells during progressive MS. Astrocytes are the most abundant glial cell in the central nervous system (CNS) and are highly responsive to immune stimuli. Upon stimulation with interferon gamma (IFN-γ), astrocytes undergo significant transcriptional changes. The most highly upregulated pathway in astrocytes after IFN-γ stimulation is antigen presentation. This pathway includes genes which compose the immunoproteasome, a large protein complex which degrades polyubiquitinated proteins into small peptide fragments. Previous work in our laboratory has shown that expression of the immunoproteasome is increased in white matter lesions in MS patients, and inhibition of the immunoproteasome exacerbates an animal model of MS, experimental autoimmune encephalomyelitis (EAE). Variants in the immunoproteasome gene *PSMB8* are also associated with increased risk of developing MS. In this study, we showed that *PSMB8* undergoes alternative splicing specifically in white matter lesions in MS. We identified a novel alternative splicing isoform in which the second intron is retained, resulting in a frameshift and predicted to undergo nonsense-mediated RNA decay. This novel isoform is significantly upregulated in white matter lesions. *In vitro*, we demonstrate that this frameshift leads to increased cellular stress through the formation of excess processing bodies (P-bodies). Overall, this indicates a potential role for alternative splicing during progressive MS, as genes critical to cellular function may be splicing poorly resulting in decreased functional proteins.

Disclosures: **B.C. Shaw:** None. **J.L. Williams:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.01/W12

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

Title: Effects of some heavy metals on brain ultrastructure and function in animal model

Authors: *T. BIKASHVILI, L. GELAZONIA, M. MIKADZE;
I.Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Experimental animal models are important for study pathogenesis of any human disease. The present study demonstrates the effect of some heavy metals on brain ultrastructure and function in rats caused by direct exposure to this metal and via their parents. Our experiments reveal that 68 mg/L and 136 mg/L Sodium (meta) arsenite in drinking water causes changes in offsprings, specifically the locomotion activity was significantly reduced and they have a tendency to depression, also revealed reduced learning ability compared to control ones. Our morphological and ultrastructural analyses revealed that astrocytes are more susceptible to

this metalloid than neurons. While the number and ultrastructure of neurons was slightly altered, astrocytes expressed notable ultrastructural changes in the prefrontal cortex and hippocampus. Pediatric lead poisoning has deleterious effects on the development of widespread brain areas that are involved in cognitive, communication, and social functioning. Two different doses of Lead acetate in drinking water (0.05%; 0.2%) were used in our experiments. Behavioral studies were performed using Social and Anxiety-related behavioral tests, which can reveal the core symptoms of autistic behavior. Studies have shown that prenatal exposure to even relatively low levels of lead results in lifelong reductions of intellectual functions and behavioral disorders and it has been suggested that lead poisoning could be a possible risk factor for ASDs. Trace amounts of manganese (Mn) are essential for good health, but overexposure to this element has been associated with neurotoxicity. Intoxication with manganese compounds ($MnCl_2$ - 20mg/ml) has a significant impact on the aggressive behavior and emotional state of animals. Decreased locomotor activity is observed in female rats. Disorders in the learning process are more pronounced in male individuals. Accumulation of manganese ions from areas of the brain is particularly pronounced in the hippocampus and cerebral cortex. Changes in the number of neurons from the hippocampal region were observed in the CA3 field and the dentate gyrus. Pyknotic nucleus, plasma membrane disruption and cytoplasmic vacuoles were observed in swollen neurons and they were surrounded by activated gliocytes. It's worth to mention that in the cortex the majority of damaged neurons were apoptotic while in subcortical nuclei –neurons were mainly necrotic. Ultrastructural analyses demonstrated that all cell types in cortex and nucleus caudatus represent destructed mitochondria, widened neurons' vacuolar system profiles, increased number of lysosomes and degeneration of axonal endings.

Disclosures: T. Bikashvili: None. **L. Gelazonia:** None. **M. Mikadze:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.02/W13

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

Support: KEITI 2021003310003

Title: Glutaraldehyde exposure-induced developmental neurotoxicity in neuron/astrocyte co-cultured cells and zebrafish

Authors: *H.-N. OH, W.-K. KIM;
Korea Inst. of Toxicology, Daejeon, Korea, Republic of

Abstract: Glutaraldehyde (GA) is a biocide used as a disinfectant, preservative, and fixative in various industries. The development toxicity, genotoxicity, carcinogenicity, and acute or chronic toxic effects of glutaraldehyde (GA) are well-reported but its effects on developmental neurotoxicity (DNT) have not been examined. To investigate the DNT of GA, we used SH-

SY5Y human neuroblastoma cells and human astrocytes co-culture model, and zebrafish. Cell Counting Kit-8, lactate dehydrogenase assay, and high-content screening revealed that GA reduced neurite outgrowth at non-cytotoxic concentrations. Relative quantitative real-time PCR results showed that GA downregulated the expression of the neurodevelopmental genes, and upregulated the expression of the astrocyte markers. As an *in vivo* study, a zebrafish embryo toxicity test showed that GA adversely affected the early development of zebrafish embryos, resulting in reduced survival, irregular hatching, and reduced heart rate. Furthermore, the width of the brain and spinal cord was decreased, and the myelination of Schwann cells and oligodendrocytes was reduced by GA in transgenic zebrafish lines. These findings provide evidence of GA-induced DNT *in vitro* and *in vivo* and highlight the need for caution regarding the potential neurotoxicity of GA.

Disclosures: H. Oh: None. **W. Kim:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.03/Web Only

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

Title: Dose-dependent effect of polyphenols on Drosophila climbing ability, motor activity, and lifespan after diffuse nervous system injury

Authors: *M. M. MENDOZA, T. A. TOGASHI, E. GIANG, R. HARTMAN;
Dept. of Psychology, Loma Linda Univ., Loma Linda, CA

Abstract: Polyphenols such as ellagic acid are secondary metabolites that protect plants against environmental insults (e.g., ultraviolet light, extreme temperatures, and predators). Consumption of polyphenols may confer health benefits, including neuroprotection, due to their antioxidant and anti-inflammatory properties. Nevertheless, they may also have hormetic properties, such that, polyphenols may elicit health benefits in low-to-moderate doses, but may become detrimental at higher doses. Our lab has demonstrated the neuroprotective effects of polyphenols several in human, rodent, and fly studies, but we have yet to systematically determine minimal, optimal, and maximum dosages. The present study investigates the impact of different doses of dietary ellagic acid (EA) on a high-throughput *Drosophila* model of diffuse nervous system injury. *Drosophila* diets were prepared with water (control) or water containing EA in concentrations mimicking levels found in graded dilutions of pomegranate juice (10%, 50%, 100% [full strength], 150%, and 200%). Half of the flies were injured using the High Impact Trauma (HIT) device, and the other half (shams) were uninjured. Compared to females, males exhibited more climbing and locomotive behaviors, but shorter lifespan. Injury reduced climbing behavior, locomotor movement, and lifespan in both sexes. In sham flies, low, but not high, doses of EA led to increased climbing behavior and lifespan, and the group that received the lowest dose of EA demonstrated the highest climbing performance. For injured flies of either

sex, all EA doses attenuated the reduction of lifespan but had no significant effect on climbing or overall motor activity. These findings highlight the importance of determining the optimal dose in all future experimental explorations of polyphenol efficacy. Although we demonstrated that all of the EA doses used in this study reduced the deleterious effects of injury on lifespan, lower doses were generally more beneficial for the sham flies. Therefore, "more is better" may not be the best approach to dietary treatments. These results also suggest that other sources of environmental stress may interact with the efficacy of polyphenol prophylactic treatments. Future research is planned to determine the biochemical pathways activated and/or inhibited at low and high doses of EA.

Disclosures: M.M. Mendoza: None. **T.A. Togashi:** None. **E. Giang:** None. **R. Hartman:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.04/W14

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

Support: NIH Grant ES030742

Title: Quality of maternal care and environmental enrichment influence cognitive and behavioral effects of developmental lead exposure in rats

Authors: *J. S. SCHNEIDER[1], C. WILLIAMS[1], S. ZAFAR[1], J. JOO[2], B. E. HIMES[2]; [1]Pathology and Genomic Med., Thomas Jefferson Univ., Philadelphia, PA; [2]Biostatistics, Epidemiology and Informatics and Ctr. of Excellence in Envrn. Toxicology, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Developmental lead (Pb) exposure results in sex-related behavioral/cognitive impairments that can persist into adulthood, in humans and in animal models. While all children are potentially at risk from environmental exposure to Pb, members of low socioeconomic status (SES) communities are at higher risk of exposure and have potentially more severe adverse outcomes from these exposures compared to higher SES cohorts. Children from low SES families, in addition to having a higher risk for Pb exposure, are also prone to living in less stimulating environments and have more negative or low quality interactions with caregivers. The present study was performed to investigate the extent to which environmental and behavioral factors (quality of maternal care and richness of the postnatal environment) may potentially interact to modify adverse effects from developmental Pb exposure. Long Evans females were randomly assigned to Control (no Pb exposure), Early Postnatal (EPN: birth through weaning) Pb exposure or Perinatal (PERI: 14 days pre-mating through weaning) Pb exposure groups. From postnatal days (PND) 2-9, maternal care behaviors of dams were observed and dams were classified as either low or high maternal care based on amounts of

licking/grooming and arched back nursing behaviors. At weaning, pups were randomly assigned to enriched (6 animals per large enclosure containing a variety of toys, running wheels, climbing and nesting materials, and tunnels that were changed three times per week) or non-enriched (standard housing, 3 animals per cage) environments. At approximately PND 55, animals began trace fear conditioning and associative memory was tested on days 1, 2, and 10 post conditioning. In Control (non-Pb-exposed) males and females, there were no significant effects from maternal care or enrichment on task performance. EPN females and PERI males in the LMC-non-enriched groups had significant memory impairments at day 10 testing that were not observed in HMC-non-enriched males or females. In all instances, enriched animals had no deficits, regardless of maternal care status. In a separate study, we assessed the influence of the same environmental and behavioral factors on male aggression as measured in a resident intruder paradigm. In this test, there were no significant influences of maternal care status or enrichment in PERI males. However, EPN LMC-non-enriched males showed significantly more aggressive behavior compared to HMC-non-enriched males and enriched males regardless of maternal care status. These results show important modulatory influences of maternal care and housing environment on Pb-induced cognitive/behavioral dysfunction.

Disclosures: **J.S. Schneider:** None. **C. Williams:** None. **S. Zafar:** None. **J. Joo:** None. **B.E. Himes:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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Program #/Poster #: PSTR202.05/W15

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

Support: JSPS Grant-in-Aid for Scientific Research (19H01152 and 23H00522 to M.K., F.M.)

Title: Developmental exposure to 2-chloro-3,7,8-tribromodibenzofuran impaired neurobehavioral development in mice

Authors: ***F. MAEKAWA**[1], E. KIMURA[1], G. SUZUKI[1], N. URAMARU[2], M. KAKEYAMA[3]; [1]Natl. Inst. for Envrn. Studies, Tsukuba, Japan; [2]Nihon Pharmaceut. Univ., Saitama, Japan; [3]Lab. for Systems Neurosci. & Preventive Med., Waseda Univ. Fac. of Human Sci., Tokorozawa, Japan

Abstract: It has been reported that developmental exposure to harmful chemicals affects the development of cognitive function. In mouse models of neurodevelopmental disorders, it has been reported that ultrasonic vocalization (USV), an indicator of social communication exhibited by pups to their mothers, is abnormal, and our previous studies evaluating the effects of dioxin exposure suggest that USV may be useful in assessing the developmental neurotoxicity of toxic chemicals. Our previous studies on the exposure effects of dioxins suggest that they may be

useful in assessing the developmental neurotoxicity of toxic chemicals. In the present study, we examined the effects of fetal and lactational exposure to 2-chloro-3,7,8-tribromodibenzofuran (TeXDF) or 1,2,3,7,8-pentabromodibenzofuran (PeBDF) on the USV. USV of pups born to dams exposed to TeXDF (8 or 40 µg/kg body weight) on gestational day 12.5 showed that the duration and frequency of USV vocalizations were significantly lower in the 40 µg/kg body weight exposure group than in the control group on days 3-9 postnatal period. By contrast, USV of pups born to mothers who received PeBDF (35 or 175 µg/kg body weight) were not affected. To examine whether developmental exposure leads to behavioral deficits in adults, we analyzed exploratory behavior in a novel environment using the IntelliCage, a fully automated testing device, and found no significant differences in either TeXDF or PeBDF. Other behavioral impact metrics that can be analyzed with the IntelliCage are currently under investigation.

Disclosures: **F. Maekawa:** None. **E. Kimura:** None. **G. Suzuki:** None. **N. Uramaru:** None. **M. Kakeyama:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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

Support: JSPS KAKENHI Grant 20K07971

Title: Microglial changes of the delayed carbon monoxide encephalopathy rat model

Authors: *S. OCHI[1], T. NISHIHARA[1], S. BOKU[2], J. IGA[1], S.-I. UENO[1];
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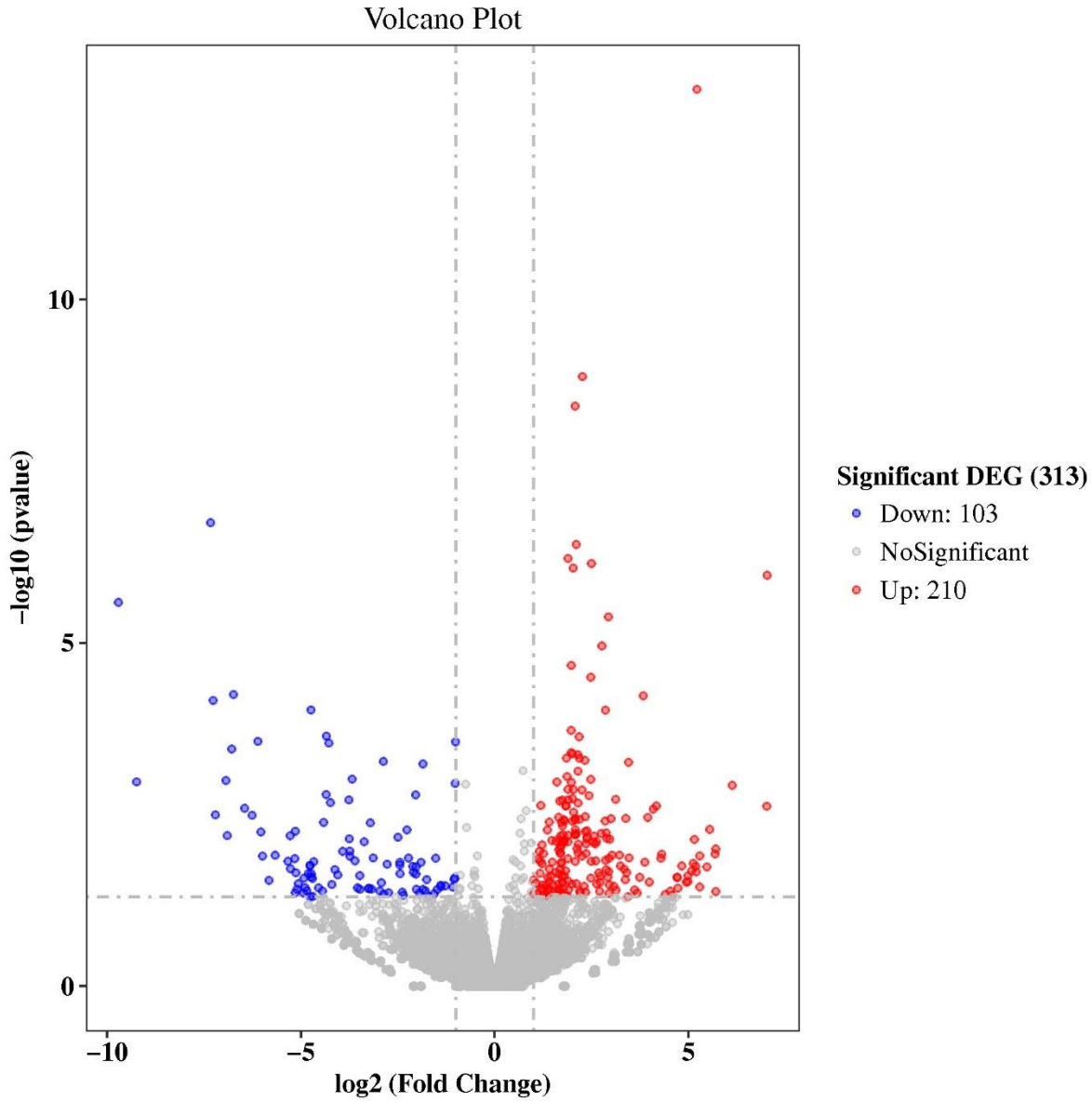
Abstract: Delayed carbon monoxide (CO) encephalopathy occurs following recovery after several weeks from acute CO poisoning. However, the mechanism of delayed neuronal injury remains unknown. Previously, we reported that the rat model of delayed CO encephalopathy showed cognitive impairment and hippocampal cell death, especially in the lesions of dentate gyrus (DG). Furthermore, delayed CO encephalopathy caused the impairment of neural precursor cells and also the number of microglial cells and the mRNA expressions of several neurotrophic factors in the hippocampus were decreased. Microglia is known to play important roles in the adult neurogenesis and promoting neurotropic factors. Therefore, in the current study, we hypothesized that changes of microglia in the hippocampus may be affected in delayed CO encephalopathy and investigated the RNA-seq of microglia in delayed neuronal CO poisoning. Wistar male rats (6 weeks old) were exposed to 1000 ppm CO for 40 min and then 3000 ppm for 20 min until they lost consciousness. If they did not lose consciousness in this 60 min, rats were exposed to 10000 ppm until they lost consciousness. Behavioral effects on learning and memory function were measured by the passive-avoidance test in controls and CO

treated rats until 3 weeks. The latencies in the CO models were significantly shorter than control group. We used magnetic cell sorting to obtain microglial cells from the hippocampus with the CD11b/c-microbeads antibody, and microglial RNA was analyzed using RNA-seq between CO group (n =3) and control group (n= 3). RNA-seq analyses revealed that those RNAs, 210 significantly up and 103 significantly downregulated RNAs were found in the CO group compared with the control group (Figure). These results suggested that delayed CO injury might damage microglia and change the mRNA expressions of microglia, thus, the impairment of functions of microglia may have important role in the pathogenesis of delayed CO encephalopathy.



Disclosures: **S. Ochi:** None. **T. Nishihara:** None. **S. Boku:** None. **J. Iga:** None. **S. Ueno:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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Program #/Poster #: PSTR202.07/Web Only

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

Support: CONAHCyT 152842
CONAHCyT 251510
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CONAHCyT 164300

Title: Lifetime glyphosate exposure disrupts motor behavior, dopaminergic markers, and sexual hormones in C57BL/6 male mice

Authors: *I. HERNÁNDEZ, W. PORTILLO, M. MENDOZA-TREJO, F. CAMACHO, M. GIORDANO, V. RODRÍGUEZ CÓRDOVA;
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Abstract: Glyphosate (Glyph) is the active ingredient of several herbicides used worldwide. Studies in animal models have shown the neurotoxic effects of Glyph. However, its long-term effects are currently unknown. In this study, our research group determined Glyph prenatal or postnatal impact on motor behavior, dopamine, and sex hormones, in male mice. The following groups were obtained. 1) Gestation: pregnant C57BL/6 mice drank 50 mg Glyph/L in distilled water (DW) only during gestation, and at weaning, their male pups drank DW. 2) Gestation-lactation-adulthood: pregnant dams were exposed to 50 mg/L in DW during gestation and lactation stages, and male pups continued treatment for 13 months. 3) Weaning-adulthood: male mice drank 50 mg Glyph/L after weaning until they were 13 months old. 4) The control group drank DW. Locomotor activity was recorded monthly, and when mice were 13 months old, we evaluated sexual behavior, tissular levels of dopamine, its metabolites and tyrosine hydroxylase enzyme (TH), testosterone, and estradiol concentrations in serum. We found alterations in the locomotor activity in the group exposed during gestation and adult stages, while alterations in motor coordination were present in all Glyph-treated groups. Dopamine levels and metabolites were not modified in any group; however, TH striatal levels were increased in the gestation group, while TH levels in the nucleus accumbens were reduced in the group exposed during gestation-lactation and adult stages. Male mice in all experimental groups did not show alterations in sexual behavior; however, estradiol and testosterone levels increased in the group exposed during gestation-lactation and adult stages. These results indicate that lifetime exposure to Glyph produces specific disruptions in locomotor, neurochemical and hormonal markers in male mice.

Disclosures: I. Hernández: None. **W. Portillo:** None. **M. Mendoza-Trejo:** None. **F. Camacho:** None. **M. Giordano:** None. **V. Rodríguez Córdova:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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Program #/Poster #: PSTR202.08/W17

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

Support: Memorial University
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Beatrice Hunter Cancer Research Institute

Title: Chemotherapy drug 5-Fluorouracil induces functional and structural plasticity at hippocampal synapses: Insight into the mechanism of Chemobrain

Authors: *D. RIWA, C. MORDEN, M. SHAHWAN, M. HIRASAWA;
Mem. Univ., St. John's, NL, Canada

Abstract: Introduction: Chemotherapy-induced cognitive impairment, known as chemobrain, is a common side effect of cancer treatment. Chemobrain imputes impaired memory, learning, and attention that can persist for years after cessation of chemotherapy, thus adversely affecting the quality of life of cancer patients and survivors. 5-fluorouracil (5FU) is a common component of first-line chemotherapy for many cancers and is known to induce chemobrain. This study investigated the effects of 5FU on the structure and function of hippocampal synapses essential for cognitive functions.
Methods: 8-10 weeks old male C57BL/6 mice were intraperitoneally (i.p.) injected with 5FU (60 mg/kg) or saline (control). Acute effects of 5FU were determined in mice that received a single injection and assessed 1 or 7 days later. To determine cumulative effects, mice received four weekly injections mimicking clinical chemotherapy regimen, and assessed 7 or 28 days after the last injection. Field EPSP was recorded ex-vivo at the hippocampal CA3-CA1 synapse to assess the effect of 5FU on synaptic function. Hippocampal CA1 dendritic spines were assessed by Golgi-Cox staining to determine 5FU induced morphological changes. To determine whether 5FU has a direct effect on the brain, field EPSP was recorded from hippocampal slices from naïve mice incubated in 10 µM of 5FU (1-3h) or left untreated.
Results: At 1 day after a single dose of 5FU, basal fEPSP and long-term potentiation (LTP) were impaired with no change in paired pulse ratio (PPR) or fiber volley, suggesting postsynaptic changes. These 5FU effects reversed within 7 days. Repeated 5FU treatment induced similar synaptic impairment, however, the effects lasted at least 28 days. Additionally, incubation of slices in 5FU resulted in impaired LTP while reducing PPR, suggesting a direct action of 5FU on the brain. After a single or repeated 5FU i.p. treatment, when functional synaptic deficits were observed, dendritic spine density of CA1 pyramidal neurons was decreased. This was more prominent in mushroom spines, suggesting a selective loss of mature spines.
Conclusion: Our results suggest that 5FU directly impairs hippocampal synaptic function in a postsynaptic dependent manner. A single dose induces an acute, reversible impairment, while multiple doses result in long-lasting deficits. Despite the difference in time course, both treatment paradigms resulted in mature spine loss, suggesting a decrease in the number of functional synapses that could partly explain the observed impairment in synaptic function.

Understanding the cellular mechanisms of chemobrain may lead to evidence-based therapeutic strategies against chemobrain.

Disclosures: **D. Riwa:** None. **C. Morden:** None. **M. Shahwan:** None. **M. Hirasawa:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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

Support: NCCIH 1R21AT009734
James and Esther King Florida Biomedical Research Program 9JK10
Bankhead-Coley Florida Biomedical Research Program 21b07

Title: Chemotherapeutic agents doxorubicin and cyclophosphamide impair choline acetyltransferase activity in the frontal cortex and striatum of female breast tumor bearing MMTV-PyVT mice

Authors: R. BOTELHO, B. JOHNS, *R. PHILPOT;
Univ. of South Florida, Tampa, Fl, FL

Abstract: "Chemobrain" encompasses cognitive impairments associated with chemotherapy in cancer patients, particularly prevalent in women receiving breast cancer treatment. Chemotherapeutic agents can suppress ovarian function, indirectly disrupting cholinergic-mediated cognitive processes due to estradiol's role in regulating high affinity choline uptake (HACU) - crucial for acetylcholine (ACh) synthesis. This impairment can be exacerbated by tumor and chemotherapy-induced neuroinflammation, which would normally be mitigated by circulating estrogen and the cholinergic anti-inflammatory pathway.
Our previous research shows that cyclophosphamide and doxorubicin (CYP+DOX) significantly impair HACU in the frontal cortex (fCTX), striatum (STR), and hippocampus (HCC) in both non-tumor and tumor-bearing MMTV-PyVT female mice. This leads to an increase in pro-inflammatory cytokines and spatial memory deficits.
Furthering this investigation, we administered one CYP+DOX injection or four weekly injections to 32 tumor-bearing and matched non-tumor bearing MMTV-PyVT female mice. Control mice received equal volumes of saline. Three or ten days post-injection, we dissected the fCTX, STR, and HCC to measure choline acetyl transferase (ChAT) activity.
We observed a significant ChAT activity reduction in the fCTX and STR of tumor-bearing mice after one CYP+DOX injection, aligning with our previous HACU impairment findings. This implies a loss of cholinergic function and potential cholinergic neuron loss, thus further illuminating chemobrain's cholinergic underpinnings. Interestingly, these effects were absent in non-tumor bearing mice, suggesting that tumor presence may heighten chemotherapy's adverse impact.

Dietary and pharmacological interventions targeting cholinergic function may alleviate some chemobrain-associated cognitive deficits. Implementing a 2% choline diet prior to CYP+DOX treatment could maintain cholinergic function, prevent spatial memory deficits, and reduce tumor volume and inflammation. Similarly, administering M1 muscarinic receptor agonist xanomeline or M1 positive allosteric modulator VU-357017 can prevent CYP+DOX-induced spatial memory impairment without affecting the antineoplastic efficacy of chemotherapy. These promising interventions necessitate further research for understanding precise mechanisms and optimal parameters.
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Poster

PSTR202. Mechanisms of Neurotoxicity II

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

Support: Cancer Research Society
Medical Research Fund
CIHR- Canada Graduate Scholarship

Title: Chemotherapeutic drug has opposing effects on the intrinsic excitability of hypothalamic orexin and MCH neurons

Authors: *S. E. C. CAMPBELL, M. S. CHOWDHURY, L. Z. FANG, M. HIRASAWA; Div. of Biomed. Sci., Mem. Univ., St. John's, NL, Canada

Abstract: Introduction: Chemotherapy (chemo) remains one of the most common forms of cancer treatment. 5-fluorouracil (5-FU) is a cytotoxic agent used as part of the first-line chemo regimen to treat many cancers. However, 5-FU also has neurotoxic side effects, with chemo-induced fatigue (CIF) being amongst the most reported symptoms. Lateral hypothalamic (LH) Orexin (ORX) neurons have recently been implicated in symptoms of CIF, as these neurons promote arousal and energetic states, while their inhibition results in fatigue. In comparison, another adjacent cell population in the LH, Melanin-concentrating hormone (MCH) neurons, promote sleep when activated. Given the opposing roles of these cells in behavioral states, we hypothesized that 5-FU inhibits ORX neurons and activates MCH neurons, both of which would contribute to symptoms of CIF. **Methods:** To test this, *ex vivo* patch clamp electrophysiology was used to record the activity of ORX and MCH neurons from adult male C57BL/6 mice (6-10 weeks) who had received a single intraperitoneal injection of 5-FU (60 mg/kg) or saline (control). 5-FU is known to cross the blood-brain barrier, hence, the acute effect of 5-FU on ORX and MCH neuron activity were assessed by incubating brain slices for 2.5h in 5-FU (10

μM). **Results:** Our results showed that treatment of 5-FU inhibited the intrinsic excitability of ORX neurons both *in vivo* and *in vitro*. Specifically, the resting membrane potential (RMP) was hyperpolarized, latency to action potential firing was longer in response to positive current injections, and the firing rate was decreased compared to respective controls. Interestingly, while MCH neurons are localized to the same brain region as ORX neurons, *in vitro* application of 5-FU depolarized the RMP, decreased the latency to fire, and increased action potential firing rates in MCH neurons. The mechanisms of these cell-specific results are currently under investigation. **Conclusion:** This study is the first to explore the effect of chemo on electrophysiological properties of hypothalamic sleep/wake neurons, which may underlie the symptoms of CIF. These results will provide information on therapeutic targets for the side effects of cancer therapies that could aid in relieving suffering for millions of cancer survivors worldwide.

Disclosures: S.E.C. Campbell: None. **M.S. Chowdhury:** None. **L.Z. Fang:** None. **M. Hirasawa:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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Program #/Poster #: PSTR202.11/W20

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

Support: Minciencias (Colombia) Grant 11180763133

Title: Trpv4 role in neurotoxicity induced by paclitaxel and oxygen-glucose deprivation

Authors: *J. C. SÁNCHEZ, J. GUERRERO, L. F. MARTÍNEZ, L. V. MUÑOZ, A. VALENCIA-VÁSQUEZ, A. M. GARCÍA;
Fac. of Hlth. Sci., Univ. Tecnológica de Pereira, Pereira, Colombia

Abstract: Calcium homeostasis is an essential process to maintain cell viability and homeostasis. Transient receptor potential vanilloid 4 channels (TRPV4) are calcium channels that respond to diverse stimuli and are involved in several signaling pathways associated with cell survival and adaptability in noxious conditions. Here, we aimed to evaluate the effect of an oxygen-glucose deprivation protocol (OGD) and paclitaxel (PTX) exposure on cell survival and TRPV4 function in SH SY5Y cells. Cell viability assessment was assessed in the presence of PTX (1 μM) for 6, 12, 24 and 48 hours. PTX exposure produced a significant reduction in cell viability in a time-dependent manner. Exposure to GSK1016790A (GSK), a specific TRPV4 agonist, significantly decreased cell survival in the presence of PTX at 6, 12 and 24 hours. The cell survival in the presence of HC-067047 (HC), a specific TRPV4 antagonist, was not affected. However, this TRPV4 antagonist increased the cell survival at all the PTX exposure times. A significant reduction in cell viability was observed following 2-hours of OGD. After 4-hours exposition to the OGD conditions, there was no cell viability. Treatment with GSK decreased cell viability even more than OGD alone, while HC addition prevented the effect of OGD. After

the PTX treatment for 6 hours, the TRPV4 mRNA levels were significantly increased by 1.86-fold. Under the OGD conditions, TRPV4 expression was significantly increased by 2.31-fold. Using the whole-cell patch clamp technique, stimulation with GSK elicited a predominantly inward current. These currents were decreased by HC. Both PTX and OGD insult elicited an increase in the magnitude of current density. GSK significantly increased $[Ca^{2+}]_i$, measured by spectrophotometry, and this increase was inhibited by HC. When PTX was added before the addition of GSK, the Ca^{2+} response increased. HC inhibited this effect. A similar effect was observed in the OGD-exposed cells. In summary, both OGD and PTX decreased cell survival in a time-dependent manner and increased TRPV4 expression, activity, and TRPV4-dependent calcium influx. Adding TRPV4 agonists promoted cell viability decrease induced by PTX and OGD. The addition of TRPV4 antagonists diminished the deleterious effect of both PTX and OGD, which confirms the role of TRPV4 in cell death induced by these two conditions. These results contribute to a better understanding of the role of TRPV4 in hypoxic injury and chemotherapy-induced neurotoxicity. TRPV4 represents a promising therapeutic target for neuroprotection deserving of further preclinical research.

Disclosures: **J.C. Sánchez:** None. **J. Guerrero:** None. **L.F. Martínez:** None. **L.V. Muñoz:** None. **A. Valencia-Vásquez:** None. **A.M. García:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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Brain & Behavior Research Foundation Young Investigator grant
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NIGMS COBRE II
RCMI8G12MD00760
NSF PRCEN fellowship
Neuro-ID 2R25NS080687 fellowship
UPR Med Sci Campus Chancellor's Office and School of Medicine Deanship.

Title: Effects of the herbicide glyphosate on cellular activity in the insula

Authors: *T. A. SALCEDO[1], *T. A. SALCEDO[3], A. FIGUEROA[4], M. CASERES[2], O. MARTINEZ[2], D. SIERRA[2];
[1]Univ. of Puerto Rico, Bayamon, PR; [2]Univ. of Puerto Rico, Medical Campus, PR; [3]Univ. of Puerto Rico Bayamon campus, Bayamon, PR; [4]Univ. of Puerto Rico Bayamon campus, Rio Piedras, PR

Abstract: Glyphosate, the active ingredient in various commercial herbicides, is considered a contaminant that can be present in food and water. Our group aims to investigate the effects of prolonged exposure to a daily dose of 2.0 mg/kg of glyphosate, which was deemed safe by the Environmental Protection Agency (EPA). The findings of our laboratory suggest that EPA reference dose leads to an increase in anxiety-like behaviors. We examined the cellular activity in specific subregions of the insula, a brain area involved in anxiety modulation. To accomplish this, rats were exposed to glyphosate for 16 weeks. Next, rats were sacrificed, and brain tissue containing the insula subregions—agranular insula dorsal and ventral (AID/AIV), granular insula (GID), and disgranular insula (DI)—was extracted. C-Fos immunohistochemistry was conducted on the brain slices to assess cellular activity. Preliminary results indicate that glyphosate does not lead to a significantly decrease in the cellular activity in the insula ($p=0.6646$). However, there is a tendency for decreased cellular activity in the AID region ($p=0.0649$). These findings contribute to the understanding of the potential neurotoxic effects associated with glyphosate consumption. Further research directions include dissociating between the caudal insula regions, known for their anxiolytic roles, as well as the rostral (frontal) regions, known for their anxiogenic roles (Mendez-Ruette et al., 2019).

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Poster

PSTR202. Mechanisms of Neurotoxicity II

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Program #/Poster #: PSTR202.13/W22

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

Title: Enviromental and virological nanotoxic determinants of transmembrane Furin

Authors: *E. FRIDMAN;
Weill Cornell Med., New York, NY

Abstract: Sulfur waste from industrialization (i.e., sulfur dioxide) or fertilizers (i.e., glyphosate) are anthropological manipulations of the sulfur cycle accelerating global climate change. Sulfate-reducing bacteria in the ecosystem and gut of living species reduce sulfur waste up to certain limits; spillover into city's sewage is associated to gut inflammation, autoimmunity and neurodegeneration increasing at alarming rates since 2000. Wastewater fecal pollution correlates with levels of enteroviruses peptides fragments anticipating by several weeks CoV-2 cases/hospitalizations. Hypotheses for virulent neurotropic/oncogenic viruses, systemic inflammatory and neurodegeneration disrupting gut mucosa systematically involve transmembrane FUS. Yet, accurate mechanism explaining how FUS increases virulency or induces toxicity are unknown. Here, the structural biology of FUR (PDB:1P8J) was reanalyzed based on its nanomagnetic properties (i.e., amino acids and inorganic minerals charges) and results showed that the overall net charge of FUS was -235 contrasting to control transmembrane

proteins TMPRSS2, ENaC and B0AT1 displaying 0, -24 and -74, respectively. Strikingly, FUS demonstrates 81 unbonded zwitterion molecules of sulfate (SO4), not observed in control proteins, that delimit a previously unknown internal channel. Results posit FUS as an inorganic-mineral channel for zwitterions highly charged molecules (>2+) likely driven by a pH gradient-dependent mechanism. This previously unknown nanomagnetic ability of FUR may explain the contrasting virulency between CoV-1 and CoV-2 based on the furin cleavage site: while the ancestor showed a single cation (+), CoV-2 presents a highly charged C-terminal tail (>3+); as deadly neurotropic Marburg Virus, neuro-oncogenic Epstein Barr, E. choli toxin or anthrax neurotoxin. Thus, virus internalization seems assured due to FUS structural damage during chemical reduction of toxics/toxins (i.e., FUS as tRNA). Aging and degeneration are associated to FUS shorter lifespan and gut inflammation. Toxics and toxins further deplete FUS reserves at neuro-enteroendocrine cells suggesting an essential innate protection role of FUR against supercharged molecules and supramolecules disturbing the neuro-enteric peptide system (i.e., GLP-1) and gut microbiota controlling via dopamine neurotransmission (i.e., D5DR) innate immunity (i.e., Lymphocytes T NK).

Disclosures: E. Fridman: None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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Title: Weak base drugs and lysosome stress responses

Authors: *P. W. HALCROW, J. D. GEIGER;
Biomed. Sci., Univ. of North Dakota Sch. of Med. and Hlth. Sci., Grand Forks, ND

Abstract: Approximately 75% of all drugs on the market have the physicochemical property of being weak bases. Drugs with weak base properties and relatively high pKa values enter acidic organelles including endosomes and lysosomes (hereafter referred to as endolysosomes), reside in endolysosomes for extended periods of time, de-acidify endolysosomes, and induce neurotoxicity. Endolysosomes contain high levels of divalent cations and these cations are released from endolysosomes when de-acidified. Endolysosomes are considered “master

regulators of iron homeostasis", and neuronal cell death and neurodegenerative disorders continue to be linked to ferrous iron (Fe^{2+}) induced reactive oxygen species (ROS) generation via Fenton-like chemical reactions. Because endolysosome de-acidification can induce lysosome stress responses that cause Fe^{2+} to be released from endolysosomes it was important for us to determine the extent to which and mechanisms by which a functionally diverse group of weak base drugs including atropine, fluoxetine, azithromycin, metoprolol, and tamoxifen affect endolysosomes and induce neuronal cell death. Using SH-SY5Y neuroblastoma cells, U87MG astrocytoma cells, and pharmacologically relevant doses, we showed that atropine, azithromycin, fluoxetine, metoprolol, and tamoxifen (1) de-acidified endolysosomes, (2) decreased endolysosome Fe^{2+} levels (3) increased cytosolic and mitochondrial Fe^{2+} and ROS levels, (4) depolarized mitochondria membrane potential, and (5) induced cell death; effects all blocked by the endolysosome-iron chelator deferoxamine. These cell biology findings suggest that, in general, weak base pharmaceuticals can induce lysosome stress responses that might affect their safety profiles.

Disclosures: **P.W. Halcrow:** None. **J.D. Geiger:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

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

Support: DE-AC52-07NA27344 /23-LW-011

Title: Sarin-surrogate-induced neurotoxicity using a human-relevant in vitro brain model

Authors: *C. BOGGURI, A. LADD,, C. VALDEZ, H. A. ENRIGHT, T. MUNDHENK, J. CADENA, D. LAM;
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Abstract: Organophosphorus (OP) nerve agents are a chemical threat to the United States, available to the civilian population (as pesticides) and historically weaponized (e.g., sarin, soman, or VX) as chemical warfare agents (e.g., World war II, Iran-Iraq war, Syrian conflicts, Tokyo subway attack). OP exposure can lead to neurological (e.g., anxiety) and motor symptoms that can manifest into chronic illnesses (e.g., Gulf War illness and Aerotoxic Syndrome). Human-relevant technology and tools have the potential to assess and address whether potential OP therapeutics can prevent long-term consequences (e.g., neurological dysfunction) that affect the human brain, improving and accelerating the translatability from research to the clinics. At Lawrence Livermore National Laboratory, we have employed a human-relevant microphysiological system to monitor and detect (using the multi-electrode array) changes in the

electrical activity of a human-induced pluripotent stem cell derived neural networks to the sarin surrogate, 4-nitrophenyl isopropyl methylphosphonate (NIMP). NIMP is a compound known to inactivate the enzyme, Achetylcholinesterase (AChE), in a similar manner as sarin. Within 24 hours of exposure, increased spiking and bursting activity was observed in a dose-dependent manner. Following washout of the surrogate neurotoxin, altered neural and network activity was observed during the 5 days post-exposure, with Synchrony analysis revealing increased network density and strength as a result of a single NIMP exposure. Changes in neural network activity was not a result of cell viability; no difference between cultures with and without NIMP exposure. However, inactivation of acetylcholinesterase, resulting in reduced enzymatic activity in the culture system (determined using Ellman's assay) was detected at 24 hours, which may have contributed to the modulated neural network activity during NIMP exposure. Current work is evaluating the consequence of therapeutic countermeasure for NIMP-induced neurotoxicity on neural network activity, including the current standard of care, Pralidoxime (or 2-PAM), an oxime countermeasure, and LLNL-02, a novel oxime countermeasure developed at LLNL.

Disclosures: C. Bogguri: None. **A. Ladd,:** None. **C. Valdez:** None. **H.A. Enright:** None. **T. Mundhenk:** None. **J. Cadena:** None. **D. Lam:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.16/W25

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

Title: In vivo neuropathology: Detecting site-specific changes in neuroinflammation following treatment with low doses of known neurotoxins

Authors: *D. S. ATHREYA[1,2], S. KEDHARNATH[2], A. PHADKE[2], P. P. KULKARNI[1,2], D. MADULARU[2], C. F. FERRIS[1,2,3];
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Abstract: Twenty-five percent of small molecules in drug development for CNS indications fail in clinical trials due to complications with neurotoxicity. The consequences are two-fold: 1) loss of time and money in bringing new drugs to market, and 2) the unwitting exposure of patients in clinical trials to neurotoxic side effects, and in the extreme case of BIA 10-2474, death and brain damage to healthy volunteers. In vivo Neuropathology (IVN) is a non-invasive MRI procedure for identifying site-specific changes in BBB permeability and neuroinflammation across the entire brain. To this end, we tested the neurotoxin trimethyltin (TMT), a gold standard for CNS toxicity; MK-801, an NMDA antagonist; kainic acid, a glutamate agonist; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); and BIA 10-2474, a FAAH antagonist. All drugs were given in small doses either once (TMT, MK-801, kainic acid) or over several consecutive days (BIA 10-2474, MPTP). Each experimental group consisted of two male and two female rats. Diffusion

weighted imaging (DWI) was used to follow changes in brain gray matter microarchitecture in rats at 3, 7, and 28 days. Measures of apparent diffusion coefficient (ADC) were used as a surrogate marker of brain cytotoxic edema caused by neurotoxin-induced neuroinflammation. The use of a 3D MRI rat atlas allowed for the quantification of ADC values in over 400 different brain areas. Multiple brain areas were identified as sites of putative neuroinflammation and cytotoxic edema for each of the toxins at each time point. Putative sites of neurotoxicity and BBB permeability were subsequently confirmed by traditional postmortem histology. The ability for IVN to follow disease progression in the same rat over time, identifying specific brain areas at risk for neurotoxicity, is a huge advantage over traditional CNS toxicology methods. IVN offers an alternative to the traditional tests for assessing CNS neurotoxicity, can minimize the cost, expedite the process, and identify subtle changes in site-specific brain areas.

Disclosures: D.S. Athreya: None. **S. Kedharnath:** None. **A. Phadke:** None. **P.P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **D. Madularu:** None. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.17/W26

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

Support: 2021R1A2C2006110
2021M3E5D9021364
2019R1A5A2026045

Title: Suppression of macrophage-mediated neurotoxicity by cell cycle signal regulation

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Abstract: After an ischemic stroke, neurotoxicity is triggered by infiltrating macrophage and exacerbate secondary degeneration in the penumbra lesion. However, infiltrating macrophages produce neurotrophic factors and have neuroprotective effects to improve the regeneration. Therefore, it would be important to regulate macrophage phenotypes to drive regeneration while concurrently curbing secondary degeneration. The present study sought to examine function of infiltrating macrophages phenotype on regeneration or degeneration in CNS neuorns. When BMDM CMs were treated to cultured cortical neurons, neurites showed degeneration, indicating

BMDM CM has neurotoxicity to CNS neurons. We previously reported that neuron-macrophage interaction can drive macrophage to pro-regenerative phenotype. Surprisingly, the CNS neurotoxicity of BMDM CMs were sharply reduced by the BMDM + neuron cocultures. To understand the mechanism of neurotixity in macrophages, we performed bulk RNA-seqeuncing and confirmed that cell cycle-related pathways were increased in co-cultured macropahges. Treatment of cell cycle inhibitors to macropahge significantly increased neurotoxicity. Also, Inhibition of Aurora Kinase, cell cycle-related genes, increased inherent neurotixicity. Therefore, thid results suggests that infiltrating macrophages cause degeneration for CNS neurons and neuron-macrophage interaction might change the macrophage pheotype to regeneration without degeneration by cell cycle-dependent pathways.

Disclosures: **Y. Seo:** None. **S. Jee:** None. **M. Kwon:** None. **B. Kim:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.18/W27

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

Support: NRF-2022R1A2C1011996
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22RB1130
2020-0-01343

Title: Toluene induces neurotoxicity with cognitive impairment in the normal adult mice

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Abstract: Toluene, a typical volatile organic compounds (VOCs) is an environmental toxin which induces neurotoxicity. Although long-term exposure to toluene is known to cause neurodegeneration in the brain, leading to a brain malfunction including cognitive deficit, the mechanisms of the direct neurotoxicity of toluene and other VOCs on the central nervous system (CNS) are not clearly understood. In this study, we aim to determine the behavioral effects of toluene and to discover the cellular mechanism of toluene toxicity in CNS. To determine the cellular mechanism of toluene toxicity, adult normal mice were administrated with toluene (0, 300, and 600 mg/kg, i.p.) for consecutive 12 days. The effects of toluene exposure were detected in the hippocampus and the frontal cortex of normal mice using transcriptome profiling focused on the mitochondrial biogenetics and neurotrophic mechanisms. The toluene exposed mice to a higher dose (600 mg/kg) showed behavioral changes including cognitive impairment in Morris

water maze test. The toluene exposed mice also showed the increased number of Iba1-positive microglia and mitochondrial dysfunction, compared to the vehicle group. These data suggest that the cellular mechanism of the environmental toxin, toluene, can be explained by mitochondrial dysfunction, cellular apoptosis, and neuroinflammation, etc.

Disclosures: **E. Seo:** None. **B. Song:** None. **S. Yu:** None. **H. Hu:** None. **M. Jang:** None. **S. Hwang:** None. **H. Seo:** None.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.19/W28

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

Support: NC3Rs KTP Grant

Title: A joint behavioural- and -imaging-based approach in larval zebrafish for identifying novel therapies against nerve agents

Authors: *J. KEARN[1], M. WINTER[2], M. PARKER[3];
[1]Defence Sci. and Technol. Lab., Salisbury, United Kingdom; [2]Univ. of Exeter, Exeter, United Kingdom; [3]Univ. of Surrey, Guildford, United Kingdom

Abstract: Nerve agents have been used in chemical warfare and function by inhibiting acetylcholinesterase, causing excessive cholinergic signalling and a range of neurotoxic effects, including seizures and muscle paralysis. Current medical treatments include atropine to counter muscarinic receptor over-activation and benzodiazepines to reduce excessive CNS excitability. The likelihood of recovery from exposure is dependent on factors such as time, with seizures becoming refractory to therapy with delayed treatment. There is a need to improve our medical therapies in the event of the release of nerve agents. Typically, rodent models have been used to assess medical therapies against nerve agents. The use of rodents is low throughput and involves the use of invasive electrodes to record CNS activity. Alternative models are required for the testing of therapies to reduce reliance on traditional animal models. Larval zebrafish are considered a non-sentient replacement for traditional animal models. Here, we have developed and utilised a behavioural- and imaging-based approach to quantify seizure-type activity in 4 days post-fertilisation larval zebrafish in the presence of nerve agents. In addition, we have tested standard medical treatments against nerve agents to validate this approach. On exposure to the cholinesterase inhibitor donepezil in light conditions, larvae exhibit concentration- and time-dependent hyperactivity, characterised by rapid looping motions. The benzodiazepine diazepam reduced hyperactivity, suggesting the increased movement relates to seizure-type events. Interestingly, this hyperactivity response is absent in larvae exposed to the nerve agents sarin, soman and VX. A light/dark assay was used to determine effects on stimulated movement. Donepezil and nerve agents elicited a concentration-dependent reduction in activity, with relative

potency consistent with that seen in mammals. Co-application of atropine ameliorated this reduced activity, confirming the cholinergic basis of the observed agent effects. In addition, we have adapted a light sheet imaging approach for fluorescence-based quantification of neural activity with the strain elavl3:GCaMP6s to allow single-plane confocal imaging of neural activity in agent-exposed larvae. Donepezil-exposed larvae exhibit clear spiking and increases of fluorescence consistent with seizures and this is ameliorated by co-application of diazepam. Future work will include imaging of nerve agent-exposed larvae for the purposes of comparison. Our results demonstrate the utility of combined behaviour and imaging approach for screening of novel therapies against nerve agents.

Disclosures: **J. Kearn:** A. Employment/Salary (full or part-time):; Dstl. 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.; NC3Rs. **M. Winter:** A. Employment/Salary (full or part-time):; University of Exeter. **M. Parker:** A. Employment/Salary (full or part-time):; University of Surrey.

Poster

PSTR202. Mechanisms of Neurotoxicity II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR202.20/X1

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

Support: NIH Grant R44MH119621

Title: 2d and 3d ipsc-derived platforms for neurotoxicity screening demonstrate compound effects on calcium activity, synapses, viability and cell proliferation

Authors: M. HASAN, ***K. L. GORDON**, C. G. RINES, N. SUAREZ, A. PASCUA, A. SMITH, P. MCDONOUGH, J. PRICE;
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Abstract: Neurotoxicity is a leading cause of drug failures preclinically and clinically. Safety testing to identify neurotoxicity for drug candidates in animal models is costly, labor intensive, and low throughput. The development of predictive *in vitro* systems that are more high throughput, better represent human disease, and reduce the use of animals in testing are desired. Human induced pluripotent stem cell models (hiPSC) are a promising avenue for developing neuronal models with multiple CNS cell types to develop predictive assays for compound toxicity. In this study, we developed and validated three platforms for neurotoxicity screening using iPSC-derived models: neural progenitor cells (NPC), two-dimensional (2D) neuron-astrocyte-microglia tri-culture models, and three-dimensional (3-D) neural spheroid models. We use single cell analysis methods to test compounds such as chemotherapy agents, effectors of channel activity, environmental toxicants, and anti-retroviral drugs on viability, calcium, synapse

numbers, or cell proliferation. For our NPC platform, we demonstrate decreased NPC viability and reduced cell proliferation after treatment with anti-retroviral compounds currently prescribed for HIV. Our 2D platform and 3D platforms have been developed with iPSC derived neurons, astrocytes and microglia for testing changes in calcium dynamics and synapses. For analysis of calcium activity, we loaded 2D or 3D cultures with fluorescent calcium dyes for calcium imaging using Vala Science's Kinetic Image Cytometer (KIC), followed by single cell analysis of the calcium transients. We observed changes in calcium activity after treatment with a chemotherapy drug (carboplatin) and channel modifying compounds (FPL64176). We routinely use fixed endpoint analysis of synapse count with antibodies recognizing pre- and post-synapses to look at changes in synapses after treatment. We are also developing methods using an AAV construct expressing GFP-tagged PSD-95 to allow for longitudinal investigation of synaptic changes after treatment with compounds, such as the environmental toxin lead. These various platforms can also be utilized to study mitochondria or ER toxicity elicited by compounds, and are relevant for studying neurotoxicity in both neurodevelopment as well as on developed intact networks.

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Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.01/X2

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

Support: NIH Blueprint for Neuroscience R01 AA027074
Bluefield Project to Cure FTD
NIA R01 AG068290

Title: Concurrent loss of C9orf72 and Progranulin synergistically promotes neuroinflammation and neurodegeneration

Authors: *N. JAHAN[1], R. LU[2], J. CHOI[3], E. J. HUANG[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two ends of a disease spectrum. In support of this idea, the majority of familial ALS and FTD cases are caused by hexanucleotide (CCCCGG) repeat expansion in the first intron of *chromosome 9 open reading frame 72* (*C9orf72*) gene. Furthermore, mutations in another FTD gene, *Progranulin* (*GRN*), increase the risk of ALS. In addition to the potential genetic interactions, there have been reports where concurrent mutations in both *C9orf72* and *GRN* genes lead to early disease-onset and more severe neurodegeneration in patients with familial form of FTD-ALS spectrum disease.

Since previous studies show that C9orf72 and PGRN play important roles in endolysosomal trafficking and loss of C9orf72 or PGRN leads to abnormal microglial activation, we hypothesize that simultaneous deletion of C9orf72 and PGRN may further aggravate neuroinflammation and neurodegeneration. To test this hypothesis, we generated an aging cohort of *wild type*, *Grn*$^{-/-}$, *C9orf72*$^{-/-}$, and *Grn*$^{-/-}$*;C9orf72*$^{-/-}$ mice, and showed that *Grn*$^{-/-}$*;C9orf72*$^{-/-}$ mice have significantly shortened lifespan compared to *Grn*$^{-/-}$ or *C9orf72*$^{-/-}$ mice. Furthermore, brain pathology examination and bulk and single-cell transcriptomic analyses reveal that *Grn*$^{-/-}$*;C9orf72*$^{-/-}$ mouse brain have more pronounced and widespread age-dependent microgliosis, innate immune activation, lysosomal and phagocytic defects, disruption of blood brain barrier, and increase in B cell receptor signaling due to the up-regulation of Fcgr2b receptor. In addition to the profound neuroinflammation phenotypes, *Grn*$^{-/-}$*;C9orf72*$^{-/-}$ mice exhibit more severe peripheral immune defects, including splenomegaly, lymphadenopathy, glomerulonephritis, and elevated autoantibodies. Together, these results support that concurrent loss-of-function mutations in both *C9orf72* and *GRN* genes most likely result in aggravated neuroinflammation and defects in peripheral immune system that synergistically promote neurodegeneration.

Disclosures: **N. Jahan:** None. **R. Lu:** None. **J. Choi:** None. **E.J. Huang:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.02/X3

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

Support: R01AG061288

Title: Microglia innate immune response contributes the antiviral defense

Authors: *H. QIAO;
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Abstract: During sepsis, pathogens and host immune cells can weaken choroid plexus (ChP) barrier and enter the brain, causing cerebral dysfunctions known as sepsis-associated encephalophagy. Here, we used human cerebral organoid(CO) and ChP organoid to model herpes simplex virus type 1(HSV-1) infection and found ChP epithelial cells were highly susceptible to HSV-1. However, the current CO and ChPO models lack the presence of a functional innate immune system, e.g., microglia, which play critical roles in coordinating immune responses and inflammatory CSF cytokines. We developed a new microglia-containing CO (M-CO) models microglia-containing ChPO (M-ChPO) models by incorporating human embryonic stem cell (hESC)-derived microglia with CO and ChPO, separately. Microglia could effectively limit HSV-1 infection in both M-CO and M-ChPO and protect epithelial barrier in ChP organoids. In conclusion, this new M-CO and M-ChPO model more closely recapitulates

the human immune response and allows us to address the significance of microglia and its innate immune pathway in HSV-1 infection, and beyond. <!--EndFragment-->

Disclosures: H. Qiao: None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.03/X4

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

Support: NIH NINDS Grant 1F31NS132407-01
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National Institute of Aging Boston University AD Center (P30AG072978)
Department of Veterans Affairs Biorepository (BX002466)
Nick and Lynn Buoniconti Foundation

Title: Repetitive head impacts induce a pro-inflammatory SPP1 microglial phenotype that may underlie chronic traumatic encephalopathy pathogenesis

Authors: *M. L. M. D. BUTLER[1], N. PERVIAZ[2], Y. WANG[2], J. D. CAMPBELL[2], A. C. MCKEE[3], J. D. CHERRY[4];
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Abstract: Chronic traumatic encephalopathy (CTE) is a debilitating neurodegenerative tauopathy associated with repetitive head impacts (RHI) endured through contact sport play, military service, or domestic violence. Although we are making enormous strides in understanding the later stages of neurodegeneration following head trauma, there are still many questions regarding the early events that precede pathology. Previous studies have suggested that microglia become reactive following RHI and might contribute to the initial tau pathology in CTE. However, the exact glial phenotypes are unclear, and better characterization is essential for the development of novel biomarkers or therapeutics to treat young individuals in the early stages of disease. Therefore, to investigate the early microglial responses to RHI and CTE, we performed single-nucleus RNA sequencing of post-mortem dorsolateral frontal cortical tissue from 28 individuals (1) without RHI exposure or CTE, (2) with RHI exposure but no CTE, and (3) with low-stage CTE, all under the age of 50. Our analyses demonstrate that RHI induces a pro-inflammatory microglial phenotype characterized by the expression of the phagocytic and degeneration-associated marker, SPP1. This SPP1-expressing population significantly

upregulates pro-inflammatory genes such as IL-1β, TLR2, and HIF1α, and gene ontological analysis identified “response to cytokine stimulus” and “TGF-β signaling” as two of the highest upregulated terms. Immunohistochemical labeling of SPP1, tau, and microglia/macrophage marker Iba1 in individuals with CTE demonstrated that Iba1-positive cells colocalize with SPP1 and are associated with regions of tau deposition. Additionally, SPP1-expressing glia were also found in individuals with exposure to RHI but no ptau pathology, suggesting they might be involved prior to tau deposition. Overall, our data demonstrates that SPP1 microglia might be an important part of the early inflammatory cascade following RHI and during CTE. These results pave the way for targeted investigation of neuroinflammatory CTE diagnostic biomarkers and therapeutic targets for RHI-exposed individuals during life.

Disclosures: M.L.M.D. Butler: None. **N. Perviaz:** None. **Y. Wang:** None. **J.D. Campbell:** None. **A.C. McKee:** None. **J.D. Cherry:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.04/X5

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

Title: Preventive effect of anti-HMGB1 nAb on the onset of distal infraorbital nerve chronic constriction neuropathy in female mice.

Authors: *S. MA[1,2], Y. NAKAMURA[2], T. KOCHI[2,3], K. HISAOKA-NAKASHIMA[2], D. WANG[4], K. LIU[4], W. HIDENORI[4,5], M. NISHIBORI[4,6], N. MORIOKA[2];
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Abstract: Post-traumatic trigeminal neuropathy (PTTN) is a kind of chronic pain caused by damage to the trigeminal nerve. In a previous study, we reported that pretreatment with the neutralizing antibody against high mobility group box-1 (HMGB1) prevented the development of PTTN in distal infraorbital nerve chronic constriction injury (dIoN-CCI) in male mice. However, trigeminal neuropathy is reported to have a high incidence in females. Also, the mechanisms of how HMGB1 evokes the PTTN are unclear. Hence, the current study investigated the effects of HMGB1-neutralizing antibody on pain-like behaviors in a female mouse model of chronic pain caused by trigeminal nerve injury.Under anesthesia, two 3-0 silk sutures were tied loosely around the dIoN in female ddY mice (6 weeks). The Anti-HMGB1 nAb was administered to the area around the injured nerve immediately after nerve injury (100 ng, 10 μL) and 2 days (100 ng, 50 μL) after IoN injury, and an equal volume of control IgG was infused around the IoN. Nociceptive-like behaviors were evaluated by measurement of hypersensitivity

to cold stimulation and von Frey test. On surgery day 13, acetone drops were applied to the surgical side of the face of mice, and the reaction time of mice was measured within 60 seconds. Von Frey filaments were used to measure mechanical pain in the left face and whisker pad of mice on the day 14 days after modeling. The intensity of the stimulus filaments was set at 0.16 g, 0.4 g, 0.6 g, 1.0 g, 1.4 g, and 2.0 g. Each stimulus was given 5 times. The number of positive reactions produced by the left side of mice was counted. Macrophage accumulation around the injured IoN and microglial activity in spinal trigeminal nucleus caudalis (Sp5c) were determined by immunohistochemistry.In female mice, the dIoN-CCI significantly induced mechanical and cold hypersensitivity, which responses were prevented by the pretreatment with HMGB1-neutralizing antibody. Moreover, the nerve injury increased macrophage accumulation around IoN and activated microglia in Sp5C. The Pretreatment with HMGB1-neutralizing antibody inhibited these responses of both inflammatory cells. These data suggest that regardless of gender, HMGB1 plays a crucial role in developing the PTTN after the nerve injury. Thus, the anti-HMGB1 nAb could be a novel therapeutic target for inhibiting the onset of PTTN in male as well as female mice.

Disclosures: S. Ma: None. **Y. Nakamura:** None. **T. Kochi:** None. **K. Hisaoka-Nakashima:** None. **D. Wang:** None. **K. Liu:** None. **W. Hidenori:** None. **M. Nishibori:** None. **N. Morioka:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.05/X6

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

Title: In vitro microglia models as a screening platform to support neuroinflammation drug discovery programs

Authors: A. ROZO, P. MALKO, ***D. M. LEITE**, A. ROBERTS, T. ROSENSTOCK, T. PHILLIPS, S. THOMSON, N. MIRZA;
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Abstract: Neuroinflammation is of growing interest for the treatment of central nervous system disorders and has received significant funding in recent years. Although novel biological targets have been identified that modulate neuroinflammation, whether they are druggable requires robust and validated Hit-to-Lead screening and Lead optimisation programs to identify development candidates. *In vitro* cellular models that faithfully capture fundamental biology and disease constructs are necessary in drug discovery programmes. To achieve these goals, Sygnature have validated rodent and human *in vitro* microglia models - the resident immune cells of the brain. Both embryonic and adult microglia were isolated from dissociated and digested rat brain tissue by positive magnetic selection using CD11b/c microbeads, while human microglia were differentiated from monocytes (monocyte-derived microglia, MDMi) using a

cytokine cocktail. Rodent and human models were characterised by cell surface markers, and ability to recapitulate microglia phenotype and function by measuring phagocytosis, cytosolic calcium, and oxygen consumption. P2X7 receptor pharmacology was used to study receptor signalling in microglia. Rat embryonic and adult microglia expressed IBA1, TMEM119, and $P2Y_{12}R$, showing a characteristic microglial morphology with no other non-neuronal cells present in cultures. MDMi showed a greater expression of microglial-specific markers (TMEM119, $P2Y_{12}R$) compared to monocytes ($p<0.05$, Mann-Whitney U test) with a morphology reminiscent of microglia. Microglia models demonstrated phagocytosis using both pHrodo-labelled E. *coli* and mouse brain fractions. Exposure of microglia to cytochalasin B (10 μM) and BzATP (P2X7 receptor agonist, 300 μM) over 8 hours triggered a significant reduction in phagocytosing cells ($p<0.05$, One-Way ANOVA). Co-incubation of BzATP with a selective P2X7 receptor antagonist (A804595, 500 nM) reversed the effect of BzATP alone eliciting an increase in cells phagocytosing either pHrodo-labelled E. *coli* or brain fractions. In further validation studies, BzATP elicited a concentration-dependent increase in cytosolic calcium in MDMi with an E_{max} of 58 ± 9% of the maximal response induced by carbonyl cyanide-p-trifluoromethoxy phenylhydrazone. Thus, we show well validated *in vitro* microglia models that are (i) amenable to high-throughput screening campaigns that allow identification of novel targets and drugs, and (ii) which can provide deeper mechanistic understanding of a drug's effects by measuring multiple differential endpoints.

Disclosures: **A. Rozo:** 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.; Sygnature Discovery. **P. Malko:** 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.; Sygnature Discovery. **D.M. Leite:** 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.; Sygnature Discovery. **A. Roberts:** 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.; Sygnature Discovery. **T. Rosenstock:** 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.; Sygnature Discovery. **T. Phillips:** 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.; Sygnature Discovery. **S. Thomson:** 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.; Sygnature Discovery. **N. Mirza:** 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.; Sygnature Discovery.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.06/X7

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

Title: Fosgonimeton, a small-molecule positive modulator of neurotrophic hepatocyte growth factor system, inhibits LPS-mediated neuroinflammation in BV2 microglia

Authors: *W. WU, L. HELTON, S. REDA, S. FECHTNER, R. TAYLOR, K. J. CHURCH; Athira Pharma Inc., Bothell, WA

Abstract: INTRODUCTION. Neuroinflammation is a pathological hallmark of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and ALS. Microglia are the predominant resident immune cells of the brain and play a key role in the onset and progression of neuroinflammation. Previously, we have shown that fosgonimeton, a small-molecule positive modulator of the neurotrophic hepatocyte growth factor (HGF) system, decreased lipopolysaccharide (LPS)-induced pro-inflammatory cytokine release in macrophages, and attenuated LPS-induced deficits in vitro and vivo (Johnston et. al, 2022). Here, we utilized LPS-activated microglia to investigate the mechanism by which fosgonimeton induces anti-inflammatory effects in vitro. METHODS. LPS-stimulated BV2 microglia were treated with the active metabolite of fosgonimeton, fosgo-AM, for 24 hours and mRNA levels of proinflammatory mediators (TNF-α, IL-1β, IL-6, iNOS, Cox-2 and NLRP3) were measured via QuantiGene multiplex assay. Nitric oxide (NO) production was determined using Griess reagent. To identify the molecular mechanisms by which fosgo-AM regulates LPS-induced neuroinflammation, we utilized homogenous time-resolved fluorescence to assess the phosphorylation status of several signaling effectors implicated in LPS-mediated TLR4 signaling including MAPK (ERK, p38 and JNK), AKT and NF-κB/STAT3. We also investigated the effect of fosgo-AM on LPS-induced mitochondrial dysfunction via mitochondrial membrane potential dye. Furthermore, we examined the effect of fosgo-AM on reactive oxygen species (ROS) production using redox-sensitive fluorescent probe DCFDA. RESULTS. Fosgo-AM decreased mRNA expression of all pro-inflammatory mediators (TNF-α, IL-1β, IL-6, iNOS, Cox-2 and NLRP3), and NO production. Moreover, fosgo-AM inhibited LPS-induced activation of ERK, p38, JNK, AKT, NF-κB, and STAT3. Fosgo-AM also increased mitochondrial membrane potential and reduced ROS production following LPS challenge, suggesting that fosgo-AM attenuates LPS-induced mitochondrial dysfunction and oxidative stress. CONCLUSIONS. These studies revealed multiple mechanisms by which fosgonimeton may promote anti-inflammatory activity. Our data show that fosgo-AM significantly attenuates LPS-induced 1) production of inflammatory mediators, 2) TLR4 signaling cascades, and 3) oxidative stress and mitochondrial dysfunction. Overall, our data support the therapeutic potential of fosgonimeton for the treatment of neuroinflammation-associated diseases. Fosgonimeton is currently under investigation in clinical trials for mild-to-moderate AD (NCT04488419; NCT04886063).

Disclosures: W. Wu: A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

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Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.07/X8

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

Title: Increased GFAP expression associated with the thyroid stimulating hormone receptor (TSHRhyt/+) mouse suggests the potential for inflammation and neurodegeneration in the CNS.

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Howard Univ., washington, DC

Abstract: The thyroid stimulating hormone receptor (TSHR) and G protein-coupled receptor 37 (GPR37) belong to the Class A type of G-coupled protein receptors, which share unique features of ectodomain shedding. GPR37 possesses neuro- and glial-protective properties against oxidative stress and is preferential to the stability of dopaminergic neurons; abnormalities in GPR37 can lead to endoplasmic reticulum (ER) stress and neurodegeneration. Although TSHR is localized in different regions of the brain and reportedly enriched in microglial populations, little is understood about its precise functional role in neuro-glial stability. The TSHRhyt/+ mouse contains a Pro556Leu mutation in the 4th transmembrane domain of TSHR, altering TSH ligand binding that causes mild to severe hypothyroidism (hyt). In certain cases deficiencies of dopaminergic neurons are observed in this mouse, suggesting that the (TSHRhyt/+) point mutation may also be linked to mechanisms that compromise neuronal or glial stability. Here, we report that western blot analysis of hippocampus and cortical brain tissues of wild type (+/+) and heterozygous (hyt/+) mice revealed no significant difference in TSHR expression. In contrast, glial fibrillary acidic protein (GFAP) and proliferating cellular nuclear antigen (PCNA) expression significantly increased in hippocampal tissues of heterozygotes versus wild types and no change in GFAP or PCNA expression was observed in cortical tissues. These observations suggest that the TSHR may play important roles in regulation in astroglial stability, adult

neurogenesis, neuroplasticity, and neural precursor cell morphology of the hippocampus. Current efforts are aimed to determine cell-type specific expression of TSHR, GPR37, and other markers of cortical and hippocampus tissues of the TSHRhyt/+ mouse.

Disclosures: C. Muniz: None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.08/Web Only

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

Support: National Science Foundation #1926990
Florida High Tech Corridor

Title: Ai-based quantitative assessment of stimulated microglia cells using low-power images

Authors: *P. R. MOUTON[1,2], H. MORERA[2], G. DENHAM[1,2], J. RIANO RINCON[2], L. HALL[2], D. GOLDGOF[2], G. J. HARRY[3];
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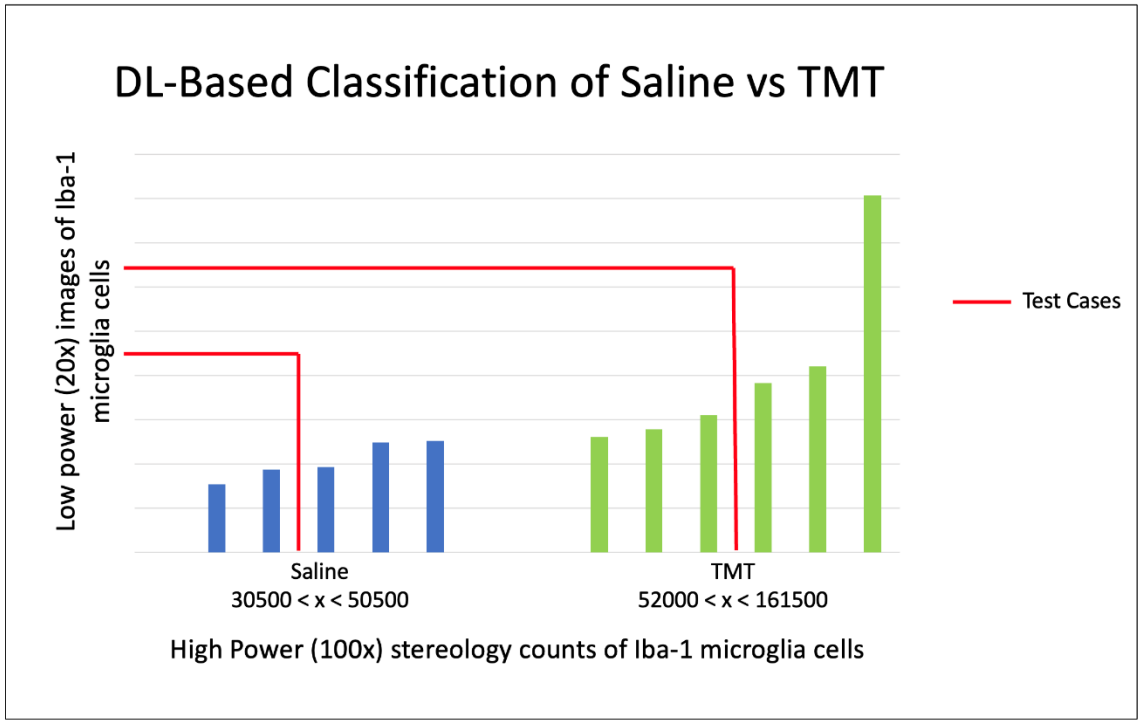
Abstract: Emerging data show microglial cells play a more complex role than previously thought in brain development and repair, neurotoxic reactions, neurological diseases and mental illnesses. To better understand the diverse phenotypic responses of microglia cells, we applied a novel, rapid and fully automatic deep learning (DL) method for assessing morphologies and cell numbers in tissue sections. Twenty-four hours after treating adult male CD-1 mice with the neurotoxicant trimethyltin chloride (TMT, 2.2 mg/kg, i.p.), TMT caused microglia activation, elevated inflammatory cytokines and neuronal death in dentate gyrus followed by morphological shifts over the next 7 days with a mix of amoeboid and process-bearing cells and diminished cytokine responses. For quantitative studies, mice were sacrificed 48 hrs and 9 days post-TMT (n=6) or saline (n=5); sagittal brain sections cut through an entire hemisphere; sections immunostained for Iba-1; and the total number of Iba-1 cell bodies (Total N_{Iba1}) counted in hippocampus (HPC) by manual stereology with high sampling stringency (coefficient of error ~ 10%). About 100-150 low-mag (20x) images of HPC were automatically collected and labeled with ground truth (GT), i.e., Total N_{Iba1} for that case. We used these GT-labeled low-mag images to train a neural network with a leave-one-out strategy where all mice served as unlabeled test cases, i.e., not in the training set. The DL model showed 91% accuracy (10/11) at predicting Total N_{Iba1} for test cases *using only low-mag HPC images as input* (Figure 1 with results for 2 test cases), i.e., without cell-level counts or subjective ratings. The only incorrect prediction missed the correct class by 10 low-mag images (55 vs. 65) with full accuracy expected after tuning of the model. Data collection per case required less than 1 minute for the DL model vs.

vs. ~ 60 minutes for manual stereology, with 0% and 5-6% inter-rater error, respectively. Using the standard curve from our trained model, data can be collected from new test cases using only low-mag images from an automatic slide scanner or ordinary cell phone.



Disclosures: **P.R. Mouton:** A. Employment/Salary (full or part-time):; SRC Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SRC Biosciences. **H. Morera:** A. Employment/Salary (full or part-time):; USF. **G. Denham:** A. Employment/Salary (full or part-time):; USF. **J. Riano Rincon:** A. Employment/Salary (full or part-time):; USF. **L. Hall:** A. Employment/Salary (full or part-time):; USF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); USF. **D. Goldgof:** A. Employment/Salary (full or part-time):; USF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); USF. **G.J. Harry:** A. Employment/Salary (full or part-time):; NIEHS.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.09/X9

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

Support: NIH Grant: R01NS120322(TS)
NIH Grant: R61NS123760(CH)

Title: A multi-disciplinary approach to interrogating the relationship between inflammasome signaling and mitochondrial dynamics during ischemia/reperfusion injury

Authors: *F. J. TORRES TORRES[1,3], G. M. FOGO[2], S. RAGHUNAYAKULA[1], J. M. WIDER[1,6], K. J. EMAUS[2], E. GRULEY[1], J. LIAO[1], R. W. NEUMAR[1,6], C. H. HSU[1,6,4], T. H. SANDERSON[1,5,7,3];
[1]Emergency Med., [2]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI; [3]Neurosci. Grad. Program, [4]Dept. of Surgery, [5]Dept. of Mol. and Integrative Physiol., Univ. of Michigan Med. Sch., Ann Arbor, MI; [6]Univ. of Michigan Med. Sch., [7]Max Harry Weil Inst. for Critical Care Res. and Innovation, Ann Arbor, MI

Abstract: Blood flow and oxygen delivery are hindered in the brain during ischemia, resulting in mitochondrial reactive oxygen species (mtROS) generation and cell death post-injury. Microglia, the resident immune cells of the brain, detect damage-associated molecular patterns (DAMPs) and initiate immune responses via NLRP3 inflammasome signaling. Pro-inflammatory polarization in turn induces shifts in mitochondrial dynamics and clearance, including caspase-1-mediated cleavage of Parkin, a critical protein in the mitophagy pathway. Inhibition of mitophagy leads to the accumulation of dysfunctional mitochondria, increasing mtROS production and potentially committing the microglia to a pro-inflammatory activation. Subsequent pro-inflammatory cytokine release by activated microglia is known to be a double-edged sword; where the initial response serves to clear the injured area, overactivation can lead to aggravated injury. However, activated microglia display distinct metabolic and morphological profiles that are context-dependent, and have yet to be thoroughly investigated in the context of brain injury. To characterize the functional and metabolic changes in during injury, we first studied differences in microglial morphology through immunofluorescent staining of a porcine cardiac arrest model. Semi-automated machine learning morphological analysis detected attributes characteristic of pro-inflammatory activation, such as cell volume, surface area, sphericity and branching, when comparing injured and sham treated large animals in a model of cardiac arrest. Additionally, we detected the presence of ASC-speck formation within affected brain regions, a critical component of the inflammasome signaling pathway. This prompted us to investigate the contribution of NLRP3 signaling in microglial activation and shifts in mitochondrial oxidative stress in a cell culture model of ischemia reperfusion (I/R) injury. We exposed primary microglial cultures from MitoQC mice, a genetic sensor for monitoring mitophagy, to 6hrs of oxygen glucose deprivation and 24hrs of reperfusion (OGD/R), with or without a specific inhibitor of NLRP3 (MCC950). Enzyme-linked immunosorbent assay also showed increased release of the pro-inflammatory cytokine TNF-α under the OGD paradigm with a marked decrease in the presence of MCC950. Western Blot has also shown increased NF-κB and Drp1(S616) phosphorylation, as well as caspase-1 cleavage as a result of OGD. Ongoing studies are focused on identifying the timing and necessity of mtROS-dependent activation of the NLRP3 inflammasome pathway.

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Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.10/X10

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

Title: Glibenclamide treatment following subarachnoid hemorrhage promotes M2 microglia polarization

Authors: *C. TOSUN[1], V. GERZANICH[2], M. J. SIMARD[3];
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Abstract: The sulfonylurea receptor 1-transient receptor potential melastatin 4 (Sur1-Trpm4) channel plays an important role in acute central nervous system injury and blocking this ion channel has been shown to be neuroprotective in several animal models of brain injury. In this study, we bilaterally injected fresh 50 μL of autologous blood under stereotactic guidance into the subarachnoid space of the entorhinal cortex. Glibenclamide treatment was administered subcutaneously by continuous infusion of drug (or equivalent volume of vehicle for control experiments) by a miniosmotic pump following SAH injury at 200ng/hr. We have previously reported a reduction of Tnfα expression with Sur1 inhibition by both pharmacological inhibition and by Abcc8 gene suppression. Here, we document that the activated and amoeboid microglia within the entorhinal cortex express different microglial markers. Rats treated with glibenclamide express significantly elevated levels of IL-10 in Iba1-positive cells. On the contrary, these activated microglia have reduced intracellular expression of iNOS when compared to vehicle controls at 48 hours after injury. We have also observed a differential expression pattern of cell surface markers MHC class I and II, indicating a shift in microglial phenotype by potentially altering microglia-dependent neuroinflammation. Our results show that blocking Sur1 with specific antagonist glibenclamide could ameliorate the extent of injury following subarachnoid hemorrhage via switching the microglial phenotype from M1 to M2 by reducing the associated neuroinflammation. This dynamic regulation of microglial polarization following injury holds a great potential of novel therapeutic interventions after devastating complications after brain injuries with bleedings. Further work is underway to elucidate the regulatory molecular mechanisms of how microglia alter their phenotype in a more controlled *in vitro* culture system.

Disclosures: C. Tosun: None. **V. Gerzanich:** None. **M.J. Simard:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.11/X11

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

Support: Conacyt 1223198
PAPITT IA202120
PAPITT IA221622

Title: Comparative Analysis of Morphology in Morphine Self-Administration and LPS-Induced Reactive Microglia in a Wistar Rat Model

Authors: *D. MEDINA SÁNCHEZ, C. CARRANZA-AGUILAR, L. TRUJILLO VILLARREAL, M. S. SERRANO RÁMIREZ, D. A. ELIZARRARÁS HERRERA, E. A. GARZA VILLARREAL;
B03, Inst. de Neurobiología, UNAM, Queretaro, Mexico

Abstract: Comparative Analysis of Morphology in Morphine Self-Administration and LPS-Induced Reactive Microglia in a Wistar Rat Model. Morphine is an opioid that has been proven to induce neuroinflammation, and the microglia have been recently implicated in opioid dependence and withdrawal. Therefore, evidences suggest a theory that the morphine-induced neuroinflammation in regions implicated in opioid addiction as nucleus accumbens (NAc) and ventral tegmental area (VTA) would be suffering changes by the microglia related to the development of opioid addiction. Because the complexity and the several reactive morphological types (ameboid or ramified), the morphology of the microglia is considered a spectrum. A better understanding of morphine induced microgliosis may contribute to the comprehension of the variability of cellular activity implicated in the opioid addiction. Herence, we propose that morphine self-administration may elicit similar morphological changes in microglia as observed with LPS-induced activation, suggesting that morphine acts as stimulant for microglial reactivity in a Wistar rat model. For this we used a morphine self-administration (0.1 mg/kg, MO+/LPS-, n=3), neuroinflammation positive control (1 mg/kg unique dose, MO-/LPS+, n=3) and negative control (saline, MO-/LPS-, n=3). The intrajugular morphine self-administration was performed in a conditioning box with 2 phases: maintenance (FR1) and acquisition (PR 9-4) phases. For evaluation of the cellular density and morphological features of microglia in NAc and VTA, we used immunofluorescence staining using antibodies targeting the microglia marker (Iba-1). Subsequently, high-resolution images were acquired using a LSM 745 Confocal microscope. The image and statistical analysis was made with ImageJ2/Fiji Rstudio, respectively. As a result, the M0+/LPS- group showed more reward infusions than the MO-/LPS- control. The morphological analysis has shown a difference between the cellular density in the treatments ($p = 0.0004$ Kruskal-Wallis), where MO+ and MO-/LPS+ showed more cellular density than the MO-/LPS- group (Dunn's Test. $p = 0.0034$, $p = 0.0012$ respectively). Also, the microglia morphology has qualitative differences between morphine and LPS groups. The LPS has more ramified and bushy phenotype correlating with a reactive microglia. Meanwhile the morphine has several phenotypes, such as ramified, hyper-ramified, primed, hypertrophied, bushy, rod and amoeboid.The chronic exposure to morphine produces microgliosis, which is accompanied by heterogeneous microglial phenotypes. This suggests that the process appears to be more intricate or multifaceted.

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Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.12/X12

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

Support: Research Grants Council (GRF/RGC)
Health and Medical Research Fund (HMRF)
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PolyU Strategic Importance Project

Title: Homozygous and heterozygous TAK1-deficiency differentially affect microglial responses in the rd10 mouse model of retinitis pigmentosa

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Abstract: Retinitis pigmentosa (RP) is a group of inherited retinal diseases characterized by progressive loss of photoreceptors. Microglia-mediated neuroinflammation has been implicated in the pathophysiology of RP and its progression. Previous studies have suggested that the transforming growth factor-β-activated kinase 1 (TAK1) plays a pivotal role in regulating chronic and acute CNS inflammation. However, it is unclear whether TAK1 is a key regulator in pro-inflammatory cytokine-driven signaling pathways in RP. In this study, we investigated the role of TAK1 in regulating neuroinflammatory responses in the rd10 mouse model of RP. We found that TAK1 was upregulated in activated microglia of rd10 retinas. To specifically investigate the function of microglial TAK1, we generated a mouse strain with conditional deletion of microglial TAK1 by crossing $CX3CR1^{CreER/+}$/rd10 mice with $TAK1^{fl/fl}$ mice. Following tamoxifen treatment, we found that homozygous TAK1-deficiency (rd10; $CX3CR1^{CreER/+}$; $TAK1^{fl/fl}$) triggered microglial cell death, while heterozygous TAK1-deficiency (rd10; $CX3CR1^{CreER/+}$; $TAK1^{fl/+}$) did not affect microglia viability but dramatically reduced microglia activation by decreasing microglia proliferation and migration to the outer nuclear layer.We observed that microglia -specific homozygous or heterozygous TAK1-deficiency dramatically reduced release of proinflammatory factors and preserved the structure and function of photoreceptors in rd10 retinas. Moreover, proteomic analysis using LC-MS/MS demonstrated significant changes in proteins related to retinal degeneration, inflammatory response, synthesis of ROS, and phagocytosis in the rd10 background, and heterozygous TAK1-deficiency downregulated the proteins related to neuroinflammation and phagocytosis. More importantly,

we identified the signal transducer and activator of transcription 3 (STAT3) as a potential downstream effector of TAK1 signaling in regulating microglial neurotoxicity. Our in vitro experiments confirmed that inhibiting TAK1 using 5z-7-oxozeaenol significantly reduced the production of proinflammatory factors by regulating the phosphorylation of STAT3 after LPS stimulation in BV2 cells. Our findings suggest that TAK1 is essential for microglia survival and microglia activation. Targeting TAK1 may be a promising therapeutic strategy for alleviating neuroinflammation and photoreceptor degeneration in RP.

Disclosures: **J. Zhang:** None. **W. Yang:** None. **B. Lin:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.13/X13

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

Support: Novartis (MBAF312A_FVTD007)

Title: Siponimod reduces proinflammatory activation of microglia in in vitro and in vivo models of multiple sclerosis and favours expression of alternatively activated microglia

Authors: *N. HEITMANN[1,2], S. FIENE[2], A.-C. GUDE[2], R. GOLD[2], S. FAISSNER[2]; [1]Ruhr Univ. Bochum, Bochum, Germany; [2]Ruhr-University Bochum, St. Josef-Hospital, Bochum, Germany

Abstract: In progressive forms of multiple sclerosis (MS), microglia (MG) are contributors to chronic inflammation. The second generation sphingosin-1-phosphate receptor modulator siponimod (sipo), licensed for secondary progressive MS, exerts presumably modulatory properties on microglia. We hypothesized that prophylactic sipo in *in vitro* and *in vivo* models of progressive MS might reduce the proinflammatory activation of MG favouring a neuroprotective environment.We induced experimental autoimmune encephalomyelitis (EAE) in CX3CR1CreER/iDTR mice (n=5-9 per group) to perform a conditional microglia knock out (MG^{ko}). Sipo or vehicle was administered orally in oil from day 0. MG^{ko} was induced in the chronic phase of EAE, mice were sacrificed during subsequent repopulation of MG. Immune cells were analyzed by flow cytometry and qPCR. Primary MG (pMG) were derived from postnatal mixed glial cultures and preincubated with sipo followed by different stimulations (Interferon-gamma, lipopolysaccharide, iron, un-/stimulated splenocytes) for 24 hours. Level of stimulation and phenotyping was assesed by MTT assay, immunocytochemical stainings and flow cytometry (n=3 in at least triplicates). Repopulating MG in MG^{ko}-EAE were characterized by lower expression of cx3cr1 and P2RY12 and upregulation of inflammatory markers (CD86, MHC-II; qPCR: IL-1beta, TNF-alpha). Sipo reduced expression of CD86 and MHC-II in repopulating MG compared to repopulation under vehicle. Additionally, sipo elicited upregulation of alternative activation markers (CD206, CD163; qPCR: Arg-1) during

repopulation. To rule out that this could be an indirect effect by sipo preventing lymphocyte migration to the brain with less MG interaction, we conducted *in vitro* assays to test for a direct protective effect of sipo on MG. Sipo prevented IFN-gamma mediated activation based on MTT assay and immune labelling (CD86, MHC-II, CD163). Sipo could prevent upregulation of activation markers in pMG following co-culture with naïve and stimulated splenocytes but failed to prevent activation by chronic EAE-derived splenocytes. Sipo modulates microglia during repopulation in MG^{ko}-EAE characterized by a less pro-inflammatory, alternatively activated, potentially phagocytic phenotype. Direct effects of sipo on MG were supported by cell culture analyses. Our findings suggest that sipo has both direct and indirect modulatory properties on microglia in *in vitro* and *in vivo* models for MS with implications for putative neuroprotection during progression.

Disclosures: **N. Heitmann:** A. Employment/Salary (full or part-time):; St. Josef-Hospital, Bochum, Germany. **S. Fiene:** None. **A. Gude:** None. **R. Gold:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva Pharmaceutical Industries Ltd., Biogen Idec, Bayer Schering Pharma, Genzyme, Merck, Serono, Novartis. F. Consulting Fees (e.g., advisory boards); Baxter, Bayer, Biogen, Genzyme, Novartis, Roche, Sanofi, TEVA, Merck, Janssen-Cilag. **S. Faissner:** 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.; Ruhr-University Bochum, DMSG, Stiftung für therapeutische Forschung, Lead Discovery Center GmbH, Novartis. F. Consulting Fees (e.g., advisory boards); Biogen, BMS, Celgene, Janssen, Merck, Novartis, Roche.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.14/X14

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

Support: NIH Grant 1141572-1-78985

Title: Inflammatory Signals Alter Iron Metabolism at the Blood Brain Barrier

Authors: *S. L. ROSENBLUM, D. J. KOSMAN;
Biochem., Univ. at Buffalo, Buffalo, NY

Abstract: Chronic inflammation is a major feature of several diseases. The inflammatory response involves cytokines release from activated monocytes triggering the release of acute phase proteins (APPs) from the liver. These systemic signals subsequently induce changes throughout the body. In the case of chronic inflammation, this signaling is continuous and pathogenic. Neurodegenerative disorders such as Alzheimer's Disease (AD) and Multiple Sclerosis (MS) often present with chronic inflammation. Systemic chronic inflammation in these

same diseases can be accompanied by toxic brain iron accumulation (BIA). Whether systemic chronic inflammation can lead to BIA is a significant unknown as both of these pathologic phenomena have been seen in neurodegenerative disorders. The blood brain barrier (BBB) is composed of a layer of brain microvascular endothelial cells (BMVEC) with support from other cells of the CNS, such as astrocytes and microglia. This barrier separates the systemic circulation from the abluminal, or brain, space. This novel project will be examining how the BBB deciphers chronic inflammatory signals, as this is the major site responding to systemic inflammation and regulating the transport of iron into the brain. Iron transport is mediated by two uptake proteins, ZIP8 and ZIP14, and one efflux protein, ferroportin (FPN). Hepcidin, an APP induced by inflammation, is well known to knockdown function of FPN. Therefore, this project also examines how IL-6 alters the hepcidin-FPN axis in hBMVEC. Western blot experiments suggest that treating hBMVEC with IL-6 for 2 hours does not change protein expression of ZIP8 and ZIP14. To examine changes in transporter localization, a ZIP14-GFP fusion plasmid was expressed in HEK cells and in the presence of LPS, calcium, and IL-6, more of this protein was seen at the plasma membrane compared to control. This correlated to an increase in ^{55}Fe uptake in ZIP14-GFP HEK cells and those treated with IL-6. Lastly, IL-6 increased ^{55}Fe apical to basal flux and decreased basal to apical flux, suggesting inflammatory signals have effects on iron flux across an hBMVEC barrier. IL-6 treatment was shown to induce hepcidin expression by qPCR and ELISA in hBMVEC. Treating with an inhibitor of hepcidin, fursultiamine, iron flux across the hBMVEC barrier was increased, suggesting a role for hepcidin in hBMVEC iron flux. Overall, this project proposes to highlight the mechanistic details connecting chronic inflammation and BIA, to provide information for the development of therapeutics to prevent toxic brain iron overload in associated pathologies.

Disclosures: S.L. Rosenblum: None. **D.J. Kosman:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.15/X15

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

Support: NIH Grant K01 NS129895-02
NIH Grant P30AI050409
Burroughs Wellcome

Title: Non-classical estrogen signaling via GPER suppresses LPS-induced neuroinflammationEstrogen Receptor Signaling in macrophages suppresses HIV-associated neurotoxic activity

Authors: *K. DAVIS[1], J. DE VASTY[2], K. WILLIAMS[2];
[1]Spelman Col., Chicago, IL; [2]Envrn. and Hlth. Sci., Spelman Col., Atlanta, GA

Abstract: Lipopolysaccharide (LPS) is a component of the cell wall of gram-negative bacteria and stimulates an acute inflammatory response and oxidative stress in human macrophages. Previous studies have used broad anti-inflammatory drugs to block the toxic effects of macrophage-induced inflammation, but these drugs have failed during clinical trials. We propose that utilizing the endogenous antioxidant mechanisms of macrophages may be more advantageous. Estrogen signaling has been shown to be neuroprotective through the classical estrogen receptors, estrogen receptor alpha and beta. However, the novel transmembrane receptor, G-coupled estrogen receptor (GPER1) has not been fully studied. We hypothesized that activation of GPER1 will reduce LPS-induced neuroinflammation. To test this hypothesis, we blocked GPER1 with a specific antagonist (G15) prior to LPS and GPER1 agonist, G-1 treatment. We assessed various downstream inflammatory and oxidative stress markers via western blot. Results showed a reduction in overall inflammation and oxidative stress in LPS-stimulated macrophages treated with G-1 compared to LPS-stimulated macrophages, alone, in a GPER1-specific manner. We also collected conditioned medium from macrophages after 1 day of stimulation. Conditioned media was then exposed to rat cortical neurons to assess neurotoxicity. LPS-conditioned media reduced neuronal numbers in culture. This neuronal loss was rescued in a conditioned medium from macrophages co-treated with G-1, in a GPER1 manner. Therefore, we hypothesize that targeting the GPER1 may be a possible therapeutic avenue for suppressing neuroinflammation in neurodegeneration disorders.

Disclosures: **K. Davis:** None. **J. De Vasty:** None. **K. Williams:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.16/X16

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

Support: WV-Inbre Grant P20GM103434

Title: Loss of mitoNEET leads to neuroinflammation and cognitive changes

Authors: *G. N. WILSON[1], K. MONAGHAN[2], J. HUBER[2], W. J. GELDENHUYS[2];
[1]West Virginia Univ., Morgantown, OH; [2]West Virginia Univ., Morgantown, WV

Abstract: MitoNEET (CISD1) is an [2Fe-2S] cluster containing protein located on the outer mitochondrial surface. The iron-sulfur cluster is redox active and is thought to act as pH and redox sensor for mitochondria, playing a role in bioenergetic regulation. The literature suggests that loss of mitoNEET leads to general mitochondrial dysfunction and iron dysregulation. Due to both of these factors playing a role in neuroinflammation, we evaluated the effect of loss of mitoNEET on neuroinflammation and cognition. CISD1 -/- mice were used at various timepoints through 12 months of age with the littermate controls. Brains were fixed and evaluated for immunohistochemical markers of neuroinflammation, including Iba1, GFAP, and silver staining

for neurodegeneration. We utilized a battery of behavioral assays, including spontaneous alternation in a Y-maze, spontaneous activity in an open field, rotarod, passive avoidance, and novel object detection. Our initial results indicated a significant increase in neuroinflammation, with increased levels of Iba1 and GFAP in the brains of mitoNEET knockout (KO) mice compared to the wild type control. Also, we found an increase in silver staining with mitoNEET KO. Lastly, we found significant changes in passive avoidance and other behavioral measures, indicating an age-dependent decrease in learning and memory capabilities of these KO mice beyond what is observed in age-matched controls. Taken together, we show here for the first time the increased presence of neuroinflammation in a mitochondrial mouse model alongside behavioral change. Further study is needed to gain understanding of the role mitoNEET plays and its putative role as drug target in cognition.

Disclosures: G.N. Wilson: None. **K. Monaghan:** None. **J. Huber:** None. **W.J. Geldenhuys:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.17/X17

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

Support: USDA Intramural

Title: Extracts from green leafy vegetables and berries, and their major polyphenolics, reduce inflammatory stress signals in HAPI rat microglial cells, in vitro

Authors: D. R. FISHER, S. N. MARSCHALL, T. ZHENG, *B. SHUKITT-HALE; USDA-ARS Human Nutr. Res. Ctr. on Aging, Boston, MA

Abstract: Diets supplemented with green leafy vegetables and berries have been shown to slow age-related cognitive decline. Kale and elderberry, for example, contain an array of bioactive phenolic compounds that may play a protective role due to their potent antioxidant and anti-inflammatory activities. Among these compounds, kaempferol is a flavonol found in green leafy vegetables and cyanidin-3-glucoside (C3G) is an anthocyanin found in elderberry. The purpose of this study was to determine if kale and elderberry, and their main flavonoid components, would be efficacious in reducing inflammatory stress signaling in microglial cells. Rat microglial (HAPI) cells were pretreated for 0.5-4 hours with kale or elderberry freeze-dried extracts (0-1.0mg/ml, Futureceuticals, Momence, IL), or the compounds kaempferol (0-100uM) or C3G (0-10uM) Sigma-Aldrich, St. Louis, MO). The cells were then stressed with 0 or 200ng/ml lipopolysaccharide (LPS) overnight and assessed for changes in nitric oxide production and iNOS expression. All compounds reduced LPS-induced nitric oxide production and iNOS expression in HAPI microglia in a concentration- and time-dependent manner ($p<0.05$). However, higher doses and longer treatment durations negatively affected cell viability. The

effects of the compounds on LPS-induced TNF-alpha and COX-2 production are currently being assessed. Therefore, these data suggest that kale and elderberry, and the flavonoid compounds in them, may be effective in reducing inflammatory stress-mediated signals, and this attenuation of inflammation may be important in age-related health maintenance.

Disclosures: D.R. Fisher: None. **S.N. Marschall:** None. **T. Zheng:** None. **B. Shukitt-Hale:** None.

Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.18/X18

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

Support: NIH/Arkansas INBRE P20GM103429

Title: Ferrostatin alters ATP production and activation of cultured microglia

Authors: *N. MCNAUGHTON[1], A. SCOTT[2], L. GENRY[3], D. DONLEY[4];
[1]Biol., Harding Univ., Nixa, MO; [2]Chem. and Biochem., [3]Biol., [4]Harding Univ., Searcy, AR

Abstract: Free radicals drive oxidation/reduction(redox)reactions that coordinate intracellularsignaling. Buildup of radicals and/ordysregulation of redox pathways causes oxidative stressthat damages cells. Microglial cells are Central Nervous System resident immune cells that aresensitive to oxidative stress at both the cellular and tissue level. Inflammation is associated withthis stress in microglia and their response to disease-associated stimuli such as amyloid beta(Aβ).Microglial dysfunction from long-term oxidative stress is implicated in the potentiation ofneurodegenerative diseases such as Alzheimer's Disease (AD)and is associated with elevatediron. However, the interaction between redox-active iron, oxidative stress, and microglialinflammation is unclear. To study this relationship, we treated cultured microglia with ferrostatin,a transferrin receptor inhibitor and putative radical scavenger, with and without Aβ stimulation.We found that ferrostatin decreases radical generation and suppresses activity of thepro-inflammatory enzyme, inducible nitric oxide synthase. This indicates that ferrostatin maypush microglia towards an anti-inflammatory state. Metabolism is a determinant of activationstate:pro-inflammatory states favor glycolysis and immuno-regulatory states favor oxidativephosphorylation. Consistent with suppression of inflammation, ferrostatin decreased Aβ-inducedglycolysis. Ferrostatin also increased basal oxidative phosphorylation and decreased maximalrespiratory capacity. These results suggest that iron acts as a regulator of microglialinflammatory state, perhaps via regulation of mitochondrial respiration. The exact mechanism(s)of how iron contributes to disease-associated inflammation in microglia is unclear;however, ourresults indicate that iron dysregulation promotes inflammation and modulates responses toextrinsic stimuli in part by altering the response to stimuli such as Aβ. Future research willelucidate how iron-sensitive genes impact metabolic balance in microglia.

Overall, our work
details the relationship between iron and microglial inflammatory responses and provides insightinto how iron, oxidative stress, and inflammation intersect to produce disease-associatedinflammation.

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Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.19/X19

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

Title: Comparison of human primary microglia and human iPSC derived microglia cells as in vitro models for microglia activation

Authors: *M. BSIBSI[1,2], K. LO[1], M. ZANELLA[1], L. GEERTS[1], S. KOSTENSE[1], J. DEGROOT[1], D. F. FISCHER[1], T. OOSTERVEEN[2], M. VLAMING[1];
[1]Charles River, Leiden, Netherlands; [2]Bit.bio, Cambridge, United Kingdom

Abstract: Microglia are resident immune effector cells in the CNS and play an essential role in neuroinflammation, ischemic and neurodegenerative disease. Therefore, microglia cells are considered a potential therapeutic target for neurodegenerative diseases. To fully understand the role of microglia, the preferred strategy would be to study primary human microglia isolated from post-mortem human brain tissue. Microglia can be isolated from both control and diseased human brain tissue with confirmed neuropathology. However, the obvious limitation on brain collection and yield of isolated cells restricts the ability to perform screening studies. Induced pluripotent stem cells (iPSCs)-derived microglia, may provide a suitable alternative for screening studies and large-scale compound validation. Yet, to effectively use iPSC-derived microglia, one must characterize the extent to which these cells faithfully represent biological processes in primary brain tissue. Here, we compared the gene expression and cytokine release from primary human microglia cells obtained from tissue provided by the Netherlands Brain Bank and iPSC-derived microglia. Exposure of primary and iPSC-derived microglia to LPS resulted in increased TNF-α secretion in a concentration and time dependent manner. LPS-mediated TNF-α secretion was strongly inhibited by dexamethasone. Priming of primary and iPSC-derived microglia primed with LPS and treatment with nigericin, a potent inflammasome activator, resulted in robust secretion of IL-1β and IL-18. Furthermore, nigericin induced IL-1β and IL-18 release was blocked by the inflammasome inhibitor MCC950 in both cell types. In addition, similarly to in house differentiated microglia, commercially available iPSC-derived microglia (bit.bio) showed a strong expression of specific markers as well as cytokine response upon LPS-treatment. Taken together, we successfully demonstrated that primary and iPSC-derived microglia respond similarly to LPS and nigericin treatment. For these reasons, these cell types could serve as a reliable tool for evaluating the potency and efficacy of prospective drugs for multiple

neurological diseases associated with microglia activation, such as Alzheimer's and Parkinson's Disease.

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Poster

PSTR203. Neuroinflammation: Microglia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR203.20/X20

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

Support: CIHR Grant PJT-173550

Title: Role of PARP-1/TRPM2 in regulation of modality-specific microglial phagocytosis

Authors: ***A. LAMONT**, M. F. JACKSON, T. KAUPPINEN;
Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Chronic neuroinflammation contributes detrimentally to the pathology of Alzheimer's disease (AD), wherein synaptic transmission is diminished, leading to severe cognitive decline. Microglia immune cells drive a number of neuroinflammatory responses, such as release of pro-inflammatory mediators and clearance of beneficial targets (such as pathogens), via phagocytosis. In AD, build-up of excessive amyloid-beta (Aβ) may lead to uncontrolled phagocytosis of aberrant targets such as healthy neurons or synapses. Previous work has shown that the microglial nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1), a driver of pro-inflammatory cellular functions, may also be involved in phagocytic regulation. Inhibition of PARP-1 has been shown to suppress aberrant phagocytosis (neuron targets) while not impacting beneficial phagocytosis (Aβ). However, phagocytosis of both targets has not been examined in the context of amyloidopathy to mimic AD. PARP-1 has also been shown to act in concert with Ca^{2+} permeable TRPM2 (Transient Receptor Potential Melastatin 2) channel, and inhibition of PARP-1 and TRPM2 can suppress nitric oxide (NO) release. In this study, we investigate whether amyloidopathy influences microglial phagocytosis of fluorescently labeled Aβ (FAM-Aβ) and if so, the extent to which PARP-1/TRPM2 signalling is involved. We used primary microglial cultures harvested at DIV 7-9. In basal conditions approximately 60% of microglia took up FAM-Aβ within 8 hours, with no significant change with inhibition of PARP-1 or TRPM2. Microglial 20 hour pre-treatment with Aβ oligomers (AβO) induced amyloidopathy and reduced percentage of cells taking up FAM-Aβ, as well as the amount of engulfed FAM-Aβ per cell ($P<0.05$, $N=4$). Inhibitors for PARP-1 (PJ34, 500 nM), or TRPM2 (JNJ, 1 μM) did not restore amount of FAM-Aβ engulfed. However, inhibition of PARP-1 and TRPM2 attenuated amyloidopathic inflammatory signalling (reduced release of NO and pro-inflammatory cytokines, while promoting release of anti-inflammatory cytokines) ($P<0.05$, $N=5$), microglial morphological transformation, and proliferation. This data suggests that PARP-1 and TRPM2 do

not have a role in uptake of Aβ in homeostatic conditions. Further, amyloidopathy compromises microglial ability to engulf Aβ, independent of PARP-1 or TRPM2. Thus, beneficial outcomes we report from inhibition of PARP-1/TRPM2 seen in AD mouse models are occurring without any changes in phagocytic Aβ clearance.

Disclosures: A. Lamont: None. **M.F. Jackson:** None. **T. Kauppinen:** None.

Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.01/X21

Topic: C.08. Ischemia

Support: Florida Department of Health # 20K09

Title: Post-stroke cognition is worsened by electronic cigarette exposure in male and female rats

Authors: *H. PRADHYUMNAN[1], S. H. PATEL[2], O. F. ALONSO[3], H. M. BRAMLETT[4,3,5], A. P. RAVAL[2,5];
[2]Dept. of Neurol., [3]Miami Project to Cure Paralysis, [4]Dept. of Neurolog. Surgery, [1]Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; [5]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Smoking is a preventable risk factor for stroke and battery-operated nicotine delivery systems known as electronic cigarettes (EC) have gained popularity in the past two decades. EC heat and aerosolize a solution of nicotine and chemicals forming harmful toxicants such as formaldehyde hemiacetal in the process. Understanding about the effects of EC vaping on stroke outcome is limited. This study investigated the effects of a 16-day EC exposure on stroke outcome and neurotransmitter metabolism in male and female rats. Sprague-Dawley rats (2-3 months old) of both sexes were randomly assigned either to air or EC (5% nicotine Juul pods) exposure using the EcigAero-TM Aerosol Exposure Apparatus. Rats were exposed for 16 nights. Per night, rats were exposed to 16 episodes of EC. Each episode consisted of 2 seconds of EC puffs followed by 8 seconds of air over the period of 8 minutes. After 16 days, the rats were divided into two cohorts. The first cohort of rats underwent brain collection for unbiased metabolomic (Metabolon Inc.) and cotinine level analyses (USDTL) after exposure. The second cohort of rats were subjected to transient middle cerebral artery occlusion (tMCAO; 90 min) or sham surgery and survived for 21 days. Beginning on day 14 post-surgery, rats were tested for learning and memory capacity using the Morris water maze followed by perfusion-fixation and brain collection for histopathological analysis. EC exposure significantly increased infarct volume in female, but not male animals. Water maze data indicated worsened post-stroke cognition in EC groups compared to air groups in rats of both sexes. The worsened cognition due to EC was more prominent in female than male animals. Metabolomic analysis indicated that EC exposure resulted in significant increases ($p<0.05$) in phenylalanine, tryptophan, and glutamate

metabolites, and both increases ($p<0.05$) and decreases ($p<0.05$) in histamine and tyrosine metabolites in the brains of female and male rats. EC vape exposure, even for as short as two weeks, impacts the metabolism of neurotransmitters, induces cognitive deficits, and worsens stroke outcomes in a sex dependent manner. Future studies investigating the impact of long-term EC usage are required to understand the chronic effects of vaping on the brain.

Disclosures: H. Pradhyumnan: None. **S.H. Patel:** None. **O.F. Alonso:** None. **H.M. Bramlett:** None. **A.P. Raval:** None.

Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

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Topic: C.08. Ischemia

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22TPA970253 AHA
CCDS Malcolm Feist Cardiovascular Research Postdoctoral Fellowship

Title: Protein arginine methyltransferase 4 can mediate cerebral circulation via adenosine receptor pathway in Alzheimer's disease

Authors: *M. S. B. UDO[1], G. A. CLEMONS[2], C. T. CITADIN[2], W. C. CARR[1], V. TESIC[1], H. W. LIN[1];
[1]Neurol., [2]Cell. Biol. & Anat., LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: Alzheimer’s disease (AD) is one of the most common neurodegenerative disorders covering about 70% of all cases of dementia. Recent studies suggest focal and global cerebral ischemia as one of the causes of AD development through which transient ischemic brain injury leads to massive neuronal death. Derangements in cerebral blood have been observed alongside tau and β-amyloid accumulation. Currently, there are no effective treatments available for AD or AD-related dementias, therefore, innovative therapies are needed. Our group has previously demonstrated that protein arginine methyltransferase 4 (PRMT4) is related to hypoperfusion in the brain of aged female 3xTg-AD mice (AD mouse model) and its inhibition (via TP064) can improve brain perfusion and memory. This enzyme methylates arginine residues to cause modifications in protein expression and function. Adenosine A2 receptor (A_2AR) plays a crucial role in brain vascular autoregulation and neurovascular coupling (NVC). PRMT4 and A2AR are both overexpressed in AD patients as well in aged 3xTg-AD mice. Since PRMT4 can interfere with protein expression and function our goal is to investigate PRMT4 as a regulator of A_2AR-mediated cerebral blood flow in the AD brain. Aged (12-month-old) male and female 3xTg-AD mice were treated or not (control) with TP064 (PRMT4 inhibitor - 30mg/kg) or Istradefylline

(A_2AR antagonist - 2mg/kg) for 7 days (i.p.) and we examined brain perfusion via laser speckle microscopy, blood-brain barrier (BBB) integrity and neurovascular coupling (NVC) through intra-vital two-photon microscopy, and memory task performance using T-maze, novel object recognition and new contextual learning. Our results suggest that specific inhibition of PRMT4 can also reduce the expression of A_2AR in the brain of treated mice. A_2AR antagonism increased the brain perfusion of aged AD mice, although male mice showed a more preeminent increase than females. Since female mice have increased expression of PRMT4 than males, our results suggest that the brain perfusion difference observed may be due to the overexpression of this enzyme. Thus, inhibition of PRMT4 can be related to reduced A_2AR expression and better brain perfusion. Istradefylline treatment also showed improved NVC and increased ZO-1 and occludin proteins, suggesting better BBB integrity. Treated mice also presented better functional performance. Taken together, our results suggest that PRMT4 and A_2AR are both involved in NVC and brain perfusion in AD pathology becoming new potential targets for AD treatment.

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Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

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Program #/Poster #: PSTR204.03/X23

Topic: C.08. Ischemia

Support: NIH Grant NS109588
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Title: Post-ischemic ubiquitination at the postsynaptic density reversibly influences the activity of ischemia-relevant kinases

Authors: L. DHAWKA[1], V. PALFINI[1], E. HAMBRIGHT[1], I. BLANCO[1], C. POON[1], A. KAHL[1], U. RESCH[2], R. BHAWAL[3], C. BENAKIS[1], V. BALACHANDRAN[1], S. ZHANG[3], C. IADECOLA[1], ***K. HOCHRAINER**[4];
[1]Weill Cornell Med., New York, NY; [2]Med. Univ. of Vienna, Vienna, Austria; [3]Cornell Univ., Ithaca, NY; [4]Cornell University: Weill Cornell Med. Col., New York, NY

Abstract: Posttranslational modifications with ubiquitin alter protein function and stability, thereby regulating cell homeostasis and viability, particularly under stress. Ischemic stroke induces detergent-resistant protein ubiquitination at the periphery of the ischemic territory, wherein cells remain viable (Hu 2001, Hochrainer 2012). Revealing the identity of ubiquitinated proteins, their cellular location, and the functional consequences of ubiquitin modification may

shed light on the role of ubiquitination in ischemic injury. Here, we employed a proteomics approach to identify proteins ubiquitinated following ischemic stroke. Cerebral ischemia was induced in male C57BL6J mice by middle cerebral artery occlusion (MCAO) using an intravascular filament. Detergent-insoluble fractions were prepared at 1hr reperfusion and ubiquitinated proteins in the ischemic neocortex were identified by ubiquitin-enrichment and subsequent nanoLC-MS/MS. We detected increased ubiquitination of 198 proteins, of which, according to GO enrichment analysis, many localize to the postsynaptic density (PSD) in neurons (FDR=$5.14x10^{-33}$, n=60 mice/group), which was biochemically confirmed by Western Blot ($p<0.0001$, n=5 mice/group). A significant number of ubiquitinated PSD proteins exhibit kinase activity (FDR=$1.02x10^{-23}$, n=60 mice/group) and include the kinases CaMKII, PKC, Pyk2 and Cdk5, whose aberrant activities contribute to post-ischemic neuronal death (Aronowski 1992, Aronowski 2000, Yan 2015, Gutierrez-Vargas 2017). We measured kinase activities at the PSD following stroke and found that CaMKII, PKC, and Cdk5 activities were decreased (for all: $P<0.0001$, n=6-9 mice/group), while Pyk2 activity was increased ($P=0.0213$, n=4 mice/group). This was accompanied by the hypo- and hyper-phosphorylation of downstream targets, respectively (for all: $P<0.05$, 4-5 mice/group). To test whether ubiquitination was accountable for changes in kinase activities, we removed bound ubiquitin by incubation of lysates with recombinant USP2 deubiquitinase. Removal of ubiquitin restored kinases' activity to pre-stroke levels (CaMKII: $P=0.0026$, n=6 mice; PKC: $P=0.0012$, n=8 mice; Cdk5: $P=0.0057$, n=6 mice; Pyk2: $P=0.0241$, n=4 mice), identifying ubiquitination as the responsible molecular mechanism for post-ischemic kinase regulation. These findings unveil a previously unrecognized role of post-ischemic ubiquitination in the regulation of essential kinases involved in ischemic injury, which could open a potential new avenue for the treatment of ischemic stroke.

Disclosures: **L. Dhawka:** None. **V. Palfini:** None. **E. Hambright:** None. **I. Blanco:** None. **C. Poon:** None. **A. Kahl:** None. **U. Resch:** None. **R. Bhawal:** None. **C. Benakis:** None. **V. Balachandran:** None. **S. Zhang:** None. **C. Iadecola:** None. **K. Hochrainer:** None.

Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.04/X24

Topic: C.08. Ischemia

Support: NIH Grant NS122808

Title: A single episode of hypoglycemia increases stroke risk in insulin-treated diabetic rats

Authors: *A. REHNI, S. CHO, A. P. RAVAL, K. R. DAVE;
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Abstract: Diabetes is a widespread disease responsible for a major part of global healthcare expenditure and mortality and morbidity worldwide. Diabetes is one of the prominent risk factors

for cardiovascular disease. Intensive antidiabetic therapy increases the risk of hypoglycemia in subjects with diabetes. Exposure to hypoglycemia increases stroke risk in subjects with diabetes[1]. Acute hypoglycemia (AH) exposure produces a prothrombotic effect[2]. We have previously shown that recurrent hypoglycemia (RH) exposure (one episode / day for 5 days) increases stroke risk in insulin-treated diabetic (ITD) rats. Next, we studied the effect of a single episode of hypoglycemia on stroke risk. Thus, in the present study, we determined how long the effect of AH on stroke risk lasts in young ITD rats of both sexes. Rats rendered diabetic using streptozotocin were treated with insulin 2-3 weeks after diabetes induction. ITD rats were randomly assigned to either ITD + AH + Glucose (hyperinsulinemic euglycemia: control) or ITD + AH groups (hyperinsulinemic hypoglycemia). Chi square test confirmed that the AH-exposed female group and the control group were balanced in terms of the proportion of rats at different stages of the estrous cycle. Stroke risk was evaluated using an in vivo model of thrombosis. Either 1 or 3 days after AH (for 3h), the jugular vein was connected to the carotid artery by a shunt containing a suture, and blood was allowed to flow for 15 minutes. The suture was then withdrawn and weighed to quantify thrombosis. The clot weights in the ITD + AH + Glucose and ITD + AH male groups quantified 1 day after hypoglycemia were 14 ± 1 mg (n = 7) and 20 ± 3 mg (n = 9), respectively. The clot weight in the male ITD + AH group was 48% greater ($p<0.05$) than in the control group. The clot weights in the ITD + AH male rats quantified 3 days after hypoglycemia (18 ± 1 mg, n=6) were not significantly different from the control group (16 ± 1 mg, n=7). Next, we confirmed if AH exposure also increases stroke risk 1 day after hypoglycemia in females. Clot weights in the ITD + AH + Glucose and ITD + AH female rats quantified 1 day after hypoglycemia were 15 ± 1 mg (n = 8) and 22 ± 2 mg (n = 9), respectively. The clot weight in the female ITD + AH group was 47% greater ($p<0.05$) than in the control group. Our data shows that AH increases stroke risk in ITD rats of either sex on day 1 post-exposure. In the future, we plan to identify the mechanism by which RH exposure increases stroke risk in diabetic rats. References: 1) Ann.N.Y.Acad.Sci. 2018;1431(1):25-34.; 2) Diabetes Care. 2018;41(12):2625-2633. Acknowledgement: NIH (NS122808).

Disclosures: **A. Rehni:** A. Employment/Salary (full or part-time):; University of Miami. **S. Cho:** A. Employment/Salary (full or part-time):; University of MiamiUniv of Miami School of Medicine. **A.P. Raval:** A. Employment/Salary (full or part-time):; Univ of Miami School of Medicine. **K.R. Dave:** A. Employment/Salary (full or part-time):; Univ of Miami School of Medicine.

Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.05/X25

Topic: C.08. Ischemia

Support: AHA 23IPA1054546
AHA TPA 1076915

Title: Tenascin C and Toll-like receptor-4 Interaction in Ischemic Stroke Pathogenesis

Authors: *B. CHELLUBOINA[1], V. ARRURI[1], S. MEHTA[3], T. CHO[1], R. VEMUGANTI[2]; [2]Neurolog. Surgery, [1]Univ. of Wisconsin, Madison, WI; [3]Neurolog. Surgery, Univ. of Wisconsin Madison, Madison, WI

Abstract: We recently demonstrated that Tenascin C (TNC) induced after acute stroke promotes inflammation. We currently evaluated the long-term outcomes and downstream mechanisms of TNC interaction with its putative endogenous receptor (Toll-like receptor-4; TLR-4) after stroke. Adult C57BL/6J mice subjected to transient middle cerebral artery occlusion (MCAO) were treated with TNC siRNA at 5 min of reperfusion. TNC knockdown decreased the infarct volume (assessed by T2-MRI) on 1 day of reperfusion and improved the motor function recovery (rotarod test and beam walk test)/cognitive function recovery (Morris Water Maze) between days 0 and 30 of reperfusion. Furthermore, the interaction of TNC with TLR-4 was investigated using wild-type (WT) and TLR-4 knockout (TLR-4 KO) mice subjected to MCAO for 1h and followed by administration of either TNC siRNA or Neg siRNA at 5 min after reperfusion and the interaction was assessed 24h after reperfusion. On 1 day of reperfusion, TLR-4 KO mice showed smaller infarcts than WT mice, and the infarct size was further reduced in TLR-4 KO mice with TNC knockdown. The TNC-TLR-4 interaction was investigated by coimmunoprecipitation 1 day after reperfusion. Our investigations showed that TNC knockdown after stroke showed long-term protection and improved post-stroke cognition. TNC knockdown reduced brain damage in TLR-4 KO mice, and coimmunoprecipitation confirmed the TNC and TLR-4 interaction. Our investigation concludes that TNC inhibition after stroke promotes long-term recovery, and TNC mediates post-stroke pathogenesis by partially interacting through TLR-4.

Disclosures: B. Chelluboina: None. **V. Arruri:** None. **S. Mehta:** None. **T. Cho:** None. **R. Vemuganti:** None.

Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.06/Y1

Topic: C.08. Ischemia

Support: NIH Grant R35NS132184
Department of Neurological Surgery, University of Wisconsin-Madison
Predoctoral fellowship, American Heart Association

Title: Circadian alignment of intermittent fasting is crucial for cerebral ischemic tolerance

Authors: *S. JEONG[1], A. CHOKKALLA[1], H. JEONG[3], V. ARRURI[1], B. KIM[1], T. V. ARUMUGAM[4], B. B. BENDLIN[1], R. VEMUGANTI[1,2,5];
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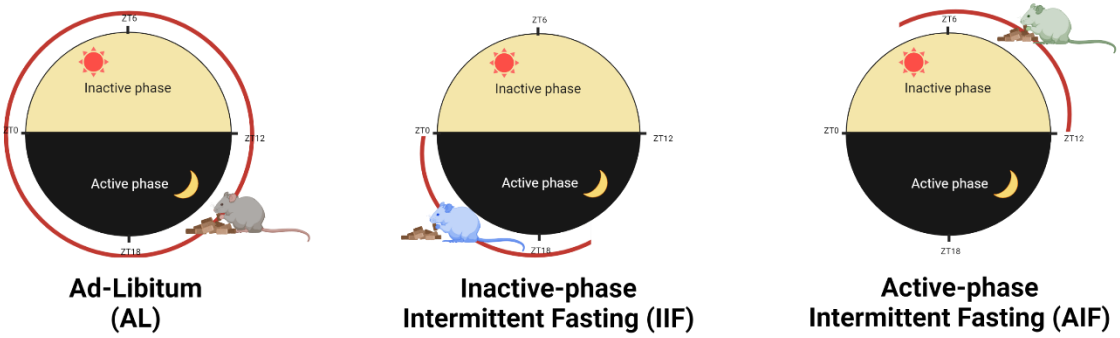
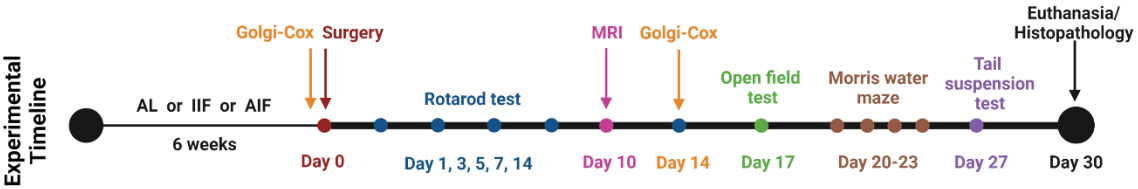
Seoul, Korea, Republic of; [4]La Trobe Univ., Melbourne, Australia; [5]William S. Middleton Mem. Veteran Admin. Hosp., Madison, WI

Abstract: The beneficial effects of prophylactic intermittent fasting (IF) on brain health are extensively highlighted in neurological diseases, particularly after stroke. IF preconditions the brain, promote neurogenesis and plasticity, and improves cognitive and motor functions after a stroke. Although the duration of fasting was shown to elicit different levels of neuroprotection after ischemic stroke, the impact of fasting time with respect to the circadian cycles is not yet explored. We currently tested if circadian alignment plays a role in IF-induced differential ischemic tolerance and to elucidate the differentially altered mechanisms. Circadian cycle-dependent IF paradigms were established in adult male and female C57BL/6J mice by fasting for 16 hours either during daytime (inactive phase IF) or nighttime (active phase IF). Following 6 weeks of active or inactive IF, mice were subjected to transient middle cerebral artery occlusion. Post-stroke tolerance was assessed by measuring brain damage (gray and white matter integrity) and neurobehavioral deficits (motor function, cognition, depression, and anxiety) from days 1 to 30 of reperfusion. Furthermore, transcriptomic profiling was performed to elucidate the putative mechanisms responsible for differential ischemic tolerance in the active versus inactive phase. Mice subjected to active phase IF showed better post-ischemic functional recovery compared to mice undergoing inactive phase IF, compared with ad libitum fed cohort. Moreover, circadian cycle-dependent IF is associated with broadly different transcriptomic alterations in the peri-infarct cerebral cortex. Together, our data indicate the regulation of brain preconditioning by the time of feeding and emphasize a crucial link between circadian cycle-dependent IF and ischemic tolerance.





Disclosures: **S. Jeong:** None. **A. Chokkalla:** None. **H. Jeong:** None. **V. Arruri:** None. **B. Kim:** None. **T.V. Arumugam:** None. **B.B. Bendlin:** None. **R. Vemuganti:** None.

Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.07/Y2

Topic: C.08. Ischemia

Support: AG052375
AG068155
AG070443
GM109098

Title: Endothelial cell deletion of tissue-nonspecific alkaline phosphatase exacerbates neurovascular unit dysfunction and alters circulating extracellular vesicle profiles in experimental ischemic stroke

Authors: *B. L. CLARY[1,2], C. B. BESWICK[2], B. PACHECO PEREIRA[2], D. KOMANDOORU[4], C. G. HERNANDEZ[2], B. D. KIRBY[2], M. AL-HUMAIRE[2], D. C. NWAFOR[2], D. DAKHLALLAH[2], C. M. BROWN[3];
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Abstract: Tissue-nonspecific alkaline phosphatase (TNAP) is an ectoenzyme that is known for its anti-inflammatory properties. Although TNAP is one of the most abundant enzymes in brain cerebral microvessels, its role in health and disease remains unclear. Our laboratory has established that cortical TNAP enzyme activity is diminished in cerebral microvessels in mouse models of ischemic stroke as well as models of sepsis and Alzheimer's disease. We have also employed pharmacological and genetic approaches to demonstrate that inhibition or conditional deletion of TNAP, respectively, in brain endothelial cells decreases vascular integrity. Therefore, we hypothesized that the loss of endothelial cell TNAP exacerbates post-stroke blood-brain barrier (BBB) integrity. To test this, we generated a transgenic mouse with condition of deletion of *Alpl,* the gene that encodes for TNAP, in endothelial cells (VEcKO mice) and compared the data to the littermate controls (*Alpl* $^{fl/fl}$ mice). Six-month old male and female mice were subjected to photothrombotic stroke and euthanized at seven days post-injury. No sex differences were observed. Cresyl violet staining and neurological scores confirmed that all stroke-injured mice exhibited larger infarct sizes and sensorimotor impairment compared to the sham counterparts; however, no differences were observed between genotypes. To assess vascular integrity, Perl's Prussian blue staining was used to quantify microhemorrhages in the stroke penumbra. These results showed that microhemorrhages were more abundant in the cortical penumbra in VEcKO mice compared to Alplfl/fl controls. We also observed that astrogliosis in the cortical penumbra was also elevated in in VEcKO mice compared to controls. To determine

whether the loss of endothelial cell TNAP contributed to systemic post-stroke dysfunction, we quantified the size and concentration of plasma extracellular vesicles (EVs) via particle tracking analysis and found that EV size was decreased and EV concentration was increased in VEcKO mice compared to littermate controls independently of stroke. Ongoing studies will investigate the cellular origin of circulating EVs as well as the functional properties of these EVs that regulate vascular integrity in brain endothelial cells. These results demonstrate that preservation of brain endothelial cell TNAP activity is essential for maintaining BBB integrity in ischemic stroke.

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Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.08/Y3

Topic: C.08. Ischemia

Title: Extracellular-vesicle mediated pyroptosis leads to gut-brain axis dysfunction after stroke in a mouse model of Alzheimer's Disease

Authors: *N. A. KERR[1], J. SANCHEZ-MOLANO[1], N. H. JOHNSON[2], J. DE RIVERO VACCARI[1], R. W. KEANE[2], H. M. BRAMLETT[1], W. DIETRICH[1];
[1]Neurolog. Surgery, [2]Physiol. and Biophysics, Univ. of Miami, Miami, FL

Abstract: Extracellular-vesicle mediated pyroptosis leads to gut-brain axis dysfunction after stroke in a mouse model of Alzheimer's Disease

Nadine A. Kerr, Juliana Sanchez-Molano, Nathan H. Johnson, Juan Pablo de Rivero Vaccari, Robert W. Keane, Helen M. Bramlett, W. Dalton Dietrich

Stroke is a known risk factor for Alzheimer's disease (AD). Both stroke and AD have similar risk factors, but the most common is age. Although several studies have highlighted that stroke is a risk factor for AD, there is less information that AD may be a potential risk factor for stroke. Gastrointestinal complications exist in both AD and stroke patients. Furthermore, stroke and AD patients with gut complications often present worsened neurological outcomes. Our laboratory has demonstrated that serum-derived extracellular vesicles (EVs) from mice that have undergone photothrombotic stroke (PTS) contribute to inflammasome-mediated cell death by pyroptosis in the gut after stroke. The goal of this study was to examine the role of EV-mediated pyroptosis in the bi-directional gut-brain axis after PTS in aged 3xTg mice and wildtype (WT) controls. Twelve-month 3xTg and WT male and female mice underwent PTS using a YAG laser. Lesion volume analysis was then performed at 1-month after PTS, showing increased infarct volume in the 3xTg-PTS mice compared to WT-PTS mice. At 24 hours after PTS, intestinal and cortical tissue was collected for western blot analysis for the following inflammasome proteins: caspase-

1, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), interleukin-1β, and Gasdermin-D (GSDMD). Results showed a significant increase in inflammasome proteins in both intestinal and cortical tissue in 3xTg- PTS mice compared to WT- PTS mice. Gut permeability was measured 72 hours post PTS using a FITC-dextran assay and results demonstrated that gut permeability was significantly increased in the aged 3xTg-PTS mice compared to WT- PTS mice. Moreover, Rotarod, Open Field, and Novel Object Recognition testing also showed that motor neurocognitive function was worsened in 3xTg-PTS mice compared to WT- PTS mice up to 2-week after ischemia, and stool-derived EVs isolated from 3xTg- PTS and sham mice presented increased levels of caspase-1 and Amyloid-β compared to WT-PTS and sham mice. Lastly, injection of stool-derived EVs collected from 3xTg-PTS mice into 3xTg-naïve mice induced inflammasome activation and pyroptosis in the cortex. Taken together, these results indicate an important role for EV signaling and pyroptosis in disruption of the bidirectional gut-brain axis after stroke in aged 3xTg mice in an animal model of stroke.

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Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.09/Y4

Topic: C.08. Ischemia

Support: R35NS132184

Title: Microrna mir-7 abundance in the post-stroke brain is regulated through the interactive actions of a network of rnas

Authors: *S. MEHTA, B. CHELLUBOINA, R. VEMUGANTI;
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Abstract: MicroRNA miR-7 abundance in the post-stroke brain is regulated through the interactive actions of a network of RNAs

Suresh L Mehta,[1] Bharath Chelluboina[1] and Raghu Vemuganti[1, 21]Department of Neurological Surgery, [2]William S. Middleton Veterans Administration Hospital, Madison, WI, USA

ABSTRACT: Transient focal ischemia rapidly and extensively alters the expression of many classes of noncoding RNAs (ncRNAs), including microRNAs (miRNAs), which play essential roles in regulating post-stroke brain damage and functional outcome. Particularly transient focal ischemia induces a sustained downregulation of miRNA miR-7, leading to derepression of its target α-synuclein (α-Syn), which promotes neuronal death. In the brain, circular RNA CDR1as regulate the abundance and function of mature miR-7. However, transient focal ischemia also significantly downregulated CDR1as levels in the peri-infarct cortex between 3h to 72h of

reperfusion, but there was no change in levels of primary miR-7 (pri-miR-7), suggesting that the decreased levels of mature miR-7 may be attributed to increased degradation after transcription. Thus, how miR-7 is regulated in the post-stroke brain is not known. Using bioinformatics analysis, we found a conserved high complementarity between long ncRNA Cyrano and miR-7. We further observed that Cyrano was upregulated and could access the free miR-7 in the ischemic brain leading to its degradation, whereas Cyrano deletion increases miR-7 levels in the ischemic brain. Thus, an intricate network of ncRNAs in the brain controls miR-7 abundance and function after a stroke.

Disclosures: S. Mehta: None. **B. Chelluboina:** None. **R. Vemuganti:** None.

Poster

PSTR204. Ischemia: Therapeutic, Interventional and Translation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR204.10/Y5

Topic: C.08. Ischemia

Support: Florida Department of Health # 20K09

Title: Altered phosphatidylcholine metabolism and ischemic stroke outcome in female rats

Authors: *S. H. PATEL[1], I. SAUL[1], K. R. DAVE[1], M. A. PEREZ-PINZON[1], A. P. RAVAL[1,2]; [1]Peritz Scheinberg Cerebral Vascular Dis. Res. Labs. (CVDRL), Dept. of Neurol., Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; [2]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Phospholipids are major structural lipids of the eukaryotic membrane. Phosphatidylcholine (PC) accounts for over 50% of total phospholipids in the cell and maintenance of PC homeostasis is critical for membrane integrity and cellular functions. A study demonstrated accumulation of PC metabolites in the brain of female rats due to nicotine (N) and oral contraceptives (OC) exposures. It is well documented that simultaneous usage of N and OC magnifies women's risk for stroke while combined N+OC exposure leads to severe ischemic brain damage in female rats. Since women tend to give up nicotine and OC prior to pregnancy, it is important to know the effects of N and OC withdrawal on the brain PC metabolism. Here, we hypothesize that N alone or in combination with OC withdrawal fails to rescue PC metabolism in the brain of female rats. Adult female Sprague-Dawley rats (n=8/group) were randomly exposed to either saline, N (4.5 mg/kg), OC, or N+OC in combination for 16-21 before being withdrawn from N+OC and allowed to recover for 30 days. Cortical tissue was then collected for unbiased global metabolomic (Metabolon Inc), qPCR and western blot analysis. A separate cohort of rats were exposed to either saline or N followed by N withdrawal (NW) for 30 days. At the end of 30-day recovery, rats were exposed to either transient middle-cerebral artery occlusion (tMCAO; 90 min) or sham surgery. Post-stroke cognition was tested with contextual fear conditioning at one month following tMCAO and subsequently the brains were collected for infarct

quantification. Pathway enrichment analysis indicated significant ($p<0.05$) increases in phosphatidylcholine (PC) metabolite levels such as 1-stearoyl-2-oleoyl-GPC, as well as fatty acids bound to carnitine viz palmitoleoylcarnitine accumulating even after N+OC withdrawal. Furthermore, fear-conditioning data revealed a significantly lower freezing time in all NW groups when compared to the saline group, implying that spatial memory deficits persist even after 30 days of NW. Lastly, the observed infarct volume was 16% ($p<0.05$) higher in the 30-day NW groups respectively, when compared to the saline group. Even after 30 days of N and OC withdrawal PC metabolomic changes fail to improve in the brain. The observed PC accumulation could inhibit post-translational palmitoylation of membrane proteins and synaptic vesicle formation, respectively, thus exacerbating ischemic brain damage in female rats by way of defective synaptic plasticity and neurotransmission.

Disclosures: **S.H. Patel:** None. **I. Saul:** None. **K.R. Dave:** None. **M.A. Perez-Pinzon:** None. **A.P. Raval:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.01/Y6

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant K22NS125179

Title: Formalizing preclinical TBI research with the Brain Injury Knowledge Ontology (BIKO) for traumatic brain injury

Authors: *M. C. SURLES-ZEIGLER[1], T. SINCOMB[3], F. T. IMAM[3], T. GILLESPIE[4], C. DIXON[5], J. S. GRETHE[2], A. R. FERGUSON[6], M. E. MARTONE[3];
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Abstract: Traumatic brain injury (TBI) is an insult to the brain from an external force and is a significant cause of morbidity and mortality in the United States. However, no effective clinical therapeutics currently exist for this injury. Although several therapies and procedures have been deemed successful in preclinical TBI research, they have yet to translate to human patients. One major hurdle to improving the efficacy of translational TBI treatment is the difficulty in recording and comparing methodological variance between and among published research studies in the same species, not to mention variance across multiple species required for translation. The initial steps to address these knowledge gaps include identifying methodological variance between studies and recording these differences into a machine-readable format. To support this, we are developing the Brain Injury Knowledge Ontology for TBI (BIKO) to create

standardized language to describe methods and outcome measures used within preclinical and clinical TBI therapy studies. BIKO complies with Open Biological and Biomedical Ontology (OBO) community best practices. It uses multiple knowledge sources to increase reuse and interoperability, including pre-existing terms within community ontologies, preclinical and clinical Common Data Elements (CDEs), augmented with new terms extracted from the literature as necessary. WebProtege, Protege, and the RDFLib python package were used to structure and build the ontology. The first version of BIKO focuses on formalizing the terminology to differentiate (1) the main preclinical TBI models (Controlled Cortical Impact Model, Fluid Percussion Model, Blast Injury Model, and Weight-Drop Model) based on major phenotypes and (2) behavioral assessment used to understand injury deficits. BIKO will provide a machine-readable way to represent the methodologies used in TBI therapeutic studies. BIKO will facilitate assembling a knowledge base about preclinical TBI models from the literature and other resources. As proof of concept, BIKO is being deployed for the PRE Clinical Interagency reSearch resourcE for Traumatic Brain Injury (PRECISE-TBI) Model catalog, a federally led interagency (VA, NIH, DoD) effort to develop queryable online resource for preclinical TBI model literature metadata.

Disclosures: **M.C. Surles-Zeigler:** None. **T. Sincomb:** None. **F.T. Imam:** None. **T. Gillespie:** None. **C. Dixon:** None. **J.S. Grethe:** None. **A.R. Ferguson:** None. **M.E. Martone:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.02/Y7

Topic: C.10. Brain Injury and Trauma

Support: DoD Grant W81XWH2211006

Title: Understanding the importance of stress as a pre-existing condition to traumatic brain injury

Authors: *J. B. STRICKLER[1], N. BOWYER[2], P. VANDEVORD[1];
[1]Biomed. Engin. and Mechanics, [2]Virginia Tech., Blacksburg, VA

Abstract: According to The American Institute of Stress, 77% of people experience stress that affects their mental health. There is considerable overlap between symptoms of stress and post-concussive symptoms such as: anxiety, irritability, impulsivity, attention deficits, depression, and insomnia. Despite the ever-rising incidence of stress and anxiety related disorders, the neurological changes as a result of stress have been commonly excluded in the study of other neurological diseases and injury. By treating the neurological effects of stress as a “preexisting condition” we hypothesize that exposure to an acute unpredictable stress (AUS) prior to a blast-induced traumatic brain injury (bTBI) will result in differential molecular and behavioral changes when compared to stress or bTBI alone.

Our model used male Sprague-Dawley rats (~300g) that were randomly assigned to one of 4 groups: unstressed-uninjured control (Con), stress only (AUS), bTBI only (BL), or stress + bTBI (AUS+BL). AUS consisted of 3 days of variable, unpredictable stress which includes restraint stress (day 1), predator urine exposure (day 2), and forced swim (day 3). AUS was followed by blast exposure consisting of 1 blast per day for 3 days. All behavioral testing occurred 27-30 days post injury (DPI). Open field (OF) and elevated plus maze (EPM) were used to assess anxiety-like behavior while 3-chamber sociability (3-CS) and social novelty (3-CSN) were used to assess depressive-like behavior. Amygdala and hippocampal tissue were extracted at 30 DPI after the last round of behavioral testing. To determine differential gene expression patterns between exposure groups the tissue was process and mRNA collected for qPCR.
At 1-month post-injury, the combination of AUS and bTBI was sufficient to cause behavioral changes. The EPM results indicated that AUS+BL animals behaved more similarly to the AUS only animals with increased time per entry into open arms. OF testing revealed AUS+BL had an additive affect when it came to anxiety-like behavior with only these animals representing a significant decrease in number of entries and total time spent in the center of the arena. AUS+BL animals also showed significantly different behavior from BL only animals in both the 3-CS and 3-CSN tests. The combination of AUS+BL also had an additive affect when it came to astrocytic reactivity and microglial activity in both the amygdala and hippocampus. Our data suggests that the neurological changes driven by stress could play a significant role in the behavioral and molecular outcomes of bTBI.

Disclosures: **J.B. Strickler:** None. **N. Bowyer:** None. **P. VandeVord:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.03/Y8

Topic: C.10. Brain Injury and Trauma

Support: I01RX002705

Title: Tbi induces chronic hippocampal-amygdala circuit alterations during a contextual fear conditioning paradigm

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Abstract: Traumatic Brain Injury (TBI) and Post-Traumatic Stress Disorder (PTSD) are two of the most common conditions affecting Veterans. However, little is known about the comorbidity of these two disorders, or how they interact and progress following injury. To study the effect of injury on fear memory circuits during acquisition and extinction, we subjected rats to lateral fluid percussion injury (FPI), creating unilateral mild to moderate TBI. Animals were run through

contextual fear conditioning (CFC) at a chronic time point 6 months post injury (n=7) or sham surgery (n=8). Two rats from each group were implanted with drivable, active 64 channel probes in the hippocampus (HC) and amygdala to obtain electrophysiology recordings during CFC. We saw profound changes in the electrophysiology of the chronic post TBI animals.Our data indicate that single unit activity was greatly affected at this time point, with dramatic increases in the HC, and a loss of activity in the amygdala. HC power in the theta range remained significantly decreased as at acute points. In the amygdala, there was corresponding loss of theta as well as a loss of gamma in the 40-80Hz range, which was increased post acquisition in both sham and injured animals. Oscillatory coherence between HC and amygdala differed in injured rats at theta, and was greater after acquisition, suggesting loss of HC theta is compounded by a desynchronization of theta between HC/amygdala. Following acquisition there was also a loss of coherence between HC and amygdala in the gamma range, which may be significant for encoding as well. The total loss of phase amplitude coupling between theta and gamma seen in HC acute data was also confirmed at this chronic injury timepoint. In addition, PAC can be calculated between structures, for example to see how much gamma amplitude in the amygdala is modulated by the phase of HC theta oscillations. PAC between HC theta phase and amygdala low-gamma amplitude (~30Hz) was present in the sham animals and strengthened post acquisition. A profound difference is present in the injured animals, where theta phase was coupled to high gamma (~85Hz) amplitude instead, which decreased post acquisition.Overall, changes in oscillatory behavior both within and between HC and amygdala suggests a loss of coordination of theta and gamma at chronic time points post TBI. In combination, behavioral alterations in CFC extinction, and changes in HC, amygdala, and their interactions, suggest an underlying substrate for dysfunction in limbic circuitry in chronic TBI. Profound changes in theta-gamma coupling, as well as its downstream effects, suggest that neuromodulatory restoration of PAC in the HC may aid extinction.

Disclosures: **E.R. Halter:** None. **E. Mirzakhalili:** None. **C. Adam:** None. **J.A. Wolf:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.04/Y9

Topic: C.10. Brain Injury and Trauma

Support: NIH NINDS K08NS101122
UVA Children's Hospital

Title: Motor Deficits Following Neonatal Hypoxic-Ischemic Brain Injury

Authors: *M. MARLICZ[1], *M. MARLICZ[2], W. MATYSIK[3], S. LEE[3], J. KAPUR[4], J. BURNSED[5];
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Abstract: Motor deficits following neonatal brain injury, from cerebral palsy to subtle deficits in motor planning, can have detrimental impact on children's lives yet often remain underreported. Rodent models of neonatal hypoxia-ischemia (HI) allow better understanding of underlying mechanisms of motor deficits and novel neuroprotective strategies testing. Here we test complex motor performance and learning in a mouse model of neonatal HI.We induce HI on postnatal day (p) 10 C57Bl6 mice through unilateral carotid ligation & 60 min 8% O2, while sham mice only undergo incision + anesthesia. Complex motor performance and learning is assessed on p30 using the accelerating rotarod and complex running wheel tasks. For rotarod, mice are placed on rotating cylinders, speed gradually increases. Over 3 training days of 10 trials/day, we compare latency to fall across trials and days to assess short and long-term performance. For the complex running wheel, mice have continuous access to a simple wheel (gross motor test) followed by a complex wheel (complex motor test, pattern of spokes removed) x4 days each. Avg time/wheel rotation is measured across days for each portion of the task. Results show significant differences in rotarod performance between HI and sham groups on days 1, 2 & 3 (n=19, day1 p=0.0146, day2 p=0.0088, day3 p=0.0347; unpaired t-test). Sham mice exhibit greater improvement across days compared to HI mice (n=7, p=0.0046; linear regression). There are no differences in simple running wheel performance between 2 groups (n=5/group, day1 p=0.7, day 4 p=0.6; unpaired t-test). On the complex wheel, HI mice perform slower than sham on day1 & 2 (n=5/group, day 1 p=0.02), but by day 4 are similar (p=0.67). Overall, we demonstrate significant deficits in complex motor performance and learning in young adult mice exposed to neonatal HI, with no gross motor deficits present. This model reflects motor deficits observed in humans who experience neonatal HI. Ongoing work examines changes in our model's motor circuit to further understand the underlying mechanisms of motor deficits in this population.

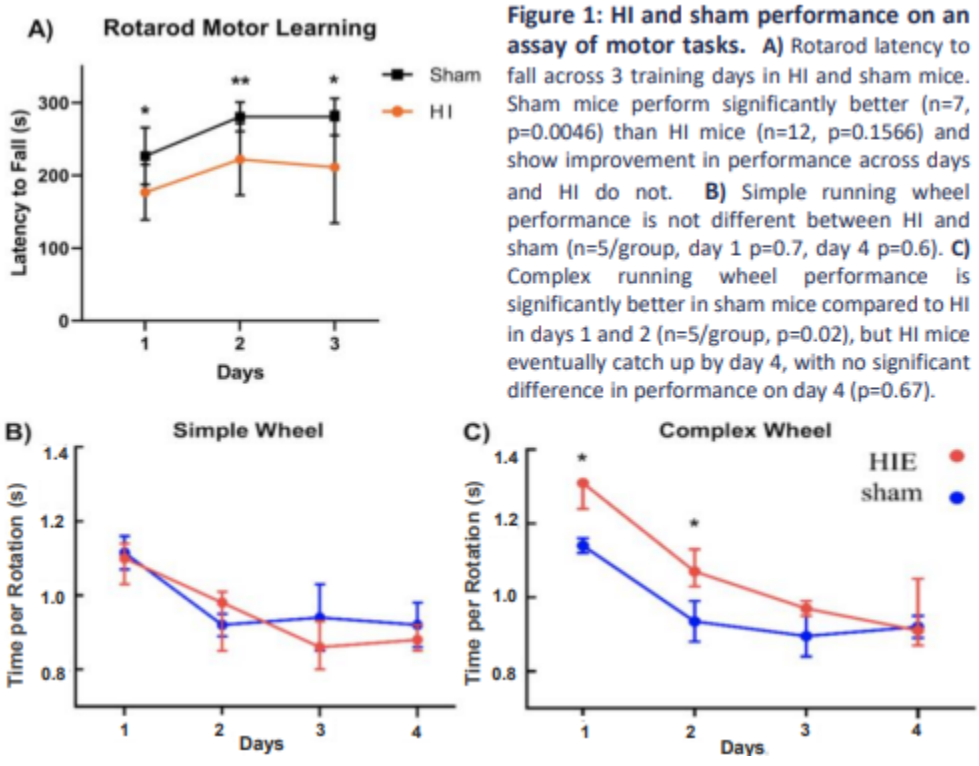

Figure 1: HI and sham performance on an assay of motor tasks. **A)** Rotarod latency to fall across 3 training days in HI and sham mice. Sham mice perform significantly better (n=7, p=0.0046) than HI mice (n=12, p=0.1566) and show improvement in performance across days and HI do not. **B)** Simple running wheel performance is not different between HI and sham (n=5/group, day 1 p=0.7, day 4 p=0.6). **C)** Complex running wheel performance is significantly better in sham mice compared to HI in days 1 and 2 (n=5/group, p=0.02), but HI mice eventually catch up by day 4, with no significant difference in performance on day 4 (p=0.67).

Disclosures: **M. Marlicz:** None. **M. Marlicz:** None. **W. Matysik:** None. **S. Lee:** None. **J. Kapur:** None. **J. Burnsed:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.05/Y10

Topic: C.10. Brain Injury and Trauma

Title: Traumatic brain injury: Delayed appearance of functional deficits after closed head diffuse axonal injury in mice

Authors: *M. I. SCHEUBER, L. RODRIGUEZ PERIS, S. MARTINS, H. SHAN, M. E. SCHWAB;
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Abstract: Closed skull severe concussion is the most frequent form of traumatic brain injury (TBI) with external forces leading to the acceleration and deceleration of the brain, causing small lesions across multiple brain regions. We established a closed-head axonal injury model in adult C57Bl/6J mice (modified Marmarou model) using a vertical weight drop on the laterally fixated head of the mice supported by a sponge. Comparable to clinical symptoms in humans, we found transient as well as long lasting functional deficits in motor ability and coordination (narrowing beam and rotarod), sociability and social novelty (3 chamber setup, measuring interaction preference between a paper ball and a mouse, followed by familiar vs. unfamiliar mouse), emotional and anxiety behavior (elevated plus maze with open and closed arms), and spatial orientation, learning and long-term memory as well as memory plasticity (Barnes maze). Interestingly, we found major differences in the time course of the manifestations of the deficits: After a stroke or a spinal cord injury, behavioral deficits are immediate, and their maximal extent is seen at acute, e.g. 3-7 day time points. Very much in contrast, we found no pronounced functional deficit within the first days post-TBI induction (2-4 days), but rather a gradual decrease in their cognitive and even locomotor abilities reaching the largest deficit around 14 up to 35 days post-injury. These findings indicate that the functional deficits after a diffuse axonal TBI are caused by mechanisms which are different from the large mechanical or ischemic lesions. They may include inflammatory cascades leading to local tissue impairments, small vessel deficits leading to local ischemias and problems of neuronal or synaptic survival and/or maladaptive processes of surviving neurons resulting in circuit disruptions. Ongoing histological analysis of the brains 7 days and 21 days post-TBI induction will give further insight into the localization and development of anatomical changes after injury.

Disclosures: **M.I. Scheuber:** None. **L. Rodriguez Peris:** None. **S. Martins:** None. **H. Shan:** None. **M.E. Schwab:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.06/Y12

Topic: C.10. Brain Injury and Trauma

Support: R01 NS120099
R37 HD059288

Title: Effect of sex and mild traumatic brain injury on anxiety behaviors with and without a branched chain amino acid dietary therapy

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Abstract: Every year, over 2.5 million Americans are affected by traumatic brain injury (TBI). Many of these victims also experience psychiatric comorbidities such as anxiety disorders, with female patients experiencing more of these pathologies following injury. Despite this discrepancy by sex, there is a significant research bias in favor of male animal subjects in neuroscience which restricts our understanding of the sexual dimorphism in the mechanism(s) and behavioral consequences of TBI. While there is some animal research on sex-based differences in TBI, the results are limited and inconsistent. To help fill this knowledge gap, the goal of this project is to investigate the potential correlation between sex and anxiety behavioral differences following TBI. This scientific question was examined by using a well-known mouse model of TBI known as lateral fluid percussion injury (LFPI), followed by a rodent behavioral test using both male and female subjects called the Elevated Plus Maze (EPM). This behavioral paradigm can operationalize anxiety behavior and the project examines the effect of sex and injury condition on the presence and degree of these behaviors. The results of this study revealed differences in anxiety within the female animals. That is, female rodents spent a lesser proportion of total time in the open arms of the maze which represents more psychiatric symptoms for anxiety due to more exposure in these areas, and injured female animals further demonstrated more anxiety than the sham cohorts. This is consistent with prior research in the Cohen Laboratory. In the second part of this project, given the differences between female animals only, strictly female mice were placed on a branched chain amino acid dietary therapy. The EPM results revealed that female sham animals had no difference in anxiety from female injured animals all on diet, but when comparing injured females on therapy to those without it, there are more inconsistent differences from the literature. In addition, in both parts of this research, there were no differences in motor capabilities across any of the study groups represented by average velocity and total distance travelled in the maze.This indicates that the presence of motor deficits in injured animals is not a confounding factor. Since the overall number of animals used in these experiments is relatively small, directions for future research could involve further attempting to find differences in experimental groups. This could occur by increasing the number of animal subjects or investigating the dietary methodology more in-depth, among exploring additional behavioral-paradigms such as cue-based fear conditioning.

Disclosures: **A.S. Cohen:** None. **M.L. Fersten:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.07/Y13

Topic: C.10. Brain Injury and Trauma

Support: NICHD Grant R37HD059288
NICHD Supplement 206180424-S

Title: Catherine Ubri

Authors: *C. UBRI[1,2], A. FARRUGIA[2], A. COHEN[2];
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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability in children and adults in the United States. 10-15% of mild TBI (mTBI) survivors develop neuropsychiatric disorders such as posttraumatic stress disorder, making them a significant public health concern. Notably, an inability to suppress fear and override fearful memories lies at the core of many neuropsychiatric disorders. This ability, known as fear extinction, is essential to mental health. Fear extinction requires learning and remembering that a fear-evoking object or situation is nonthreatening after it is repeatedly presented without an aversive consequence, thereby creating a retrievable extinction memory. The ability to retrieve fear extinction memories relies on the infralimbic cortex (IL) subregion of the mPFC. Data suggests that the potentiation of IL neurons is necessary for fear suppression and the retention of fear extinction memories. While previous research shows fear extinction is impaired after mTBI in both humans and rodents, little is known of how the IL responds to mTBI. Using a well-established mouse model of mTBI, this work aims to determine whether mTBIs disrupt the IL neurocircuitry responsible for fear extinction. Preliminary data in male mice suggests that during the fear extinction retrieval test session, injured mice freeze more than sham. We further predict injured mice will show reduced IL network activity, a failure to generate long-term potentiation, and reduced excitability in IL neurons. This work begins to outline the mechanism of injury-induced fear-based neuropsychiatric disorders, and lays the groundwork for the development of a treatment for mTBI survivors.

Disclosures: **C. Ubri:** None. **A. Farrugia:** None. **A. Cohen:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

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Topic: C.10. Brain Injury and Trauma

Support: DOD Grant TP210043
New Jersey Commission on Brain Injury Research CBIR20PIL004
New Jersey Commission on Brain Injury Research CBIR19IRG025

Title: Sex differences in risk/reward decision making and prefrontal catecholamine regulation following repetitive mild traumatic brain injury

Authors: *C. KNAPP[1], E. PAPADOPOULOS[1], S. B. FLORESCO[2], B. D. WATERHOUSE[1], R. L. NAVARRA[1];
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Abstract: Mild traumatic brain injury (mTBI) can disrupt cognitive processes leading to increased risk-taking behavior. Currently, little is known regarding the effects of repetitive injury (rmTBI) on risk/reward decision making or whether these outcomes are sex specific. Here we examined how rmTBI affects risk/reward behavior in rodents using a well-established probabilistic discounting task (PDT). Rats were trained in the PDT, exposed to sham surgery (no mTBI), a single injury, or a series of three closed-head control cortical impact injuries within 1 week, and then tested for 4 weeks. RmTBI increased risky choice preference in females, but not males, during the first week post-injury. However, following initial uncertainty of reward delivery, rmTBI produced chronic increases in reaction times in males only. The medial (mPFC), orbitofrontal (OFC), and anterior cingulate (ACC) regions of the PFC play prominent roles in risk/reward decision making. Previous reports have demonstrated catecholamine imbalances within the PFC following TBI, which may underlie TBI-induced behavioral outcomes. To further investigate our observations and potential mechanisms of catecholamine imbalance within the PFC, Western blotting was used to measure levels of catecholamine synthetic, packaging, degradation, and reuptake transporter proteins in PFC sub-regions 48 hours following rmTBI. In the OFC, rmTBI altered protein levels that suggest decreased catecholamine packaging, storage, & release, along with reduced clearance and degradation. Interestingly, levels of the degradation enzyme, MAO-A, were specifically reduced in female rats. This sex-specific decrease of MAO-A serves as a novel variable to further elucidate differential rmTBI-induced mechanisms of increased risk preference.

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Poster

PSTR205. Brain Injury: Behavioral Studies

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Program #/Poster #: PSTR205.09/Y15

Topic: C.10. Brain Injury and Trauma

Support: NINDS NS110898

Title: Sex differences in depression-like behavior and limbic PACAP expression following repetitive mild TBI in adolescent rats

Authors: *C. R. MARTIN, T. A. MCCORKLE, J. R. BARSON, R. RAGHUPATHI; Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Mild traumatic brain injury (TBI) is the most common type of TBI, and adolescents are particularly susceptible to concussions, because of participation in contact sports. While boys have been shown to experience cognitive deficits following mild TBI, girls are more likely to report deficits in affect such as depression and anxiety. Preclinical models of sports-related concussions have demonstrated that female, but not male, rats exhibit depression-like behaviors into adulthood following repeated mild TBI in adolescence. The present study focused on pituitary adenylate cyclase-activating polypeptide (PACAP), a stress-related neuropeptide implicated in depression and highly expressed in the limbic paraventricular nucleus of the thalamus (PVT). Adolescent male and female Long-Evans rats were anesthetized and subjected to 3 repetitive mild closed-skull impacts over 7 days (brain-injured males $n = 8$, females $n = 10$) or anesthetized 3 times without injury (sham-injured males $n = 7$, females $n = 8$). At 4 - 5 weeks post-injury, all animals were tested in an object recognition memory and forced swim test, and they were then sacrificed two days later for examination of gene expression using quantitative real-time PCR. Both male and female brain-injured rats exhibited deficits in object recognition memory ($p < 0.05$ compared to sham-injured rats); however, only female brain-injured rats displayed a significant increase in immobility/behavioral despair in the forced swim test compared to sham-injured male and female rats ($p < 0.05$). Brain-injured females also displayed a 162% increase in levels of PACAP mRNA in the PVT compared to their sham-injured counterparts ($p < 0.05$), while injured male rats only displayed a 29% increase in PACAP mRNA compared to sham-injured male rats (not significant). These data suggest that PACAP expression in the PVT may regulate depressive-like behavior following repeated mild TBI. Ongoing studies seek to evaluate the effect of repeated mild TBI in the nucleus accumbens, which is implicated in depression-like behaviors and is a major target region for the PVT.

Disclosures: C.R. Martin: None. **T.A. McCorkle:** None. **J.R. Barson:** None. **R. Raghupathi:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.10/Y16

Topic: C.10. Brain Injury and Trauma

Title: Mild traumatic brain injury impairs motor function but spares short term memory

Authors: *G. UMOREN[1], O. A. TIJANI[1], A. J. IDOWU[2];
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Abstract: Traumatic Brain Injury (TBI) affects an estimated 10 million people worldwide every year. The well-characterized Marmarou impact acceleration model creates an in vivo repetitive TBI model that accurately typifies the clinically seen effects. The cerebral cortex and the hippocampus which are crucial areas for motor and memory function are especially susceptible to TBI.
We assessed the outcomes of mild traumatic brain injury (mTBI) on locomotor activity and short term memory in female mice. Twelve female mice were divided into two groups, each group with six mice. Control mice had no mTBI induced (n=6) while the test group (n=6) were induced with mTBI using the weight drop model.
In the Open Field Test, using the Anymaze behavioral tracking software to track and analyze locomotor activity, mTBI induced mice had significant reduction in total distance covered, average speed and the number of line crossings, providing a basis for impaired motor function in the mTBI group.
Furthermore, in the Novel Object Recognition Test, using the Anymaze Behavioral Tracking Software, the percentage investigation time in the mTBI group was more than 50%, showing an improved memory function. Additionally, the mTBI group had a positive discrimination index, which represents an improved short term memory compared with the control mice. We conclude that mild traumatic brain injury impaired motor function but preserves short term memory.

Disclosures: G. Umoren: None. **O.A. Tijani:** None. **A.J. Idowu:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

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Program #/Poster #: PSTR205.11/Y17

Topic: C.10. Brain Injury and Trauma

Support: R01 = R01NS101108
PTE MERIT = I01RX003498

Title: Touchscreen visuomotor associative learning tasks for cognitive assessment following TBI in pigs

Authors: *S. PENNACCHI[1], T. ALLEN[4], O. E. FRUCHET[5], E. R. HALTER[1], M. ROSALINO[2], E. MIRZAKHALILI[2], C. D. ADAM[6], J. A. WOLF[3];
[1]Neurosurg., [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Univ. of Pennsylvania, Wayne, PA; [4]Florida Intl. Univ., Miami, FL; [5]Neurosurg., NIH, Bethesda, MD; [6]Univ. of Pennsylvania Neurosci. Grad. Group, Philadelphia, PA

Abstract: We have designed and adapted two touchscreen tasks for use with Yucatan mini pigs in which pigs are trained to associate visual stimuli with specific touch responses. Both tasks are visuomotor associative learning paradigms developed to assess cognitive changes post-traumatic brain injury (TBI). We've begun testing these paradigms in pigs that have received a cortical contusion injury (CCI) and have seen early deficits between injured and sham animals. In the Conditional Association Task (CAT), the pig is presented with one of three random stimuli, then forced to choose the right or the left box on the touchscreen. Two images are “fixed” trials in which a correct response occurs when the pig selects either the right or the left box on the touchscreen (one image is associated with left and the other to right). The third image is a conditional trial where the pig must remember the result of the previous “fixed” trial and choose the opposite response. Performance is determined by the percentage of correct responses (fixed and conditional) and time to reach criteria (70% correct on conditional trials and 95% correct for fixed trials). After reaching criteria, pigs underwent CCI or sham injury and performance was assessed over time. The sham animal quickly regained both fixed and conditional responses, but the injured animal took 20 sessions to regain the fixed trial criteria, and never again reached criteria on conditional trials. In addition to the CAT, the Delayed Match to Sequenced Sample (DMTSS) task has both a match to sample and a match to sequence portion. Pigs are trained initially to choose a random stimulus image out of a group of four image choices. Once an animal is fully trained on a simple sample matching task (>85% correct answers), there are two ways to increase the difficulty of the task. First, a variable time delay can be added between the stimulus presentation and the response screen. Second, the animal must remember and reproduce a sequence of two stimuli instead of a single stimulus image. Finally, both these items can be combined into a delayed sequence matching task. Pigs were trained on a delayed sample matching task with a variable delay from 1 to 7 seconds. Injured animals trained on the DMTSS task had a drop of over 10% in their accuracy post-injury, which took a week of training to recover. This preliminary data suggests these spatial, episodic, and frontal-hippocampal dependent memory tasks may detect changes in hippocampal/limbic circuitry after CCI.

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Poster

PSTR205. Brain Injury: Behavioral Studies

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Program #/Poster #: PSTR205.12/Y18

Topic: C.10. Brain Injury and Trauma

Support: Funding Source: National Institutes of Health (R01-NS119472 to HIC)

Title: Novel visual detection task to measure behavioral deficits in rats following traumatic brain injury

Authors: *M. A. SJOHOLM[1], J. GERMI[1], N. SESHADRI[1], P. M. HARARY[1], D. JGAMADZE[2], J. WOLF[2], H.-C. I. CHEN[2];
[2]Neurosurg., [1]Univ. of Pennsylvania - Perelman Sch. of Med., Philadelphia, PA

Abstract: Injuries to the cerebral cortex significantly contribute to the long-term neurological disability commonly seen after traumatic brain injury (TBI). Deficits in the function of sensory systems are understudied in TBI. Animal models present the opportunity to investigate the behavioral effects of these sensory deficits, setting the stage for understanding the impact of interventions on functional recovery. Here, we developed a visual detection task for rodents based on drifting grating stimulation, which are known to evoke strong visual responses. Male Long Evans rats (6-8 weeks old) were trained in an operant chamber to detect drifting grating versus grey screen visual stimuli presented on a touchscreen. Rats selected a side of the touchscreen based on the stimulus being presented and received a milk reward for correct responses. The contrast (3.125, 15, 25, 40, and 100%) and spatial resolution (5 and 12 cycles per screen) of the drifting gratings varied randomly across trials. After establishing baseline performance metrics, rats received a controlled cortical impact (CCI), fluid percussion injury (FPI), or sham craniotomy (as control) to the visual cortex (-5 mm anterior/posterior, and -2.5 mm medial/lateral from bregma). All craniotomies were 5 mm in diameter and performed on the right hemisphere. The CCI used a cylindrical tipped impactor 3 mm in diameter, operating at 2.5 m/sec, with a penetration depth of 2 mm. The FPI used an impact force consistent with a mild TBI. Rats were then tested on visual detection task 1, 2, 4, and 8 weeks after intervention followed by sacrifice to determine the histological nature of the injury. Preliminary analysis indicates decreased accuracy on the visual detection task 1 week after injury with gradual recovery at subsequent timepoints. Ongoing analysis on this dataset is investigating whether changes in drifting grating spatial resolution modify the difficulty of the task. Subsequent iterations of this study will focus on accentuating the injury effect via either increasing the injury severity or difficulty of the task (e.g., random dot kinematogram). Ultimately, we intend to apply this task to the assessment of functional recovery after TBI in response to repair interventions. While this methodology is geared toward studying visual system injury, it may be adapted to address injuries to other cortical systems such as those governing attention, memory, or learning.

Disclosures: M.A. Sjoholm: None. **J. Germi:** None. **N. Seshadri:** None. **P.M. Harary:** None. **D. Jgamadze:** None. **J. Wolf:** None. **H.I. Chen:** None.

Poster

PSTR205. Brain Injury: Behavioral Studies

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Topic: C.10. Brain Injury and Trauma

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UPR RCM Chancellor's Office
UPR Deanship of Medicine
R21ES034191

Title: Influence of closed head injury on emotional states and pain-related behaviors

Authors: L. VICENTE-RODRÍGUEZ[1], Y. ALICEA-TORRES[1], N. GONZÁLEZ-MARCANO[1], G. SERRANO-RIVERA[1], D. NAZARIO-MARTÍNEZ[1], P. VÁZQUEZ-MARTÍNEZ[1], O. MARTÍNEZ-GUZMÁN[2], *D. SIERRA-MERCADO[2];
[1]Biol., Univ. of Puerto Rico Cayey Campus, Cayey, PR; [2]Anat. & Neurobio., Univ. Puerto Rico Sch. of Med., San Juan, PR

Abstract: Concussive brain injury has received increased interest as a public health concern in the United States. Concussion results in axonal damage that disrupts communication between brain regions, inducing negative emotional states, such as increased anxiety-like behaviors. Moreover, concussion leads to the development of somatosensory impairments, namely pathological pain that occurs distant from the site of injury. The periaqueductal gray (PAG) and the dorsal raphe nucleus (DRN) are adjacent midbrain structures necessary for the processing of emotions and pain. We hypothesized that neuronal activity in these two brain regions will be affected by concussive brain injury, thus influencing emotions, including pain states. To test this idea, we used a closed head injury (CHI) model that simulates concussive brain injury in male rats. We performed platform mediated avoidance to evaluate anxiety-like behaviors. Then, we measured neuronal activity in the PAG and DRN using cFos immunohistochemistry. CHI rats showed increased time on the platform, suggesting that concussive injury increases anxiety-like behaviors. Immunohistochemistry results revealed an increased neuronal activity in the DRN, supporting the idea that concussive injury influences both anxiety and pain. To further test this possibility, we are now examining the influence of CHI on pain-related responses. Preliminary results suggest that CHI causes mechanical hypersensitivity. In conclusion, brain injury induces anxiety-like behaviors, and this negative emotional state might affect both emotional and pain processing.

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Poster

PSTR205. Brain Injury: Behavioral Studies

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR205.14/Y20

Topic: C.10. Brain Injury and Trauma

Title: Chronic Pain Development in Female Rats Following Repeated Mild Blast Neurotrauma

Authors: *A. WRIGHT[1], S. F. MURPHY[1], P. J. VANDEVORD[1,2];
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Abstract: Blast-induced traumatic brain injury (bTBI) is prevalent in active military personnel following combat and Veterans. Veterans have reported chronic headaches that are persisting months and even years following repeated blast exposures. According to the Department of Veteran Affairs, over 20% of post-9/11 servicemembers suffer from chronic headaches and migraines. Patients who sustain a mild TBI (mTBI) are reported to be at greater risk of developing chronic pathologies including headaches/pain, analgesia/opioid abuse, and behavioral deficits [Zaloshnja et al (2005)]. An active area of study is whether or not this population is developing chronic headaches due to bTBI sustained in combat. There is a need to examine the underlying mechanisms that contribute to the chronification of headaches following bTBI. The aim of this study was to determine the influence of repeated blast exposures on chronic pain development and subsequent social deficits and anxiety-like behaviors. Female Sprague Dawley (10 weeks of age) rats were exposed to a repeated bTBI (n=10) in an Advanced Blast Simulator. Animals were subjected to three blast events separated by 1 hour. Shams (n=10) underwent all of the same procedures except the blast insult. Assessments to examine pain sensitivity (Von Frey and hot/cold plate) and anxiety-like behaviors (open field test) were performed weekly and monthly, respectively following blast injury. Statistical analyses were performed using GraphPad prism version 9 software, with statistical differences between groups and multiple time points assessed using two-way ANOVA with repeated measure. P*ost-hoc* tests were applied when appropriate. The blast introduced a positive average overpressure of 19.98 psi with a positive phase duration of 2.28 ms**.** Chronic pain and anxiety-like behaviors were seen in animals following repeated blast exposure. Starting 3 weeks following blasts, there was a significant decrease in maximum force response to the Von Frey filaments of bTBI animals vs sham indicating facial sensitivity. While blast animals became significantly more sensitive to cold at 7-weeks post-blast, sensitivity to heat was the same in both groups up to 8 weeks following injury. At 1-week post-injury, blast animals spent more time in the center of the arena compared to sham animals during open field. These preliminary findings suggest that chronic pain began developing in female blast-induced animals around 3-weeks post blast. Future studies will examine pathological changes in the brain post-blast to understand the underlying changes that contribute to chronic pain development in females.

Disclosures: A. Wright: None. **S.F. Murphy:** None. **P.J. VandeVord:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.01/Z1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation 598799

Title: Investigating the role of MAP7 in promoting rVRG nerve sprouting after cervical spinal cord injury

Authors: K. A. BUCKHAULTS, B. M. CURRAN, A. C. LEPORE, S. R. TYMANSKYJ, L. CHENG, ***L. MA**;
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Abstract: Nerve regeneration can be achieved in different ways. While much of recent attention has been focused on re-growth of injured nerves, it has been recognized that functional recovery can be achieved by axon sprouting or formation of axonal branches from either injured nerves or spared nerves. However, the molecular mechanism of axon sprouting is not well understood. Here, we investigate the role of MAP7 in axon sprouting or branch regeneration, because MAP7 is a microtubule-associated protein that is enriched at branch junctions and stabilizes microtubules to prevents branch retraction (Tymanskyj and Ma, 2019, *J Neurosci*.). Using a rodent spinal cord injury model involving the respiratory function, we test whether MAP7 can promote axon sprouting after injury. Specifically, recombinant adeno associated virus-2 (AAV2) was injected unilaterally to express MAP7-EGFP or EGFP alone (as control) in the rostral ventral respiratory group (rVRG) of the medulla of adult female rats. AAV2-mCherry virus was co-injected to label rVRG axons. Four weeks after injection, half of the animals underwent spinal cord hemisection at the cervical level 2 (C2) and in the side contralateral to the injection site. After 4 weeks of recovery, animals were tested for their respiratory function using electromyography (EMG) recordings of the diaphragm, followed by perfusion for tissue collection. To visualize rVRG axons, cross sections (30μm) were cut from brain stem to spinal cord (C1-C5) and then imaged by confocal microscopy after antibody staining and analyzed using Fiji (ImageJ). To automate image analysis, several macros were developed to identify and quantify labeled rVRG axons at different cervical levels and in different spinal cord regions (gray vs white, ipsilateral vs contralateral). Systematic analysis of axonal profiles between four experimental conditions (injury vs non-injury, and EGFP vs MAP7-EGFP) is current underway. We will present data from the analysis to examine the correlation of axonal sprouting with changes in respiratory functions. These data will provide the first analysis of MAP7 in promoting nerve sprouting/branch regeneration after spinal cord injury in vivo.

Disclosures: K.A. Buckhaults: None. **B.M. Curran:** None. **A.C. Lepore:** None. **S.R. Tymanskyj:** None. **L. Cheng:** None. **L. Ma:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.02/Z2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life Spinal Cord Research Foundation
Craig H. Neilsen Foundation, Award #727694
NYS DOH SCIRB C37712GG
NYS SCIRB Postdoctoral Fellowship C33613GG
NIH/NINDS R21NS127622

Title: In vivo assessment of long-distance axon growth and guidance ability in the developing mammalian central nervous system.

Authors: *C. RUVEN[1], J. KAISER[1], P. PATEL[1], B. CAGLAYAN[1], R. KAWAGUCHI[2], V. SAHNI[1,3,4];
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Abstract: Long-distance axon navigation in the developing central nervous system (CNS) establishes long-range connectivity. This ability is lost in the adult CNS and the failure to regenerate long-distance connectivity after adult CNS injuries leads to permanent functional deficits. Established models of neonatal CNS injuries disrupt the microenvironments needed for axon growth and guidance. This limits their ability to investigate long-distance axon growth ability and therefore when and how long-distance axon growth ability is lost during development remains unknown. We established a novel microsurgical approach to transect developing corticospinal neuron (CSN) axons, without producing an overt spinal lesion and investigated long-distance growth ability of the developing corticospinal tract (CST) in neonatal mice. We identify, rather surprisingly, that this ability is not equivalently lost throughout the developmental period of CST growth into the cord. Rather, the ability is lost differentially and acutely during the period of axon guidance. At postnatal day 4 (P4), while the ability is robustly maintained at thoracic T11, it is completely abolished at cervical C2. We identify that CSN lose long-distance axon growth ability at cervical C2 even while extending axons to thoraco-lumbar segments. Our results show that the capacity of the CNS to support long-distance axon navigation is lost acutely across the developing CNS and the developmental time window for long-distance axon navigation is much shorter than other forms of axonal plasticity. The differential loss of long-distance growth at distinct spinal levels does not appear to be due to segmental differences in astrocytic or microglial activation. Our results suggest that the initial control over long-distance axon growth is differentially governed across the length of an axon by context-specific mechanisms.

Disclosures: C. Ruven: None. **J. Kaiser:** None. **P. Patel:** None. **B. Caglayan:** None. **R. Kawaguchi:** None. **V. Sahni:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.03/Z3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01NS085426 to VJT
NIH/NINDS Research Supplement to Promote Diversity in Health-Related Research to AI

Title: Investigating the role of tubulin tyrosine ligase (TTL) in regrowth of adult sensory axons after chemogenetic neuronal activation

Authors: *A. ISLAM[1], V. J. TOM[2];
[1]Dept. of Neurobio. and Anat., [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Injury to the mature nervous system often results in permanent functional deficits. One factor limiting recovery is that neurons lose the intrinsic ability for robust axon growth after development. Our lab showed that activating adult dorsal root ganglia (DRG) neurons using chemogenetics, i.e., designer receptors exclusively activated by designer drugs (DREADDs), increases the ability to regenerate axons into the spinal cord after a dorsal root crush injury in vivo. This was associated with more axons turning off white matter and into grey matter and other neurons, potential synaptic partners. This increased growth and turning may be attributed to microtubules, components of the cytoskeleton important for development, shape and motility, and organelle transport. Microtubules have two distinct domains, either stable or labile, depending on the type of post-translational modifications. While mature neurons contain a high concentration of stable (acetylated) microtubules in their axons, increasing stable microtubules may lead to forced polymerization of tubulin, causing abnormal axon growth. Meanwhile, labile (tyrosinated) microtubules are much more dynamic and are necessary for normal growth-cone motility and axon extension. We found that when adult DRG neurons are chemogenetically activated via the excitatory DREADD hM3Dq in vitro, there is an increase in labile, tyrosinated microtubules within the distal axon that mediates the improved axon outgrowth from activated neurons. We have preliminary data that axons in chemogenetically activated, hM3Dq+ neurons have increased levels of tubulin-tyrosine ligase (TTL), a protein which tyrosinates tubulin, compared to growing axons from neurons that do not express hM3Dq. We did not observe any differences in expression levels of VASH1/2, which detyrosinates tubulin, between groups. We hypothesize that TTL plays a major role in the improved axon outgrowth observed after neuron activation. We will use AAV-shRNA-TTL to knockdown TTL in hM3Dq+ DRG neurons to directly assess the necessity of TTL in axon regrowth in adult, chemogenetically activated neurons. Elucidating the mechanisms involved in axon regeneration after neuron activation will identify potential molecular targets to increase the regenerative capability of adult neurons after injury.

Disclosures: A. Islam: None. **V.J. Tom:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.04/Z4

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Role of the Mitochondria-Microtubule relationship in the axon fate after traumatic injury: an in vitro study

Authors: *T. ANDRIOT, C. DUPUIS, T. PAN, J.-M. PEYRIN, F. NOTHIAS, S. SOARES; Neurosci. Paris Seine, CNRS-UMR8246, INSERM1130, IBPS, Sorbonne university, Paris, France

Abstract: A traumatic lesion in the central nervous system (CNS) induces cellular and molecular mechanisms leading to irreversible dysfunctions. In addition to a non-permissive post-injury environment for axonal regeneration, mechanical stress also rends CNS neurons vulnerable, reflected by microtubules (MT) network strongly affected and mitochondrial damages. At molecular level, axonal degeneration is associated with energy drop, cytoskeletal destabilization and finally axonal degradation. In mean time, mitochondria repositioning associated to microtubule network, stimulates axon growth and branching by rescuing energy deficits. Thereby, mitochondria and MT are essential in determining the axon fate. However, the precise mechanism underlying the connection between mitochondria and microtubules in post-injured axons are still not well elucidated. The objective of the present study is to develop a simple *in vitro* model to perform mechanical trauma in order to decipher the processes of axonal regrowth, trough assessment of microtubules and mitochondria dynamics. Our aim is to understand the influence of microtubules-mitochondria cross talk on axon fate after a traumatic injury. We particularly focused on the corticospinal tract connecting to the spinal cord neurons. Corticospinal neurons are known to possess a poor axon regeneration capacity after a spinal cord injury. We show that we have developed an *in vitro* set-up of an oriented cortico-spinal network with evidence of functional connectivity, within microfluidic chips. This device allow the application of a mechanical injury, followed by axonal regeneration. By using a pharmacological approach to modulate microtubules state, mitochondria dynamic is analyzed, and thus axon fate post-injury. We are demonstrating how the impact of MT network can influence mitochondria dynamic to increase axonal regeneration.

Disclosures: T. Andriot: None. **C. Dupuis:** None. **T. Pan:** None. **J. Peyrin:** None. **F. Nothias:** None. **S. Soares:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.05/Z5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR

Title: Therapeutic knockdown of miR-340-5p to promote neuronal recovery following axonal injury

Authors: *M. A. HINTERMAYER[1], E. HUA[2], Y. DING[2], A. E. FOURNIER[3];
[1]McGill Univ. Integrated Program in Neurosci., Montreal, QC, Canada; [3]Dept Neurol & Neurosur, [2]McGill Univ., Montreal, QC, Canada

Abstract: The regeneration of damaged neurons in the mammalian central nervous system (CNS) is severely limited, owing to a decrease in the intrinsic regenerative capacity of neurons throughout development, the presence of growth inhibitory substances in CNS lesions, and a reduction in neurotrophic support. This paucity of regeneration leaves individuals sustaining neurotrauma or axonopathy in response to degenerative changes in the brain with prolonged impairments. In contrast, neurons in the dorsal root ganglion (DRG) retain regenerative capacity following injury, prompting inquiries into how CNS neurons can be reprogrammed to regenerate. Whereas some studies have focused on modulating individual gene targets to enhance axon regeneration, an alternative approach is to modulate multiple genes in neurons simultaneously. MicroRNAs (miRNAs) are small, non-coding RNA molecules that target and downregulate multiple mRNAs in concert, serving as powerful regulators of multi-gene programs in cells and thus potentially concurrently regulating pro-regeneration and pro-survival gene programs. We conducted a systematic literature search to identify existing single cell RNA sequencing (scRNA seq) datasets from regenerating DRGs to identify a list of 104 differentially expressed regeneration-associated genes (RAGs). We applied a novel *in silico* approach to this dataset to predict miRNAs that individually regulate large numbers of RAGs. Individual miRNAs were identified that were predicted to regulate up to 55 of the 104 DRG RAGs. *In vitro* screening in rat primary cortical neurons demonstrated that neurite regeneration is promoted by modulating the levels of four candidate miRNAs after chemical axotomy. Inhibiting miR-340-5p promoted the regeneration of the longest neurites *in vitro*. *In silico* analysis predicts that miR-340-5p regulates numerous pathways associated with cell survival, neurotrophin sensitivity, and neurite outgrowth. Inhibiting miR-340-5p upregulated TrkB, and sensitized neurons to BDNF, which improved neuronal survival following axotomy *in vitro.* This phenotype was not present when BDNF was combined with the selective TrkB inhibitor, ANA-12. The creation of a custom circularized miR-340-5p sponge facilitated potent knockdown of miR-340-5p that further improved neurite regeneration *in vitro*. *In vivo* studies using the optic nerve crush model of CNS axon regeneration are currently being conducted. Understanding the molecular basis of CNS axon regeneration failure and how it is distinguished from the regenerative-competent PNS can identify key players that can be targeted therapeutically to promote more adaptive responses to injury.

Disclosures: M.A. Hintermayer: None. **E. Hua:** None. **Y. Ding:** None. **A.E. Fournier:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.06/Z6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Supported by NS108189 to O. Steward

Title: Exploring two methods of selective labeling of the corticospinal tract (CST) after spinal cord injury in mice

Authors: *N. NABI[1], O. STEWARD[2];
[1]Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA; [2]Reeve-Irvine Res. Centyer, Univ. of California Irvine, Irvine CA, CA

Abstract: Spinal cord injury (SCI) permanently impairs voluntary motor function due to lack of regeneration of CST axons. Consequently, approaches to enhance axon regeneration often focus on the CST to restore motor function. Standard methods for tracing CST axon regeneration involve injections of tracers such as biotinylated dextran amine (BDA), but this approach requires additional surgery for tracer injections after regeneration has occurred and adds experimental variables including time constraints for tracer transport. Here, we explore two methods for tracing CST axons after SCI; one involves selective genetic labeling and the second links tract tracing with the AAV-based intervention that enables regeneration. Previous studies have characterized selective labeling of CST axons in transgenic mice expressing mu-crystallin (*Crym*) with an eGFP tag. To explore whether Crym-eGFP can be used to trace regenerating CST axons after SCI, we created a new transgenic strain by crossing Crym-eGFP mice with PTEN^{ff}/tdT mice where delivery of AAV/Cre deletes PTEN. Mice received either cervical (C5) or thoracic (T9) dorsal hemisection injuries. Mice were perfused at weekly time points post-SCI and spinal cords were immunostained for eGFP. GFP labeling persisted in proximal segments of cut axons at all time points, and eGFP-positive degeneration debris was evident caudal to the injury until approximately 6 weeks post-SCI. Persistence of eGFP in degenerating axons complicates detection of early stages of regeneration in Crym-eGFP mice. Surprisingly, in mice with T9 level injuries, eGFP-positive profiles were present in the dorsal column caudal to the injury site and in the meninges. EGFP-positive profiles were not observed in the dorsal column with C5 injuries. A possible explanation is that Crym expression is induced in meningeal cells near the lesion site, which then migrate into the dorsal column. The presence of eGFP-positive profiles caudal to a spinal cord injury presents another complication for tracing regenerating CST axons by Crym-eGFP labeling. To explore a second approach for labeling, we used transgenic PTEN^{ff}/tdT mice. Injections of AAV/Cre into the sensorimotor cortex in transgenic PTEN^{ff}/tdT deletes PTEN, which enables CST axon regeneration and tdT expression is induced in transduced neurons. To determine whether tdTomato labeling would be sufficient to selectively trace CST axons from transduced neurons, PTEN^{ff}/tdT mice received AAV-Cre injections into the sensorimotor cortex. Immunostaining for tdT revealed robust labeling of CST axons and arbors in the gray matter. An advantage of this approach is specific labeling of axons from PTEN-deleted neurons.

Disclosures: N. Nabi: None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OS is a co-founder, scientific advisor, and has economic interests in the company Axonis Inc, which is developing novel therapies for spinal cord injury and other neurological disorders..

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.07/Z7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: e Canadian Institutes of Health Research Grant PJT-162387
Wings for Life Spinal Cord Research Foundation Grant WFL-CA-13/20

Title: Impact of IL-1alpha on blood-spinal cord barrier permeability and IgG internalization in CNS-resident cells

Authors: *A. CASTELLANOS MOLINA[1], A. BOISVERT[1], A. LAROCHE[2], J. FERRY[1], C. ILLIANO[1], N. VALLIÈRES[1], M. LESSARD[1], É. BOILARD[2], N. QUAN[3], S. LACROIX[1,4]; [1]Ctr. de recherche CHU de Québec-Université Laval, Québec, QC, Canada; [2]Axe maladies infectieuses et immunitaires du Ctr. de recherche CHU de Québec-Université Laval, Québec, QC, Canada; [3]Charles E. Schmidt Col. of Medicine, Florida Atlantic Univ., Jupiter, FL; [4]Dept. de médicine moléculaire de l'Université Laval, Quebec, QC, Canada

Abstract: Spinal cord injury (SCI) can be divided into primary damage caused by mechanical tissue disruption and secondary damage initiated by the release of alarmins from necrotic cells into the spinal cord parenchyma, triggering neuroinflammation. Our recent work revealed that the alarmin IL-1alpha is released by necrotic microglia immediately after SCI and has a deleterious role in the pathology. To recapitulate these processes *in vivo*, we injected PBS (as control) or recombinant mouse IL-1alpha intra-cisterna magna (i.c.m.) to adult WT mice and performed real-time intravital imaging to observe the integrity of the BSCB (n=3/group). We found that IL-1alpha induces neutrophil infiltration and BSCB disruption within 6h after injection. Using the IL-1R1-restored (*Il1r1*$^{r/r}$) mice (n=4-5/group), which exhibit an IL-1R1 knockout phenotype that can be reversed in a cell-specific manner by Cre-mediated recombination, we restored IL-1R1 expression in endothelial cells (ECs) and found that endothelial IL-1R1 signaling drives the disruption of the BSCB. Depletion of myeloid cells using an anti-Gr1 antibody prevented BSCB leakage (n=4-5/ group). Next, we performed immunofluorescence staining against IgG with the CNS-resident cells markers NeuN (neurons), Sox9 (astrocytes), Iba1 (microglia), and Olig2 (oligodendrocyte lineage). Notably, the increased BSCB permeability was associated with the transport of immunoglobulin type G (IgG) into the spinal cord and brain parenchyma, as well as their internalization within central nervous system (CNS)-resident cells at 6, 12 and 24h post IL-1alpha i.c.m. injection in the WT mice (n=6/group). To elucidate the mechanism(s) underlying IgG internalization, we utilized Fc gamma receptor knockout (FcγR$^{-/-}$) mice (n=4/group) that lack activating FcγR (all except FcγRIIb). We also observed IgG infiltration and internalization into CNS-resident cells FcγR$^{-/-}$ 24h after i.c.m. injection of IL-1alpha, indicating that activating FcγRs do not play a role in these processes. To further explore possible interactions between neurons and IgGs, we injected i.c.m. polyclonal IgGs conjugated to Alexa Fluor 488 in adult FcγR$^{-/-}$ and WT mice (n=4-5/group). Our

data revealed close interactions between IgGs and neurons and glial cells throughout the brain and spinal cord at 1 and 4 hours post-injection in both mouse lines, indicating that CNS cells possess the capacity to sequester IgGs even under normal physiological conditions, independent of activating FcγRs. These findings have significant implications in pathologies characterized by elevated IgG levels in the CNS, such as SCI and neuroinflammatory diseases.

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Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.08/Z8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: KQTD20200820113040070
JCYJ20200109141433384
NSFC81971062

Title: Lactobacillus rhamnosus GG supplementation reduces pain and increased expression of μ-opioid receptor in the spinal cord in rats with bone cancer

Authors: *X.-J. SONG, W. YUAN, J. XIAO, H. LIAO;
Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Chronic cancer pain is one of the most unbearable symptoms for the patients with advanced cancer. The treatment of cancer pain continues to possess a major challenge. Here, we report that adjusting gut microbiota via probiotics can reduce bone cancer pain (BCP) in rats. The model of BCP was produced by tumor cell implantation (TCI) to the tibia in rats. Continuous feeding of Lactobacillus rhamnosus GG (LGG) was used to modulate the gut microbiota. Mechanical allodynia, bone destruction, fecal microbiota, and neurochemical changes in the primary dorsal root ganglion (DRG) and the spinal dorsal horn (DH) were assessed. LGG supplementation (109 CFU/rat/day) delayed the production of BCP for 3-4 days and significantly alleviated mechanical allodynia within the first 2 weeks after TCI. TCI-induced proinflammatory cytokines TNF-α and IL-1β in the DH, and TCI-induced bone destruction in the tibia were both significantly reduced following LGG supplementation. Meanwhile, we found that LGG supplementation, in addition to inhibiting TCI-induced pain, resulted in a significantly increased expression of the μ-opioid receptor (MOR) in the DH, but not in the DRG. LGG supplementation significantly potentiated the analgesic effect of morphine. Furthermore, our results showed that LGG supplementation led to an increase in butyrate levels in the colon and serum and a decrease in histone deacetylase 2 (HDAC2) expression in the DH. Feeding TCI-rats with sodium butyrate solution alone, at a dose of 100 mg/kg, resulted in decreased pain, as well

as decreased HDAC2 expression and increased MOR expression in the DH. The increased expression of MOR and decreased HDAC2 were also observed in neuro-2a cells when we treated the cells with serum from TCI rats with supplementation of LGG or sodium butyrate. This study provides evidence that reshaping the gut microbiota with probiotics LGG can delay the onset of cancer pain. The butyrate-HDAC2-MOR pathway may be the underlying mechanism for the analgesic effect of LGG. These findings shed light on an effective, safe, and non-invasive approach for cancer pain control and support the clinical implication of probiotics supplementation for patients with BCP.

Disclosures: X. Song: None. **W. Yuan:** None. **J. Xiao:** None. **H. Liao:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR206.09/Z9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 5R01NS118200

Title: Spinal cord injury directly causes pressure ulcer disrepair

Authors: *C. P. VADALA[1], A. R. FILOUS[2], S. S. PATEL[4], F. O. NOVAIS[3], J. M. SCHWAB[2]; [1]Dept. of Neurol., The Ohio State Univ. Neurosci. Grad. Program, Columbus, OH; [2]Dept. of Neurol., [3]Dept. of Microbial Infection and Immunity, The Ohio State Univ. Wexner Med. Ctr., Columbus, OH; [4]Ohio State Univ., Columbus, OH

Abstract: Background: Spinal cord injury (SCI) disrupts the communication between the central nervous and immune system. However, appropriate coordination of immune responses is needed for effective wound healing. Resolution of inflammation is an active, required process that stimulates the removal of inflammatory cells (efferocytosis) from the injury site. Deciphering the specific mechanisms underlying impaired wound healing after SCI may provide new targets to enable and support pressure ulcer (PU) closure in patients. PU in SCI patients often heal slowly while chronic wounds that affect nearly one in three patients. They are associated with increased mortality and may constitute a rehabilitation confounder. **Purpose:** This study investigates the SCI-induced effect on the immunological response at the PU site. Immune responses after SCI are well documented within the spinal cord, but data on the inflammatory PU site is scarce. **Hypothesis**: SCI directly delays wound healing and inflammation resolution programs, resulting in skewed and incomplete healing. **Methods**: We assessed immune responses at the PU site using a novel SCI-associated PU model (SCI-PU) compared to sham controls (Sham-PU). The SCI-PU model utilizes three 24-hour ischemia/reperfusion cycles (12hx12h) using magnets (50 mmHg) applied to the dorsal skin beginning three days after SCI. This creates two distinct skin lesions caudal to the SCI (lower back). Wound healing is assessed using histological methods and inflammation responses within

the PU are assessed with flow cytometry. **Results:** We found skewed biological responses at the PU site in animals with SCI. Wound healing is delayed by SCI and leads to incomplete healing up to 30 days post PU induction. **Implications:** Our data demonstrates that SCI impairs PU healing, likely due to maladaptive, systemic immune response.

Disclosures: C.P. Vadala: None. **A.R. Filous:** None. **S.S. Patel:** None. **F.O. Novais:** None. **J.M. Schwab:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.10/Z10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Grant 457328
Wings for Life Grant 19/19
Wings for Life Grant 08/20
NIH Grant 120612

Title: Acquired pneumonia impairs locomotor recovery by expanding the lesion after spinal cord injury

Authors: *A. R. FILOUS, J. M. SCHWAB;
The Ohio State Univ., Columbus, OH

Abstract: Spinal cord injury (SCI) disruptes the communication between the CNS and the immune system. As a result, theses patients become severely immune compromised, making them highly susceptible to infections. In fact, pneumonia is the leading cause of death after SCI. However, even patients that are fortunate enough to survive the initial infection suffer from long-term recovery impairments compared to patients without acute infections. The question of how infections impair recovery potential as gone unaddressed in the literature because of the standard use of prophylactic antibiotics in pre-clinical SCI models. Here, we developed a clinically-relevant model to study acquired pneumonia after SCI (SCI-AP) in a bedside-to-bench approach. At 3 days post-injury, when mice are most immunocompromised, mice are inoculated with *Streptococcus pneumoniae* directly into their lungs. Similar to humans, SCI mice exhibit higher mortality after acquired pneumonia compared to SCI only mice. Use the Basso Mouse Scale and activity box to assess locomotor recovery, we observed delayed and impaired functional recovery of hindlimb function as a result of infection. We used immunohistochemistry to understand the underlying mechanisms of this functional deficit and found that SCI-AP mice have an exacerbated lesion environment and impaired axonal regeneration compared to SCI only mice. This study identifies a novel and potent outcome-modifying factor for SCI patients. While a cure for SCI is still out of reach, infections have a severe impact on patient survival and recovery potential. By understanding the mechanisms underlying secondary damage triggered by

pneumonia after SCI, we hope to develop clinically-relevant strategies to protect recovery potential by preventing the impact of acquired infections.

Disclosures: **A.R. Filous:** None. **J.M. Schwab:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR206.11/Z11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation 891313
Advancing a Healthier Wisconsin (AHW) 5520645

Title: Inhibition of CCR5 improves functional recovery and reduces secondary tissue damage after thoracic spinal cord injury

Authors: A. BACHMANN, J. PAGE, A. RAIMONDI, S. KAFURA, ***A. KRONER-MILSCH**; Neurosurg., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Inflammation plays a dual role after spinal cord injury (SCI). While essential for early debris clearance and containment of the lesion, prolonged and exacerbated inflammation is also a major contributor to secondary tissue damage. A group of small signaling molecules, chemokines, have emerged as crucial regulators of immune cell migration and activation. We have previously demonstrated that the chemokine CCL3 contributes to impaired locomotor recovery, tissue damage and inflammation after SCI. Signaling through CCR5, one of the CCL3 receptors, has been shown to deteriorate functional outcome, neuronal survival and sprouting in mouse models of traumatic brain injury and stroke. Inhibition of CCR5 signaling, using an FDA approved inhibitor (maraviroc), mitigated neuropathic pain in a sciatic nerve constriction model and reduced inflammation in experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis.In this study, we investigated the impact of CCR5 inhibition on locomotor recovery, secondary tissue damage and inflammation in a mouse model of thoracic contusion SCI. Acute treatment with maraviroc resulted in significantly improved locomotor recovery in horizontal ladder walk and Catwalk analyses. Histological analysis of injured spinal cords revealed significantly smaller lesion sizes in maraviroc treated mice compared to controls, in addition to improved neuronal survival (NeuN+ cells). Furthermore, maraviroc treated mice had a higher density of synaptophysin positive synapses in areas surrounding the lesion compared to control mice.Our results indicate that CCR5 inhibition with maraviroc is a potential pharmacological treatment to promote functional recovery, tissue preservation and neuronal survival in a clinically relevant model of spinal cord injury. Future studies will explore the potential of maraviroc treatment initiated at clinically relevant acute and chronic timepoints after SCI.

Disclosures: **A. Bachmann:** None. **J. Page:** None. **A. Raimondi:** None. **S. Kafura:** None. **A. Kroner-Milsch:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.12/Z12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: JSPS KAKENHI Grant 21H02655
JSPS KAKENHI Grant 20K21499
HUSM Grant in Aid

Title: Inhibition of glial scar formation after spinal cord injury in Noggin conditional knockout mice and by anti-Noggin antibody treatment

Authors: *S. YAMAGISHI[1,2], S. LI[3,2], J. LI[2], Y. HAN[2], Y. PING[4], H. ARIMA[3], Y. MATSUYAMA[3], K. SATO[2];
[1]Optical Neuroanatomy, [2]Dept. of Organ and Tissue Anat., [3]Dept. of Orthopedic Surgery, [4]Dept. of Cell. and Mol. Anat., Hamamatsu Univ. Sch. of Med., Hamamatsu, Shizuoka, Japan

Abstract: Noggin is a glycosylated protein that can act as an inhibitor of bone morphogenetic protein (BMP) and has been shown in animal studies to improve functional recovery after spinal cord injury (SCI). Its mechanism of action promotes the regeneration and repair of damaged spinal cord tissue by regulating the differentiation and growth of glial cells and regulates the interaction between neurons and glial cells. However, very few studies on endogenous Noggin have been reported, and the available studies are limited to the therapeutic effects of exogenous Noggin. Therefore, we explored the expression and role of endogenous Noggin after SCI using Noggin-GFP transgenic mice and Noggin$^{lox/lox}$; Nestin-cre conditional knockout mice. The results showed that Noggin was upregulated in the spinal cord after SCI, mainly in reactive astrocytes. In addition, endogenous Noggin was found to play a key role in regulating SCI responses, promoting GFAP expression of astrocytes, and improving functional recovery. These findings suggest that Noggin may be a therapeutic target for controlling glial scar formation after SCI.

Disclosures: **S. Yamagishi:** None. **S. Li:** None. **J. Li:** None. **Y. Han:** None. **Y. Ping:** None. **H. Arima:** None. **Y. Matsuyama:** None. **K. Sato:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.13/Z13

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Gap-junction-mediated bystander transformations in the spinal cord after injury safeguards the neuronal viability and regeneration

Authors: A. PEDRONI[1], Y.-W. E. DAI[1], ***L. LAFOUASSE**[2], W. CHANG[1], I. SRIVASTAVA[1], K. AMPATZIS[1];
[1]Karolinska Institutet, Karolinska Institutet, Stockholm, Sweden; [2]Karolinska Inst., Solna, Sweden

Abstract: The adult zebrafish spinal cord displays an impressive innate ability to regenerate after traumatic insults, yet the underlying adaptive cellular mechanisms remain elusive. Here we show that while the cellular and tissue responses after injury are conserved among vertebrates, the zebrafish spinal motoneurons are remarkably resilient by remaining viable and functional. We also reveal the dynamic changes in motoneuron glutamatergic input, excitability, and calcium signaling, and we underscore the critical role of calretinin (CR) in binding and buffering the excessive intracellular calcium after injury. Importantly, we demonstrate the presence and the dynamics of a neuron-to-neuron bystander neuroprotective cooperation mediated through gap junction channels. Our findings support a model in which the intimate and dynamic interplay between glutamate signaling, calcium buffering, gap junction channels, and intercellular cooperation safeguards neuronal survival and regeneration.

Disclosures: A. Pedroni: None. **Y.E. Dai:** None. **L. Lafouasse:** None. **W. Chang:** None. **I. Srivastava:** None. **K. Ampatzis:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.14/Z14

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Physiological and behavioral changes in central neuropathic pain rats with mechanical and thermal sensitivity abnormalities after spinal cord injury

Authors: *B. AVONTS, R. FESSLER, B. DAVID;
Rush Univ. Grad. Col., Chicago, IL

Abstract: Central neuropathic pain (CNP) commonly develops in patients after spinal cord injury (SCI), causing debilitating symptoms and sensory abnormalities to traditionally non-noxious mechanical and thermal stimuli. CNP regularly presents itself around a year after injury in humans, resulting from permanent cellular and anatomical changes. Previous scientific studies have demonstrated greater efficacy of treatments when delivered preemptively, but the biological

variability in individuals has limited the number of positive outcomes. Thus, it is necessary to investigate the physiological and behavioral processes contributing to sensory changes that develop over time. Here we assess inflammation as a potential mediator of CNP, as well as gait, out to 8 weeks after injury. Using the tail flick and von Frey tests, we performed hierarchical clustering to determine the subpopulation of rats that developed thermal and mechanical sensory abnormalities. The tail flick test showed a subpopulation of hypersensitive rats significantly different than normosensitive SCI rats, that remained similar to sham rats at weeks 1, 3-8 post-injury ($p<0.05$). The von Frey test showed a subpopulation of hyposensitive rats significantly different than normosensitive SCI rats, remaining similar to sham rats at weeks 6-8 post-injury ($p<0.05$). We saw significant changes in gait with both modalities of sensory abnormalities, as well as increased levels of myeloid cells at the site of injury ($p<0.05$). We conclude further investigation may reveal acute changes in inflammation through blood serum, mediating the immune response of macrophages at both the injury epicenter and regions of the cerebral cortex involved in processing of higher functions.

Disclosures: B. Avonts: None. **R. Fessler:** None. **B. David:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR206.15/Z15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS R01NS122961
Mission Connect, a program of TIRR Foundation

Title: L-selectin Shedding Regulates Neutrophil Function, Accumulation and Neurological Recovery after Spinal Cord Injury in a Sex-dependent Manner

Authors: *M. E. LEAL[1], S. K. REID[2], V. S. TSENG[3], D. A. MCCREEDY[3];
[1]Biol., Texas A&M Chapter, College Station, TX; [2]Texas A&M Inst. for Neurosci., College Station, TX; [3]Biol., Texas A&M Univ., College Station, TX

Abstract: Inflammation after spinal cord injury (SCI) can contribute to secondary tissue damage and poor functional outcomes. Neutrophils are the first immune cell type to enter the injury site in large numbers, however, the mechanisms underlying damaging neutrophil properties are not fully understood. We have previously shown that augmenting the cleavage or shedding of L-selectin, an adhesion and signaling receptor on neutrophils, can reduce secondary injury and improve neurological recovery after SCI. To determine the effect of L-selectin shedding on neutrophil function, we utilized L(E) mice, which express a non-cleavable version of L-selectin that cannot be shed. *In vitro*, we observed a decrease in degranulation from stimulated bone marrow neutrophils from female, but not male, L(E) mice when compared to wild types (WTs). In addition, we observed more reactive oxygen species (ROS) production from stimulated

neutrophils from L(E) mice relative to WTs for both sexes. To determine if L-selectin shedding affects neutrophil responses *in vivo*, we quantified neutrophil accumulation in the injured spinal cord at 1, 3 and 35 days post-SCI in adult male and female L(E) and WT mice. Interestingly, we observed a sex and genotype-dependent difference in neutrophil accumulation at 1 day post-SCI. At 35 days post-SCI, we observed greater neutrophil accumulation in female L(E) mice when compared to WTs but no differences were observed in male mice. Coinciding with the sex-dependent differences in neutrophil responses, we observed diminished functional recovery in female L(E) mice compared to WTs, however, no difference was observed between these genotypes in male mice. To assess the effects of L-selectin shedding on long-term tissue sparing we quantified spared white matter in the injured spinal cord 35 days post SCI. We observed decreased percentages of white matter in L(E) mice when compared to WTs for both sexes combined. Our data demonstrate sex-dependent roles for L-selectin shedding in neutrophil function and long-term recovery after SCI.

Disclosures: M.E. Leal: None. **S.K. Reid:** None. **V.S. Tseng:** None. **D.A. McCreedy:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.16/Z16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NSCT Grant 109-2314-B-075-045-MY3

Title: Therapeutic potential of lactate dehydrogenase A inhibitors in painful peripheral neuropathy.

Authors: *H.-J. CHENG[1], Y.-Y. SUN[1], C.-S. SUNG[3,4], Z.-H. WEN[1,2];
[1]Inst. of BioPharmaceutical Sci., [2]Dept. of Marine Biotech. and Resources, Natl. Sun Yat-sen Univ., Kaohsiung City, Taiwan; [3]Dept. of Anesthesiology, Div. of Pain Mgmt., Taipei Veterans Gen. Hosp., Taipei, Taiwan; [4]Sch. of Med., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: Therapeutic potential of lactate dehydrogenase A inhibitors in painful peripheral neuropathy.**Hao-Jung Cheng**[1,*], Yu-Yo Sun[1], Chun-Sung Sung[2,3], Zhi-Hong Wen[1,41] Institute of BioPharmaceutical Sciences, National Sun Yat-sen University, Kaohsiung 804201, Taiwan[2] Department of Anesthesiology, Division of Pain Management, Taipei Veterans General Hospital, Taipei 112201, Taiwan[3] School of Medicine, National Yang Ming Chiao Tung University, Taipei 112304, Taiwan[4] Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung 804201, Taiwan
Neuropathic pain (NP) affects over 450 million people worldwide, posing a significant burden on society. Unfortunately, there is still no effective treatment for NP management. Previous reports found that energy metabolism might be a potential therapeutic approach for NP treatment. Normally, glycolysis can convert glucose into pyruvate, transported into mitochondria for energy

generation. However, under NP conditions, glycolysis becomes an important energy source. In this scenario, pyruvate is converted into lactate by lactate dehydrogenase A (LDHA) rather than entering the mitochondria. Consequently, lactate can accumulate in the extracellular space, leading to acidification, which contributes to nociceptive sensitization. Therefore, LDHA may play a crucial role in NP and warrant further investigation. To investigate the role of LDHA in NP, we administered LDHA inhibitors, namely FX11 and oxamate, intrathecally using an osmotic pump in rats with chronic constriction injury (CCI)-induced NP. Nociceptive tests, including mechanical allodynia and thermal hyperalgesia, were performed to assess the analgesic effects of LDHA inhibitors. Immunofluorescence staining and co-localization assays were conducted on the ipsilateral spinal cord dorsal horn (SCDH) to explore the potential mechanisms of LHDA in NP.Both FX11 and oxamate exhibited significant and dose-dependent attenuation of CCI-induced nociceptive sensitization. CCI upregulated LDHA protein expression in neuronal cells of the ipsilateral SCDH. However, intrathecal administration of LDHA inhibitors did not inhibit CCI-induced upregulation of LDHA protein in spinal neurons. Interestingly, we observed that intrathecal LDHA inhibitors prevented CCI-induced translocation of LDHA from the cytoplasm into the nucleus of neuronal cells on the ipsilateral side.Based on our research, it appears that LDHA translocation may have a significant impact on nociception. Additional studies are needed to explore the relationship between LDHA translocation and NP.

Disclosures: H. Cheng: None. **Y. Sun:** None. **C. Sung:** None. **Z. Wen:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.17/Z17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS119673

Title: Astrocytic TG2 Attenuates Functional Recovery after CNS Injury Primarily by Limiting the Ability of Astrocytes to Metabolically Support Neurons in Injury Contexts

Authors: *T. DELGADO, J. EMERSON, P. GIRARDI, G. JOHNSON;
Univ. of Rochester, Rochester, NY

Abstract: Following CNS injury, astrocytes take on reactive phenotypes that grade from neurotoxic to neuroprotective, which impact subsequent neuronal recovery processes, such as axonal regeneration. Only recently has the heterogeneity of reactive astrocyte populations begun to be described, and little is known about the contextual requirements and molecular inflection points that underlie these graded responses. Accumulating data from my lab suggests that one of these inflection points is transglutaminase 2 (TG2). TG2 is complex in that it regulates signaling of numerous molecular pathways at the cell membrane, in the cytosol, and in the nucleus; thus, it can provide multiple levels of context-dependent input into gene regulation. Importantly, when

TG2 is depleted from astrocytes, they better protect neurons in culture from oxygen-glucose deprivation (OGD), improve motor function recovery in a mouse spinal cord injury model, and better facilitate neurite outgrowth in vitro on an injury-relevant, growth-inhibitory matrix. Additionally, TG2 depletion upregulates lipid handling (lipid uptake and formation of lipid droplets) and lipid metabolism pathways in an injury-dependent manner. In a semi-ground up approach, we used non-biased genetic and proteomic screening of TG2 wild type and knock-out astrocytes to explore the functional pathways influenced by the absence of TG2. Following this -omic data and our spinal cord data showing an upregulation of lipid metabolism pathways from astrocytic TG2 knock-out, we measured differences in lipid uptake and metabolism in our astrocyte groups, which may constitute a pathway in which TG2 knock-out astrocytes can better support neuronal growth and survival during stress.

Disclosures: **T. Delgado:** None. **J. Emerson:** None. **P. Girardi:** None. **G. Johnson:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.18/Z18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Daniel and Ada Rice Foundation

Title: A Long-Term Characterization of the Spinal Cord Immune Response Following Contusive Injury in Mice

Authors: *N. WROBEL, R. G. FESSLER, B. T. DAVID;
Neurosurg., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Traumatic spinal cord injury is rapidly followed by a robust immune response within the spinal cord, consisting of activation of central nervous system resident immune cells and infiltration by peripheral immune cells. This local immune response persists chronically and is widely recognized to hold considerable sway over the progression of secondary tissue damage. As the majority of individuals with spinal cord injuries live with chronic injuries, studies examining chronic spinal cord inflammation are warranted, yet they are much fewer in number than studies of acute and subacute inflammation. We present an extensive, long-term characterization of the post-injury immune response in spinal cords of adult (10-12-week-old) female wild type (C57BL/6) mice, following a moderate (50 kdyn) spinal cord contusion at T9. One group of mice received a T9 laminectomy and spinal cord contusion (n=8/time point), while the control group remained naïve (n=6/time point). A total of 9 terminal assessment time points were included, ranging from 1 day to 6 months post-injury. At each terminal time point, relative levels of certain T cell subsets (helper T cells, cytotoxic T cells, regulatory T cells), as well as macrophages and microglia, were assessed via flow cytometry. By applying standard measures of locomotor (open-field task) and sensory (tail flick) function at each of the terminal time

points, we are able to identify correlations between behavioral recovery and the prevalence of certain immune cell types. We observed the highest levels of macrophages at 60 days post-injury and the highest levels of T cells at 6 months post-injury. Both populations remained considerably elevated over controls at 6 months post-injury.

Disclosures: **N. Wrobel:** None. **R.G. Fessler:** None. **B.T. David:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR206.19/Z19

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 4UH3NS124630-02
R01NS100531
R41TR002293
The Miami Project to Cure Paralysis
State of Florida

Title: Mechanism-of-action of a kinase inhibitor capable of treating experimental spinal cord injury

Authors: *A. BADILLO MARTINEZ[1,2], I. WILLIAMS[2], J. K. LEE[2], J. BIXBY[2,3,4], V. LEMMON[2,5,3], H. ALI[2,6,7,3];
[1]Neurosci. Grad. Program, Univ. of Miami Neurosci. Grad. Program, Miami, FL; [2]The Miami Project to Cure Paralysis, [3]Sylvester Comprehensive Cancer Ctr., [4]Dept. of Mol. and Cell. Pharmacol., [5]Inst. for Data Sci. and Computing, [6]Peggy and Harold Katz Family Drug Discovery Ctr., [7]Katz Family Div. of Nephrology and Hypertension, Univ. of Miami Sch. of Med., Miami, FL

Abstract: The limited ability of the central nervous system (CNS) to regenerate damaged axons greatly restricts recovery from spinal cord injury (SCI) and other CNS trauma. Regeneration is suppressed by the lack of neuron-intrinsic regenerative capacity and by the neuron-extrinsic inhibitory environment of the injured CNS. To address this problem, we developed a novel therapeutic strategy, in the form of a small-molecule kinase inhibitor that simultaneously targets kinases in both intrinsic and extrinsic signaling pathways. The top candidate, RO48, strongly promoted neurite outgrowth in rodent primary neurons and in human iPSC-derived neurons. RO48 also promoted axon growth and behavioral recovery in mouse models of spinal cord injury (SCI). Consequently, we are developing RO48 into a drug candidate for clinical testing. While two major mechanistic targets (S6K1, ROCK2) have been identified and validated, the full mechanism of action (MoA) of RO48 and its chemical analogs is still under investigation. We use nanoBRET assays to measure in-cell target engagement of our compounds with S6K1, ROCK2, and 3 additional candidate targets (PRKX, PRKC-γ, and PRKG1). Multiple linear

regression models are used to estimate the relative contribution of each target to the compounds' ability to promote neurite outgrowth. Modulation of cell signaling pathways downstream of target engagement is investigated using transcriptomic and phosphoproteomic analyses. Impacts on the abundance of proteins encoded by regeneration-associated genes, as well as overall protein levels, is investigated using mass spectrometry. Finally, cellular sites of action (soma/dendrites vs axons) is investigated using microfluidic chambers. These studies will help elucidate the MoA and site(s) of action of our compounds, informing the choice of future target engagement assays, biomarker identification, and drug delivery strategies.

Disclosures: A. Badillo Martinez: None. **I. Williams:** None. **J.K. Lee:** None. **J. Bixby:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on an international patent application filed by the University of Miami on the compounds described in this study. **V. Lemmon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on an international patent application filed by the University of Miami on the compounds described in this study. **H. Ali:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on an international patent application filed by the University of Miami on the compounds described in this study..

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.20

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Institute of Neurological Disorders and Stroke (NS104422)

Title: Blood serum from pain treated animals that have received a spinal cord injury (SCI) induces protein extravasation in recipient SCI animals

Authors: *S. R. PARTIPILO, P. K. BELTZ, J. W. GRAU;
Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: When the spinal cord becomes mechanically damaged (primary injury), the cellular environment undergoes prolific changes which fuel cell death and impede functional axonal regeneration (secondary injury). Our laboratory has developed an extensive line of research around the observation that nociceptive-input (pain) after a T12 contusion injury increases blood infiltration and markers of inflammation at the lesion site. In the long-term, this expands the size of the primary lesion and impairs locomotor recovery. We have yet to fully delineate the mechanisms through which nociception drives hemorrhage, but a line of recent work suggests that systemic, blood-borne factors are involved. In a preliminary experiment, 8 "donor" rats received a T12 contusion. The next day, half of the donor animals received pain-input in the

form of intermittent electrical shock to the tail and the other half received an equal period of restraint. One hour later, the animals were sacrificed, and their blood was collected and spun into serum. In the next phase of the experiment, 16 contused animals were given a 0.3mL intravenous injection of the serum from either the pain-input or the no pain-input rats. Rats that received serum from pain-input animals had increased hemoglobin and pro-inflammatory cytokines at the lesion site. Further, they showed a decrease in acute locomotor function. A second experiment sought to examine if these effects were dosage-dependent. For this experiment, blood serum was collected in a similar manner from a new set of donor rats and then administered to 36 recipient animals. Animals received an injection of serum from donors that had or had not received pain at a dosage of either 0.1mL, 0.3mL, or 0.9mL. While serum from animals that had received pain only induced a weak overall increase in hemoglobin content (p =.09), a Bradford assay revealed that these animals had a significant increase in total protein concentration at the lesion site (p=.04). This finding prompted an analysis of the Bradford data from both sets of donor animals, which revealed that pain-input caused a robust increase in total protein concentration at the lesion site. These slightly divergent results between blood serum transfer studies suggest that the increase in protein at the lesion site is not just hemoglobin and red blood cell infiltration. Analyses of past results revealed that noxious stimulation consistently enhances protein concentration at the site of injury and that this is sometimes observed in the absence of significant hemorrhage. Further work is being conducted to understand the circumstances under which this effect is observed and the protein constituents.

Disclosures: S.R. Partipilo: None. **P.K. Beltz:** None. **J.W. Grau:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Program #/Poster #: PSTR206.21/Z20

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 2021ZD0201700
82171391
20JC1419500
SHSMU-ZDCX20212000

Title: Chronic stress hinders sensory axon regeneration by altering cellular energetics

Authors: *Q. HAN, Y. RUAN, J. CHENG, J. DAI;
Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China

Abstract: Spinal cord injury (SCI) often leads to physical limitations, chronic pain, significant life changes, and social isolation, increasing the risk of chronic psychological stress and associated disorders such as anxiety and depression. Despite the recognition of the negative effects of chronic stress on SCI recovery, the mechanisms linking stress and regeneration are not

fully understood. In this study, we examined the impact of chronic stress on primary sensory axon regeneration in a mouse model of preconditioning lesions. Our findings showed that chronic stress caused mitochondrial cristae loss and oxidative phosphorylation (OxPhos) inhibition within primary sensory neurons, hindering the regrowth of their central axons. The stress hormone corticosterone was identified as a critical factor in this process, impacting satellite glial cells instead of neurons by suppressing Kir4.1 expression and leading to increased neuronal hyperactivity and elevated ROS levels. These high levels of ROS altered the shape of the mitochondrial cristae and impaired its OxPhos, which is essential for powering axonal regeneration. Our results highlight the importance of addressing psychological stress in SCI patients to promote sensory-motor rehabilitation.

Disclosures: Q. Han: None. **Y. Ruan:** None. **J. Cheng:** None. **J. Dai:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR206.22/Z21

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation 457328
Wings for Life Grant 19/19
Wings for Life Grant 08/20
NIH Grant 120612

Title: Immunological, structural, and functional changes of the lung after spinal cord injury-associated pneumonia (SCI-AP)

Authors: *A. DAS[1], A. R. FILOUS[2], K. I. STANFORD[3], L. A. BAER[3], J. M. SCHWAB[2]; [2]Neurol., [3]Physiol. and Cell Biol., [1]The Ohio State Univ., Columbus, OH

Abstract: Systemic immunosuppression is a devasting result of spinal cord injury (SCI), leaving many patients susceptible to infections such as pneumonia. SCI-associated pneumonia (SCI-AP) is an outcome modifier associated with increased mortality and disability. Despite representing a clinically relevant and per se treatable outcome modifier, the underlying characteristics of SCI-associated pneumonia (SCI-AP) in the lungs have not yet been established. Using a clinically relevant controlled model of induced SCI-AP, structural, immunological, and functional changes were monitored and quantified comparing SCI-AP with SCI-only groups. Matching clinical onset, SCI-AP was induced by inoculating the mice with *Streptococcus pneumoniae* at 3 days post-injury. All histopathological assessments were performed at acute and chronic timepoints post-injury. The presence of pneumonia in the bronchioles of the lung tissue was assessed with a *Streptococcus pneumoniae* antibody and emerging structural changes in bronchial wall thickness and apoptosis were assessed in the lower right lung. We used immunohistochemistry to assess changes in the structures of the lung and found that SCI-AP induces damage beyond SCI alone.

SCI-AP also increases apoptosis and inflammation in the lung compared to SCI alone. Functionally, mice with acquired pneumonia have impaired respiration compared to SCI only mice, as early as 12 hours after inoculation. Overall, this work demonstrates features of acute and chronic SCI-AP and its subsequent structural, immunological, and functional changes in the lung. These results indicate that SCI-associated pneumonia infection evokes damage to the lung tissue and decreases overall lung function beyond any impairments after the initial SCI. The results of this study will be used to further advance research on SCI-AP with the aim to provide new insights on effective therapeutics and/or prevention methods for patients suffering from SCI-induced immunosuppression.

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Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Program #/Poster #: PSTR206.23/Z22

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NSF GRFP
Mission Connect
NINDS R01NS122961

Title: Post-injury depletion of neutrophils impairs long-term functional recovery in a sex-dependent manner after spinal cord injury

Authors: *M. PACHECO[1], M. E. LEAL[2], D. A. MCCREEDY[3];
[1]Texas A&M Univ. Neurosci. Inst. For Neurosci., College Station, TX; [2]Biol., Texas A&M Chapter, College Station, TX; [3]Lab. Med. and Program in Immunol., Univ. of California, San Francisco, CA

Abstract: Following spinal cord injury (SCI), an inflammatory cascade ensues that can worsen tissue damage and impair long-term functional recovery. Neutrophils, the most abundant circulating leukocytes in humans, are the first immune cells to infiltrate the injured spinal cord in large numbers and have long been considered to exacerbate tissue damage and functional deficits post-SCI. Recent evidence has demonstrated sex-specific differences in neutrophil function, however, these differences have yet to be explored in the context of SCI. Here, we utilize antibody-mediated neutrophil depletion via anti-Ly6G to assess sex-dependent differences in the role of neutrophils in functional recovery following SCI. To first investigate sex-dependent effects of anti-Ly6G-mediated neutrophil depletion in mice, we administered 2.5mg/kg of either anti-Ly6G (1A8) antibody, IgG (2A3) control antibody, or vehicle intraperitoneally to male and female wildtype mice (age 12-20 weeks). Using flow cytometry, we found that antibody-mediated neutrophil depletion effectively reduced overall neutrophil numbers from circulation in

male and female mice but only significantly reduced mature neutrophils from circulation in females within 1-day post-injection. Antibody-mediated neutrophil depletion performed 1-day prior to SCI had no significant impact on locomotor recovery regardless of sex, however, neutrophil depletion immediately post-injury significantly impaired long-term hindlimb locomotor recovery in male but not female mice. Collectively, our findings indicate a sex-dependent and temporally-restricted role for neutrophils in promoting long-term recovery following SCI. Future work will explore sex-dependent differences in neutrophil function, neutrophil contribution to recovery after SCI, and mechanisms of action for the observed deleterious effects of post-SCI neutrophil depletion in males.

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Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Support: R21 NS115094/NS/NINDS NIH HHS
CTSI

Title: Excitotoxicity and acrolein damage induced by spinal cord injury - the role of GLT-1

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Abstract: Spinal cord injury (SCI) is a devastating condition that causes variable and often significant sensory, motor, and autonomic dysfunction. Despite extensive research, the limited efficacy of clinical treatments highlights the need for a better understanding of the complex pathology underlying SCI in order to identify new treatment strategies for functional restoration. SCI pathology consists of an initial mechanical injury (primary injury) followed by biochemical cascades (secondary injury) which exacerbates the extent of damage and worsens functional outcomes. Acrolein, a highly-reactive α,β-unsaturated aldehyde, has been demonstrated to play a pivotal role in secondary injury events including oxidative stress, mitochondrial dysfunction, and lipid peroxidation. Excitotoxicity is another known secondary injury mechanism in SCI that results from an increase in extracellular glutamate levels. However, the interaction between acrolein pathology and excitotoxicity after SCI has not been investigated in detail. Under normal conditions, synaptic glutamate levels are tightly regulated primarily by astrocytic glutamate transporter-1 (GLT-1) receptors. Evidence showing that GLT-1 could be compromised by other reactive aldehydes suggests its potential as a key target for acrolein-mediated dysfunction that could contribute to excitotoxicity and neurodegeneration following SCI. Thus, we sought to first

characterize GLT-1 expression and function following SCI, and then determine if acrolein mediates these findings by interacting with GLT-1. Using a clinically relevant T10 contusion model of SCI, we show that: 1) GLT-1 and acrolein levels display an inverse expression pattern as revealed by immunoblotting and immunohistochemical labeling, 2) acrolein interacts with GLT-1 as revealed by co-immunoprecipitation, and 3) the injection of exogenous acrolein to intact spinal cord tissue significantly reduced GLT-1 expression. Together, these findings suggest the causal role of acrolein in glutamate excitotoxicity following SCI by affecting GLT-1 protein expression. Furthermore, the implications of this study reveal potential targets for neuroprotective therapeutic intervention following acute SCI.

Disclosures: **R. Stingel:** None. **J. Page:** None. **S. Sun:** None. **R. Shi:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.25/Z24

Topic: C.11. Spinal Cord Injury and Plasticity

Support: JSPS KAKENHI 23K14367
JSPS KAKENHI 22H02962

Title: Astrocytic heterogeneous nuclear ribonucleoprotein U facilitates scar formation after spinal cord injury

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Abstract: Spinal cord injury (SCI) triggers the activation of multiple cellular and extracellular components at the lesion site, leading to the formation of a scar. This scar serves as a crucial structure that regulates the regeneration of neuronal networks and is primarily influenced by the activation and maintenance of astrocytes. Astrocytes emerge as a key player in the formation of glial scars following injury. Although environmental cues are known to play a significant role in astrocyte activation after injury, the intracellular mechanisms governing this process are still being investigated. In this study, by utilizing genome-wide RNA interference screening, we identified heterogeneous nuclear ribonucleoprotein U (Hnrnpu) as a critical endogenous molecule involved in the pathological state of spinal astrocytes. Our results showed that siRNA-mediated inhibition of Hnrnpu effectively suppresses primary astrocyte proliferation and migration *in vitro*. In an *in vivo* SCI model, intraspinal treatment of mice with AAV2/5-Hnrnpu shRNA under the control of the astrocytic glial fibrillary acidic protein (GFAP) promoter significantly inhibited astrocyte proliferation. These results highlight the essential role of Hnrnpu in astrocyte activation in both *in vitro* and *in vivo* assessments. Furthermore, suppression of Hnrnpu dramatically impairs astrocytic scar formation, neuronal circuit reconstruction, and

motor function recovery in injured mice. Notably, we have also found that *HNRNPU* suppression in human astrocytes leads to a reduction in signature genes associated with astrocyte proliferation and migration, wound healing, and axon guidance, suggesting a potential clinical application of modulating astrocytic Hnrnpu for promoting neuronal regeneration after central nervous system (CNS) injuries. Together, our findings reveal a fundamental role of Hnrnpu in facilitating astrocyte activation in response to injuries, and targeting astrocytic Hnrnpu presents a promising therapeutic avenue to restore neurological function following CNS injuries.

Disclosures: L. Quan: None. **R. Muramatsu:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.26/Z25

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canadian Graduate Scholarship - Master's

Title: Examining the role of fractalkine on functional and synaptic changes in a murine model of degenerative cervical myelopathy

Authors: *C. M. ZHOU[1,3], S. T. BROCKIE[1,3], M. MOVAHED[4], S. SADAT[1,3], J. HONG[1,3], M. G. FEHLINGS[1,3,2];
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Abstract: Research Rationale: Degenerative cervical myelopathy (DCM) encompasses several age-related degenerative conditions that cause a compression of the cervical spinal cord. The functional deficits experienced by DCM patients may be caused by a maladaptive elimination of synapses. Recently, the fractalkine receptor (CX3CR1), which is found on microglia, has been shown to be involved in microglial-mediated synaptic elimination. Further, deletion of the receptor results in improved functional outcomes and synapse formation after traumatic spinal cord injury (SCI).
Objective: The main objective of this study is to investigate the role of fractalkine (CX3CR1-CX3CL1) signaling on functional outcomes and synaptic elimination following DCM—a type of non-traumatic SCI. It was hypothesized that *i)* fractalkine-mediated synaptic engulfment occurs after DCM, and that *ii) Cx3cr1* deletion will attenuate the synaptic loss whilst improving functional recovery.
Materials and Methods: DCM was induced in C57BL/6 (n = 5 to 7 / timepoint) and *Cx3cr1*$^{-/-}$ (n = 5 to 7 / timepoint) mice by inserting a polyether aromatic material under the C5-C6 lamina. Animals undergoing sham surgery (n = 3 / timepoint) received material implantation and removal after 30 seconds. Synaptic elimination and functional recovery were characterized at five timepoints (0, 2, 4, 8, and 12-weeks post-DCM) using synaptic markers and CatWalk Gait

analysis.
Results: Our exploratory immunostaining revealed that our model of DCM significantly reduced the total area of the spinal cord and grey/white matter ratio over 12-weeks. We observed that wild-type animals exhibit a time-dependent and selective loss of synapses after DCM as indicated by VGlut2, PSD-95, VGat, and Gephyrin labelling as well as a significant decrease in the number of neurons ($NeuN^+$) in the ventral horn. A genetic knockout of CX3CR1 alters the time frame and type of synapses that are lost after injury and preserves neuronal integrity in the ventral horn.
Conclusion: Current data suggests that synaptic and neuronal loss occurs in wild-type animals undergoing DCM. An absence of *Cx3cr1* may be neuroprotective as it alters the time-course of synaptic loss and preserves the number of neurons after injury. Further immunostaining and behavioural analyses are needed to elucidate the contribution of the fractalkine receptor in more depth.

Disclosures: C.M. Zhou: None. **S.T. Brockie:** None. **M. Movahed:** None. **S. Sadat:** None. **J. Hong:** None. **M.G. Fehlings:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inteligex Inc.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.27/Z26

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Grant 457328
Wings for Life Grant 19/19
Wings for Life 08/20
NIH Grant 120612

Title: Acquired Pneumonia Leads to Lesion-Remote Spinal Cord Bleeding After SCI

Authors: *A. B. HUFFMAN, A. R. FILOUS, J. M. SCHWAB;
Ohio State Univ., Columbus, OH

Abstract: After spinal cord injury (SCI), patients become immunocompromised promoting increased infections. Pneumonia is among the most severe of these infections and is associated with impaired recovery and increased mortality. The mechanisms underlying these deficits are unknown. Here, using a bedside-to-bench approach, we have developed a mouse model of acquired pneumonia after SCI (Spinal Cord Injury-associated Pneumonia, SCI-AP). Using immunohistochemistry, we observed that mice with acquired pneumonia develop more substantial bleeding into the spinal cord itself compared to SCI mice without infections. We have identified these bleeds and categorized their location in 500 µm intervals from the lesion core in both ‘SCI only’ and SCI-AP mice. In SCI-AP infections, we detect additional remote bleedings

('bleeding satellites'). These findings provide a better understanding of the detrimental impact by an acquired pneumonia and may serve as basis to develop protective strategies to protect outcome 'at risk'.

Disclosures: A.B. Huffman: None. **A.R. Filous:** None. **J.M. Schwab:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.28/Web Only

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Endowment
R01NS091582
EpiMet pilot

Title: Alteration of metabolic phenotypes of intraspinal macrophages after SCI to derive reparative characteristics.

Authors: *R. KUMARI[1], *R. KUMARI[2], H. J. VEKARIA[3], O. H. WIREMAN[2], W. M. BAILEY[2], S. M. MACLEAN[2], A. N. STEWART[2], E. P. GLASER[2], P. G. SULLIVAN[3], S. P. PATEL[2], J. C. GENSEL[2];
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Abstract: Alteration of metabolic phenotypes of intraspinal macrophages after SCI to derive reparative characteristics. Spinal cord injury (SCI) activates resident microglia and recruits peripheral monocytes (collectively, CNS macrophages) into the injured nervous system that play roles in neuropathology by exacerbating neurodegeneration but also promoting repair. The average age at the time of SCI has increased to a median age of 50.5 years old in the US. Age is a key determinant of functional recovery following both clinical and experimental SCI. Sustained pro-inflammatory macrophage activation contributes to these age-related SCI deficits. Here, we hypothesize that injury-induced impairments in macrophage metabolism, and specifically oxidative phosphorylation (OXPHOS), drive pro-inflammatory macrophage activation after SCI and that age-dependent impairments in macrophage metabolism drive sustained pro-inflammatory macrophage activation. We tested this hypothesis by analyzing the bioenergetic profiles of intraspinal macrophages after T9 contusion SCI and comparing young (4-month-old; MO) versus aged (14-16 MO) mice. Spinal cords were collected at 7 days post-injury (dpi) and subjected to a magnetic bead sorting protocol to isolate intraspinal macrophage/microglia cells (CD11b+). Viable cells were plated at 50,000 cells/ well and subjected to Seahorse XF (Agilent) assay analysis and real-time levels of oxygen consumption rate (OCR) were determined. Treatment with dichloroacetate (DCA 25mM-3 hrs)- a pan pyruvate dehydrogenase PDK inhibitor- significantly increased basal OCR and ATP-linked OCR

in cultured macrophages and SCI macrophages isolated and treated ex-vivo. Additionally, basal respiration and ATP synthesis-linked respiration of intraspinal CD11b+ cells were significantly lower in aged mice compared to young mice. Our observation indicates SCI causes metabolic dysfunction in macrophages by decreasing OXPHOS which can be improved by DCA treatment. We further show that age causes metabolic dysfunction limiting macrophages' abilities to shift from glycolysis to OXPHOS. Recent advances in macrophage metabolism highlight efficient OXPHOS as key for sustaining anti-inflammatory/reparative functions. Therefore, our findings implicate macrophage metabolism as a potential contributor to pro-inflammatory activation with age.

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Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.29/Z27

Topic: C.11. Spinal Cord Injury and Plasticity

Support: T32 HL134621
R01 HL153102
SPARC OT2OD023854

Title: Distribution of cervical spinal c-Fos expression after epidural stimulation in rats with cervical spinal cord injury

Authors: *A. R. MICKLE[1], R. COFFEY[2], N. HALL[2], J. D. PEÑALOZA-APONTE[1], E. A. DALE[2];
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Abstract: While treatments to restore breathing after cSCI are lacking, epidural stimulation has shown great promise in recovering volitional control of many functions. We have previously shown that closed-loop epidural stimulation (CL-ES) reinstates ipsilesional diaphragm EMG activity in C2-hemisected (C2HS) rats [Mickle et al., 2023]. With longer stimulation periods, CL-ES further elicits lasting increases in the excitability of the respiratory motor network [Malone et al. 2022]; together, these results show great promise for the use of CL-ES to restore independent breathing. Modeling studies have explored epidural stimulation current spread, but the neuronal pathways responsible for CL-ES restoration of diaphragm EMG output after C2HS are unknown. Here, we investigate the neural populations activated by inspiratory-triggered electrical stimulation. Rats were isoflurane anesthetized and implanted with diaphragm EMG and C4 stimulating electrodes before C2-HS. Inspiratory triggered spinal motor evoked potentials of the diaphragm (sMEPs) were recorded to determine motor threshold. Rats were exposed to either

20 minutes of biphasic CL-ES at 75% threshold (n = 3) or a sham waiting period (n = 3). A third group of time-matched controls (n = 3) remained electrically naïve with no sMEPs measurements. An hour after the start of CL-ES, animals were perfused with 4% PFA, spinal cords flash frozen, and the C4 segment serially sectioned. An initial screening of c-Fos protein expression every 240-300 µM was utilized to identify the location of highest expression at which point c-Fos, ChAT, and somatostatin spatial mRNA expression was determined via RNAscope. Total c-Fos expression did not reach significant differences between groups (cell count: 242 +/- 95 naïve, 301 +/- 36 sham, 400 +/- 64 stim). However, c-Fos localization was highly lateralized in electrically naïve animals, while animals receiving electrical stimulation had a more even distribution (lower side % of total: 21 +/- 5 naïve, 44 +/- 5 stim, $p < 0.05$). Stimulation did not alter colocalization with ChAT as ~15% of ChAT neurons were c-Fos positive across groups, and only marginally increased colocalization of c-Fos with somatostatin (% somatostatin c-Fos positive: 1.5 +/- 1.5 naïve, 2.8 +/- 0.9 sham, 5.8 +/- 0.8 stim). Work to identify c-Fos expression in CtB labeled phrenic motor neurons, inhibitory/excitatory neurons, interneurons, and glia is ongoing. While a definitive neural population activated by CL-ES remains elusive, this work begins to uncover the neural underpinnings of CL-ES's increase in motor output, which will be key to tailoring this therapy for maximal benefit in the clinic.

Disclosures: A.R. Mickle: None. **R. Coffey:** None. **N. Hall:** None. **J.D. Peñaloza-Aponte:** None. **E.A. Dale:** None.

Poster

PSTR206. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR206.30/Z28

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01 NS099635
NIH grant R01 NS110707

Title: Regulation of fibrotic scar formation by interferon gamma signaling after spinal cord injury

Authors: Y.-Y. ZHENG[1], X. CHEN[1], H. XUE[1], Y. LIU[1], ***Q. CAO**[2];
[1]Ctr. for Translational Sci., FIU, Port St Lucie, FL; [2]Ctr. for Translational Sci., Florida Intl. Univ., Port St. Lucie,, FL

Abstract: Spinal cord injury (SCI) leads to formation of fibrotic scar which represents the major barrier for neural regeneration and repair. It is critical to elucidate mechanism regulating fibrotic scarring to develop novel strategies to improve the inhibitory injury environment and promote regeneration and functional recovery. Here, using genetic lineage tracing, we determined that both perivascular and meningeal fibroblasts migrated to injury site following immune cell infiltration staring 3 days after cervical segment 5 dorsal hemisection and formed persistent

fibrotic scar in the injury epicenter. Interferon gamma receptor 1 (IFNGR1) and its downstream signaling were significantly upregulated in scar-forming fibroblasts in the injured spinal cord. Importantly, fibrotic cell-specific deletion of IFNGR1 after SCI decreased the number of PDGFRbeta+ fibroblasts and the deposits of fibronectin, collagens and CSPGs and thus reduced fibrotic scarring in the injury center. Furthermore, regeneration of descending 5-HT and corticospinal tracts was significantly increased in IFNGR1 conditional deletion mice compared to control ones. Our results indicate that interferon gamma signaling plays an important role in regulating fibrotic scarring, suggesting manipulation of this signaling could be a novel approach to reduce fibrosis and alleviate the injury environment to promoting axonal regeneration after SCI.

Disclosures: **Y. Zheng:** None. **X. Chen:** None. **H. Xue:** None. **Y. Liu:** None. **Q. Cao:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.01/AA1

Topic: D.01. Somatosensation

Support: NIH Grant R01NS115963

Title: Anatomical and functional analysis of cervical ascending projection interneurons

Authors: *N. RANAWAT, F. IMAI, Y. YOSHIDA;
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Abstract: Second-order cervical ascending interneurons receive information from sensory neurons and then relay it to supraspinal region like dorsal column nuclei (DCN), ventral medullary reticular formation (MdV). In this study we aim to perform anatomical and functional analysis of excitatory and inhibitory cervical spinal interneurons projecting to supraspinal regions. Retrograde tracing using CTB and retrograde AAV illustrates that DCN projecting C5/C6 cervical spinal neurons are mainly present in lamina 4Sp-8Sp and approximately 55% neurons are Pax2 positive excitatory. Using retrograde transsynaptic Pseudotype rabies virus, we also found that the DCN projecting C5/C6 cervical spinal interneurons receives input from both ipsi and contralateral sensory neurons as well as other spinal interneurons. To visualize excitatory and inhibitory cervical spinal interneurons, we have used a Cre-dependent anterograde AAV to trace the axons and presynaptic terminals from cervical C5/C6 with vGlut2Cre (excitatory) and vGatCre (inhibitory) mice. Anatomical tracing from excitatory cervical neurons shows axon projection to thalamus, parabrachial nuclei (PBN), DCN and MdV. On the other hand, inhibitory cervical neurons only project to the DCN and MdV. Finally, we investigated whether DCN projecting cervical spinal projection neurons rely on skilled behavior. Using inhibitory DREADDs, we silenced the cervical spinal interneurons projecting to DCN in pasta handling and found that chemogenetic silencing of the cervical spinal interneurons-DCN circuit

resulted in increased pasta handling time. Thus, we propose that cervical spinal interneurons integrate sensory information and both excitatory and inhibitory neurons relay it to supraspinal region DCN. Chemogenetic silencing of this circuit can impair upper limb functions. Together, these findings suggest role of cervical spinal interneuron-DCN circuit in skilled behavior.

Disclosures: **N. Ranawat:** None. **F. Imai:** None. **Y. Yoshida:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.02/AA2

Topic: D.01. Somatosensation

Support: National Science and Technology Innovation 2030 Major Program (2021ZD0204404, 2022ZD0207300)
Lingang Laboratory (Grant No. LG202104-01-01, LG-QS-202201-03)
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Shanghai Rising-Star Program (Grant No. 21QA1401000)
the Medical Research Council (Grant No. MR/V033638/1)
Tencent Foundation

Title: Organization of somatosensory ascending pathways revealed by single-neuron projectome analysis

Authors: *W. DING[1], Y. SUN[2,3];
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Abstract: Relay of multimodal somatosensory information from the spinal cord to the brain is critical for sensory perception, but the underlying circuit organization remains unclear. We reconstructed central projections of 831 mouse spinal cord neurons, and identified 24 projectome-defined subtypes, each projecting via extensive axon collaterals to a distinct set of brain areas, predominantly located in the contralateral hemisphere. Many subtypes exhibited dorsal horn-lamina preferences in their soma location, and the total length of axon arbors of each neuron of some subtypes positively correlated with that of its dendritic arbors. Furthermore, axon projections of central relay neurons receiving spinal projections were reconstructed and 36

projectome-defined subtypes were identified, each projecting to selective brain areas in one or both hemispheres. These results on the organization of axon projections at the single-cell level provide a new framework for understanding the complex neural circuitry underlying coordinated processing of diverse somatosensory information.

Disclosures: W. Ding: None. **Y. Sun:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR207.03/AA3

Topic: D.01. Somatosensation

Support: National Science and Technology Innovation 2030 Major Program Grant 2021ZD0204404
National Natural Science Foundation of China Grant 31825013

Title: Classification, spatial distribution and projectome of diverse spinal projection neurons

Authors: *J.-K. LIN, Y.-G. SUN;
Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai, China

Abstract: Projection neurons in the spinal dorsal horn are critical for conveying different modalities of somatosensory information to the brain, but the subtypes of these neurons and their central projection patterns remain largely unknown. Using single-cell transcriptomic profiling of retrogradely labeled spinal projection neurons in adult mice, we found 11 subtypes of projection neurons, with the different laminar and mediolateral distribution. Sensory stimulus-dependent activation of immediate early genes revealed that different subtypes were preferentially involved in pain and itch processing. Further analyses showed selective molecular features and communication networks in subtypes involved in pain and itch processing. Finally, mapping of projectome of different neuronal subtypes, including newly-defined inhibitory neurons, revealed subtype-specific patterns of central targets involved in sensory and emotional processing. These results provide a comprehensive transcriptomic description of diverse subtypes of spinal projection neurons and their distinct properties in central projections and sensory processing.

Disclosures: J. Lin: None. **Y. Sun:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR207.04/AA4

Topic: D.01. Somatosensation

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Brain Canada Platform (Grant No. 255934)
Brain for Healthy Lives (HBHL, Grant No. 2473633)
CIHR (fellowship)
FRQS (fellowship)

Title: Mapping the topographical functional connections between the brain and the cervical spinal cord

Authors: *C. LANDELLE[1], N. KINANY[2,3], B. DE LEENER[4,5], O. LUNGU[1], V. MARCHAND PAUVERT[6], D. VAN DE VILLE[2,3], J. DOYON[1];
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Abstract: Introduction : The sensorimotor system is characterized by a topographical organization reflecting body space representation, with a somatotopic organization of the brain (Penfield et Boldrey, 1937), and dermatomal and myotomal arrangements in the spinal cord. Yet, little is known about the topographical organization of brain-spinal cord functional connections in humans (Vahdat et al. 2015, Kinany et al. 2023). Here, we address this gap by leveraging simultaneous fMRI of the brain and spinal cord at rest to capture brain-spinal cord functional connectivity (FC) using an information theory measure - mutual information (MI).
Methods : 31 right-handed healthy controls underwent an MRI session (3T Siemens Prismafit), consisting in simultaneous brain and cervical spinal cord acquisition of anatomical and functional images at rest. Preprocessing was done using an in-house pipeline (Landelle et al. 2023). We investigated FC using pairwise MI. Specifically, we extracted the mean time series from spinal regions of interest (ROIs) and computed their MI with brain voxels time series. The individual MI maps were entered in a non-parametric permutation-based t-test (1000 permutations). T-maps were corrected for multiple comparisons at cluster level (p-FWE < 0.05).
Results : First, we found significant FC between each spinal quadrant (right ventral, left ventral, right dorsal and left dorsal) and cortical (M1, S1, SMA, pMC) and subcortical (putamen, thalamus, cerebellum) sensorimotor areas. Most of the significant MI clusters were found in the sensorimotor cortex contralateral to spinal ROIs. Second, a topographical organization emerged from the FC between the different levels of the cord and the sensorimotor cortex. Notably, there was a rostro-caudal gradient of FC patterns, with different spinal levels preferentially connected to different parts of the sensorimotor cortex, in accordance with knowledge on body space representation.
Conclusion : Our findings reveal in vivo the topographical organization of the functional cerebro-spinal connections. They show that the brain-spine connectivity follows key features of the sensorimotor system, namely lateralization and somatotopy. This constitutes the first demonstration of a large-scale somatotopic arrangement in agreement with homunculi representations.

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Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.05/AA5

Topic: D.01. Somatosensation

Support: NIH

Title: A spinal circuit for heat sensation

Authors: ***H. WANG**, W. CHEN, Z. DONG, W. CUI, W.-J. ZOU, W.-C. XIONG, L. MEI; Case Western Reserve Univ., CLEVELAND, OH

Abstract: Neurons for noxious heat sensation in spinal cord were largely unknown. We designed a method to label the heat-activated spinal cord neurons and identified the interneuron markers they express. The heat-activated spinal interneurons are heterogynous, with some of them positive of ErbB4, SST and CCK. Functional analysis determined both the requirement and sufficiency of ErbB4+ neurons for basal heat sensation. The additive effects among ErbB4+, SST+ and CCK+ neurons suggest a population coding model for noxious heat sensation. We also got data to show that the ErbB4+ spinal neurons receive direct inputs from the TRPV1+ DRG neurons and the NRG1-ErbB4 signal at DRG-spinal cord connection participates in the heat sensation.

Disclosures: **H. Wang:** None. **W. Chen:** None. **Z. Dong:** None. **W. Cui:** None. **W. Zou:** None. **W. Xiong:** None. **L. Mei:** None.

Poster

PSTR207. Spinal Sensory Processing

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.06/AA6

Topic: D.01. Somatosensation

Support: F32NS110155
K99NS126569
R01AR063772

Title: Visualizing the spinal coding of itch

Authors: *T. D. SHEAHAN[1], C. A. WARWICK[1], A. Y. CUI[1], D. A. A. BARANGER[2], V. J. PERRY[1], A. P. MANALO[1], L. G. FANIEN[1], H. KOERBER[3], S. ROSS[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Washington Univ. in St. Louis, St. Louis, MO; [3]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA.

Abstract: Although itch is a distinct sensation, the neural circuits that encode itch remain unclear. Here, we visualize the spinal representation of itch by using two-photon Ca^{2+} imaging to identify the neurons that are engaged by diverse itch-inducing agents and inhibited by itch-relieving agents. With this approach, we characterize the neurons that integrate itch locally within the spinal cord, as well as those that relay itch to the brain. Moreover, we find that itch-inducing agents activate a population of gastrin-releasing peptide receptor neurons that show a distinct form of persistent and cell autonomous Ca^{2+} oscillations, which may represent a spinal signature of itch. Finally, we show agents that relieve itch, including clinically effective kappa opioid receptor agonists, do so by suppressing activity of itch spinal neuron networks. Our work provides a neural framework for the spinal coding of itch that can be used as the foundation to understand the mechanisms underlying pathological itch conditions in future studies.

Disclosures: T.D. Sheahan: None. **C.A. Warwick:** None. **A.Y. Cui:** None. **D.A.A. Baranger:** None. **V.J. Perry:** None. **A.P. Manalo:** None. **L.G. Fanien:** None. **S. Ross:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.07/AA7

Topic: D.01. Somatosensation

Support: R34 NS111654

Title: Targeting translaminar interneurons of the spinal cord dorsal horn for the study of somatosensory integration in chronic pain

Authors: M. E. DOTY[1], L. VULCHANOVA[2], ***A. G. J. SKORPUT**[3];
[1]Dartmouth Col., Hanover, NH; [2]Univ. of Minnesota, Minneapolis, MN; [3]Neurol., Dartmouth Hlth., Lebanon, NH

Abstract: Chronic pain is characterized by an enhanced perception of somatosensory stimuli. In the context of tactile allodynia, this hypersensitivity is driven in part by altered functioning of neural circuits in the spinal cord dorsal horn (SCDH) that regulate the integration of disparate somatosensory modalities. However, which SCDH neurons are necessary/sufficient to mediate allodynia, and how they participate in the nociceptive circuit remains unclear. Somatosensory input to the SCDH is spatially segregated into distinct laminae with noxious input synapsing into superficial laminae while non-noxious tactile input projects to deeper laminae. Excitatory interneurons of the SCDH with translaminar morphologies have long been hypothesized to serve as hubs of integration between non-noxious and noxious somatosensory modalities. However,

testing of this hypothesis has been limited by an inability to selectively identify translaminar neurons. We have discovered that continued expression of the developmental transcription factor Prox1 serves as a novel genetic marker for a molecularly distinct population of excitatory SCDH interneurons that have translaminar morphologies, and which can be classified as vertical and antenna cells. Using a transgenic mouse (ProxTom), in which expression of the fluorescent protein tdTomato is controlled by the Prox1 promoter, we are implementing tract tracing, immunohistochemistry, patch clamp electrophysiology, calcium imaging and single cell transcriptomics to characterize the function of these translaminar spinal neurons in mediating acute and chronic pain. Greater understanding of the circuits that mediate nociception, and the cellular/molecular plasticity they undergo in chronic pain states will significantly advance the search for circuit specific therapies that block the transition from acute to chronic pain while avoiding the system-wide side effects of current analgesics.

Disclosures: **M.E. Doty:** None. **L. Vulchanova:** None. **A.G.J. Skorput:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.08/AA8

Topic: D.01. Somatosensation

Title: Neurochemical organization of the internal basilar nucleus in the human spinal cord

Authors: *G. SENGUL[1], E. D. G. KIRBIYIK[2,3], E. CANDAR[2], I. DEMIRCUBUK[4];
[1]Ege Univ., Izmir, Turkey; [2]Neurosci., Ege University, Inst. of Hlth. Sciences, Dept. of Neurosci., Izmir, Turkey; [3]Izmir Tepecik Training and Res. Hospital, Dept. of Physical Med. and Rehabil., Izmir, Turkey; [4]Ege University, Inst. of Hlth. Sciences, Dept. of Anat., Izmir, Turkey

Abstract: Internal basilar nucleus (IB) is located within the limits of lamina 4 in C1-C6 segments of the ventromedial spinal dorsal horn. Primary afferent fibers from the dorsal root ganglia project to the IB, and there are central projections from median and ulnar nerves to here. A large number of IB neurons send projections to the thalamus and receive descending projections from the sensorimotor cortex. IB was reported to be present in the cat, rat, hamster, mouse, marmoset monkey, rhesus monkey, and human. A limited number of studies regarding the chemoarchitecture of the IB showed parvalbumin, calbindin, calmodulin, calretinin, and VGLUT1 immunoreactivity in the rat IB neurons. To investigate the neurochemical organization of the human IB, 10% formalin-fixed C1-C6 human spinal cord segments were cut using a cryostat at a thickness of 30 micrometers and stained using immunohistochemical markers realed to pain: calcitonin gene-related peptide, choline acetyltransferase, enkephalin, glycine, glutamate, serotonin, and substance P. IB neurons were found immunoreactive for all of these markers under light microscopy. The findings of this study reveals the neurochemical organization of the IB in the human spinal cord and suggests its involvement in pain processing.

Disclosures: **G. Sengul:** None. **E.D.G. Kirbiyik:** None. **E. Candar:** None. **I. Demircubuk:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.09/AA9

Topic: D.01. Somatosensation

Support: ANR (AAPG2021)
French Ministry of Higher Education, Research and Innovation (PhD fellowship to Elysa Crozat)

Title: Thoracic Cerebrospinal Fluid-Contacting Neurons: Novel Players in Autonomic Function Regulation?

Authors: *E. CROZAT, J. RAMIREZ-FRANCO, C. MICHELLE, J. TROUSLARD, N. WANAVERBECQ;
Inst. of Neurosciences of la Timone, Marseille, France

Abstract: The sympathetic autonomic nervous system (SANS) plays a vital role in controlling involuntary bodily functions. The activity of sympathetic preganglionic neurons (SPNs), the primary output of the SANS, is regulated by supraspinal descending pathways and local interneurons. However, the intraspinal cellular partners involved in SPN regulation remain largely unknown. Cerebrospinal fluid-contacting neurons (CSF-cNs) are located near to SPNs in the thoracolumbar segments and are strategically positioned between the cerebrospinal fluid (CSF) and parenchyma. These CSF-cNs are thought to function as chemo- and mechanosensory neurons, detecting and integrating signals relevant to body homeostasis. Therefore, they present an ideal candidate to regulate autonomic function alongside SPNs. However, their specific role and interaction within the SANS require further investigation. Using advanced molecular, histological, electrophysiological means and optogenetic techniques, the anatomical and functional connectivity between CSF-cNs and SPNs was characterized. We determined the morphology, distribution, and electrophysiological properties of SPNs across the thoracic spinal cord and our findings indicate that SPNs project towards the central canal region, where CSF-cNs are located. Notably, *punctae* originating from CSF-cNs are also observed around SPNs, suggesting reciprocal connectivity. To further investigate this circuitry, we developed viral infection of the adrenal medulla to selectively trace SPNs neural pathways. We used transgenic mice (PDK2L1-Cre::*flex*-ChR2) to combines patch-clamp recordings of SPNs with optogenetic activation of CSF-cNs in acute thoracic spinal cord slices. We developed the CRACM strategies to demonstrate functional connectivity between CSF-cNs and SPNs. Our data suppose both anatomical and functional interaction between CSF-cNs and SPNs. In the future, we will refine the observed connectivity using retrograde monosynaptic tracing with rabies virus and expect to label SPN presynaptic partners, including CSF-cNs. Further, we will need to assess the impact of

CSF-cN activity on SANS function and its influence on autonomic outflow, particularly cardiovascular tone. The observed anatomical connectivity and the future development of surgical and labeling techniques will further help in characterizing at the morphological and functional level this novel autonomic network. The results of this study provide important insights into the potential interplay between CSF-cNs and SPNs as intraspinal partners regulating autonomic function.

Disclosures: **E. Crozat:** None. **J. Ramirez-franco:** None. **C. Michelle:** None. **J. Trouslard:** None. **N. Wanaverbecq:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.10/AA10

Topic: D.01. Somatosensation

Support: FWO-SB Ph.D. fellowship 1S56719N
MSCA Postdoctoral Fellowship 101067670
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Wings for Life Spinal Cord Research Foundation WFL-BE-15/22
FWO Research Grant G096320N

Title: Two distinct inhibitory populations govern the acquisition and recall of spinal sensorimotor learning.

Authors: *S. LAVAUD[1,2], C. BICHARA[1,2], M. D'ANDOLA[1,2], S. YEH[1,2], A. TAKEOKA[1,2,3]; [1]Neuro Electronics Res. Flanders (NERF), Leuven, Belgium; [2]KU Leuven, Dept. of Neurosci. and Leuven Brain Inst., Leuven, Belgium; [3]IMEC, Leuven, Belgium

Abstract: Spinal circuits are essential for movement automaticity. The spinal cord not only executes and adjusts motor outputs by integrating information from multiple somatosensory channels but also undergoes lasting adjustments following repetitive practice in various motor tasks, even without brain inputs. However, the spinal mechanisms underlying these lasting sensorimotor adjustments remain unclear. Here, we establish a quantitative kinematic framework to characterize a spinal conditioning behavior, where spinal circuits functionally isolated from the brain learn to adapt motor output through multimodal sensory integration. Using unbiased kinematic analyses, mouse genetics, and virus-mediated loss-of-function circuit manipulations, we uncover that a class of dorsal spinal inhibitory neurons, $Ptf1\alpha^{ON}$ neurons, is crucial for this learning behavior. Using *in-vivo* high-density electrophysiological recordings in the spinal cord and optogenetics identification of $Ptf1\alpha^{ON}$ neurons in awake, behaving mice, we found a persistent, up-regulated activity of this population during learning at the single-unit level. Further analysis reveals selective modulation of Aδ/C second-order spinal neurons. Conversely, acutely silencing $Ptf1\alpha^{ON}$ neurons using the pharmacogenetic DREADD method reverses the specific

modulation of Aδ/C second-order spinal neurons. These findings demonstrate that *Ptf1α*^{ON} neurons are necessary for modulation of Aδ/C afferents and this modulation likely mediates spinal learning behavior. Additionally, we characterize whether and how the spinal cord retains previously learned behavior by subjecting mice to a recall trial after a single training session. We find that the spinal cord can retain the previously conditioned behavior. Moreover, selectively suppressing or facilitating the activity of a class of ventral inhibitory neurons, *Engrailed1*^{ON} neurons, does not affect learning but flexibly disrupts or facilitates recalling conditioned behavior. Together, these results reveal the mechanisms regulating learning and retention of learned behavior which are mediated by two molecularly and spatially distinct spinal inhibitory populations.

Disclosures: **S. Lavaud:** None. **C. Bichara:** None. **M. D'Andola:** None. **S. Yeh:** None. **A. Takeoka:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR207.11/AA11

Topic: D.01. Somatosensation

Support: German Research Foundation (DFG) DA 2322/1-1

Title: Predictive inhibition of leg proprioception in Drosophila

Authors: *C. DALLMANN, S. AGRAWAL, S.-Y. J. LEE, A. COOK, J. C. TUTHILL; Univ. of Washington, Seattle, WA

Abstract: To effectively control arms and legs, motor circuits rely on feedback from proprioceptive neurons encoding body position and movement. Feedback signals must be tuned to support different behaviors, but it has been challenging to understand how specific types of feedback are regulated in behaving animals at a cellular level. We addressed this question in the fruit fly Drosophila, focussing on proprioceptive neurons of the femoral chordotonal organ (FeCO), the largest proprioceptor in the fly leg. FeCO axons project to the ventral nerve cord, the analog of the spinal cord. To study their activity during behavior, we developed a setup for two-photon calcium imaging of the ventral nerve cord and markerless tracking of leg movements of tethered flies walking and grooming on a treadmill. We found that position-encoding FeCO axons were active across behaviors, whereas movement-encoding FeCO axons were suppressed during walking and grooming. Circuit reconstruction in an electron microscopy volume of the Drosophila ventral nerve cord revealed that the movement-encoding axons receive input from a specific group of GABAergic interneurons. The activity of these neurons suggests that they provide predictive presynaptic inhibition during active leg movements. We propose that the function of this predictive inhibition could be to suppress expected proprioceptive feedback

caused by the animal's own movement to increase sensitivity to unexpected external perturbations.

Disclosures: C. Dallmann: None. **S. Agrawal:** None. **S.J. Lee:** None. **A. Cook:** None. **J.C. Tuthill:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.12/AA12

Topic: D.01. Somatosensation

Support: CGS-D CIHR
Foundation Grant CIHR

Title: Disinhibition compromises spatiotemporal processing of tactile input by disrupting the receptive fields of spinal dorsal horn neurons

Authors: *L. MEDLOCK[1], S. A. PRESCOTT[2];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: The spinal dorsal horn (SDH) plays a crucial role in processing touch and pain signals. Different inhibitory circuit motifs in the SDH, including feedforward and lateral inhibition, influence the temporal and spatial integration of incoming signals. Spatial processing of somatosensory input is accomplished through neuronal receptive fields (RF). Specifically, spinal neurons have a center-surround RF structure formed via excitatory connections with primary afferents and inhibitory connections with other spinal interneurons. RFs have been shown to expand when synaptic inhibition is reduced, which may underlie the clinical observations that broad or dynamic stimuli produce more allodynia than punctate or static touch in patients with neuropathic pain. Despite recent findings, the impact of RF expansion on SDH neuron or circuit function remains unclear. To begin disentangling this synaptic connectivity in the spinal cord we recently built a data-driven model of the SDH circuit. Furthermore, we have incorporated the synaptic connectivity underlying RFs into our model to efficiently examine the spatial processing of different types of tactile stimuli (e.g. diffuse/punctate or static/dynamic). Using experimental electrophysiology data recorded from spinal interneurons, we fit the network model to both normal and pathological RF sizes and firing rates. To account for this increased model complexity and to allow for easier scalability of RFs in our model we used advanced optimization techniques including evolutionary algorithms. Neuropathic conditions, simulated by reductions in synaptic inhibition, disrupted feed-forward inhibition in ways that increased temporal summation of spikes and lateral inhibition in ways that reshaped RF organization and increased spatial summation of tactile input. Finally, our model made testable predictions regarding multiscale targets for combating the effects of pathological disinhibition on spatiotemporal processing in the SDH.

Disclosures: **L. Medlock:** None. **S.A. Prescott:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: D.01. Somatosensation

Support: UKRI CDT in Prosthetics and Orthotics (Grant No. EP/S02249X/1)
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Title: Cervical transcutaneous spinal cord stimulation can elicit sensations in the distal upper limb at sub-motor stimulation intensities

Authors: *R. KHARBOUSH[1], A. PASCUAL VALDUNCIEL[1], J. IBANEZ PEREDA[2], D. FARINA[1];
[1]Imperial Col. London, London, United Kingdom; [2]Univ. of Zaragoza, Zaragoza, Spain

Abstract: Sensory function loss due to amputation is a key challenge in prosthetic development, particularly for upper limb prosthetics. Peripheral sensory interfaces are only suitable for people with distal amputations. Epidural spinal cord interfaces have demonstrated success in generating sensations perceived to stem from the missing limb in individuals with various levels of amputation. However, they require invasive specialised surgery. In this work, we present a novel sensory interface with the central nervous system via cervical transcutaneous spinal cord stimulation (TSCS). To identify TSCS's ability to elicit sensations from different regions in the upper limb, we explored the effects of different TSCS parameters such as stimulation location (C5-C7, C6-T1), burst frequency (50 Hz, 100 Hz, 150 Hz) and carrier frequency (6 kHz vs 10 kHz) on the quality and location of evoked perceptions in fifteen healthy human subjects using a psychophysical questionnaire. We also compared TSCS to transcutaneous brachial plexus (BP) stimulation to unravel the potential effects of TSCS caused by spinal cord stimulation and the effects due to direct nerve stimulation. Our work demonstrates that TSCS applied at sub-motor threshold with a burst-modulated alternating current evoked sensory precepts perceived to originate from the distal upper limb in 73.3% of subjects. We also show that all twelve tested TSCS protocols elicited sensations in the distal upper limb comfortably, i.e., below the threshold of unpleasant sensation. The TSCS protocol that was found to be optimal (cathode location over C5-C7, a carrier frequency of 6 kHz and a burst frequency of 50 Hz) could elicit sensations distally in eleven out of fifteen subjects with an average subject-ranked comfort rate of 59.7%, which corresponds to "Neutral". Moreover, during TSCS, 60% of subjects reported feeling sensations stemming from the fingertips. We showed that TSCS is superior to the BP stimulation protocol used in this study, which elicited distal precepts in only 40% of the subjects. Our results

indicate a significant correlation between the stimulation location and eliciting a sensory precept in the distal upper limb ($p < 0.05$). The results of this study reveal that TSCS is a promising technique for sensory restoration that can potentially be applied to individuals with any upper limb amputation levels.

Disclosures: R. Kharboush: None. **A. Pascual Valdunciel:** None. **J. Ibanez Pereda:** None. **D. Farina:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.14/AA14

Topic: D.01. Somatosensation

Support: Max Planck Society
European Research Council (under the European Union's Horizon 2020 research and innovation programme; grant agreement No 758974)

Title: Investigation of thermal coding strategies in the human spinal cord via 7T fMRI

Authors: *A. DABBAGH, U. HORN, F. EIPPERT;
Pain Perception, Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Over the past decades, we have gained a deeper understanding of temperature detection by primary sensory neurons. Notably, a recent study (1) used in-vivo calcium imaging to study dorsal horn neuron responses to thermal stimuli in mice, revealing distinct coding strategies for heat and cold. To study these mechanisms in the human spinal cord, we aim to apply a similar approach, utilizing high-resolution 7T fMRI to assess thermally-induced hemodynamic responses.
In a first proof-of-principle experiment, we aim to measure and differentiate BOLD responses to innocuous and noxious heat and cold stimuli. However, pain thresholds for cold stimuli can vary widely between individuals (2). Moreover, 7T fMRI in the spinal cord is challenging due to susceptibility-induced inhomogeneities and physiological noise. To address these challenges, we piloted i) the psychophysical assessment of thermal stimulation and ii) the optimization of imaging sequences. We gathered behavioral data from 24 participants using thermal stimuli (range: 0°C - 47°C) of varied durations to identify suitable stimulation parameters. In parallel, we focused on achieving high-resolution imaging and improving the tSNR of functional data. Data was acquired via gradient echo EPI with a spatial resolution of 0.75 x 0.75 x 3mm. We also utilized a high-resolution T2*-weighted sequence (0.38 x 0.38 x 3mm) for gray and white matter delineation and structural T1 maps for anatomical reference.
Our behavioral piloting suggested that stimuli with a duration of 5s and temperatures of 46°C, 42°C, 18°C and 0°C are optimally suited, as they induced the expected sensations and are short enough to allow for large trial numbers to boost statistical power. Preliminary fMRI data yielded

a tSNR of 15.76 (SE = 0.70) within the spinal cord, and additional slice-specific z-shimming improved the tSNR by 26.8%. Precise gray and white matter delineation on the individual level was achieved via the high-resolution T2*-weighted structural data.
This project aims to investigate thermal coding strategies in the human spinal cord, translating findings from animal models to humans. The selected thermal stimuli and optimized 7T fMRI sequences provide a promising experimental protocol. Despite challenges at this preliminary stage, the study aims to enhance our understanding of thermal perception mechanisms.
(1) C. Ran, M. A. Hoon, X. Chen, Nat. Neurosci. 19, 1201-1209 (2016).
(2) S. Chéry-Croze, Pain. 17, 109-137 (1983).

Disclosures: **A. Dabbagh:** None. **U. Horn:** None. **F. Eippert:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.15/AA15

Topic: D.01. Somatosensation

Support: NIH Grant 1U19NS130608-01

Title: Mapping sensory circuits in the human spinal cord using immunohistochemistry

Authors: *O. C. DAVIS[1], A. J. TODD[2], T. J. PRICE[1];
[1]Ctr. for Advanced Pain Studies, UT Dallas, Richardson, TX; [2]Spinal Cord Group, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Primary afferents detect sensory stimuli and convey this information to the spinal dorsal horn. Incoming signals can be amplified or reduced through excitatory and inhibitory circuits, respectively, and the balance of excitatory to inhibitory tone in the spinal cord has a major impact on sensory thresholds and perception. A combination of the use of transgenic mouse lines and immunohistochemistry has revealed much information about the anatomy of functional spinal circuits underlying pain and itch transmission in the rodent, although the extent to which this translates in human is still relatively unknown. Antibodies to glutamate and GABA receptor subunits are unreliable in formaldehyde-fixed tissue and require antigen retrieval. To avoid this, antibodies raised against either postsynaptic scaffolding proteins or presynaptic transporters have been used to dissect neuronal circuits in rodent spinal cord. We have used a combination of confocal and electron microscopy techniques on tissue sections from male and female organ donors to show that antibodies for synaptic marker proteins can be used to reliably visualise synapses in human spinal cord. Punctate synaptic labelling was consistently seen across adult ages (18 - 64) and both sexes. Postsynaptic markers of glutamatergic synapses, Homer and GluR2, colocalised and often apposed profiles that contained the vesicular glutamate transporter 2, but not those containing the vesicular GABA transporter. Similarly, Gephyrin, a protein localised to GABAergic and glycinergic synapses in the mouse spinal cord, apposed profiles

containing inhibitory but not excitatory presynaptic markers. These can therefore be used to begin dissecting excitatory and inhibitory circuits in human spinal cord. Glutamate is the main fast neurotransmitter of primary afferents and immunolabelling for Homer has been used to identify the output targets of different subpopulations of primary afferent in the rodent. Here, we have started to investigate the anatomy of the central projections of human primary afferents. For example, we find that central terminals of CGRP-immunoreactive afferents apposed many Homer-immunoreactive puncta, suggesting that unlike in the rodent CGRP is expressed by primary afferents that form complex glomerular-like structures in the human spinal cord. The afferents also formed contacts onto dorsally directed dendrites of presumed lamina IV projection neurons. Together, these results show that antibodies raised against synaptic markers can be used to reveal neuronal circuitry underlying sensory transmission in the human spinal cord and how it changes with age, sex and in chronic pain states.

Disclosures: O.C. Davis: None. **A.J. Todd:** None. **T.J. Price:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.16/AA16

Topic: D.01. Somatosensation

Support: ERC Starting Grant 758974

Title: Task-evoked high frequency oscillations in cortex and spinal cord

Authors: *E. BAILEY, B. NIERULA, T. STEPHANI, V. NIKULIN, F. EIPPERT;
Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: High frequency oscillations (HFOs) in response to somatosensory stimulation were first observed in the 1970s in human EEG recordings as small notches on the earliest cortical somatosensory evoked potential (SEP). Recent concurrent EEG and single-unit recordings in primates suggest HFOs may be a non-invasive marker for cortical population spiking. Here, we examined whether such HFOs can be non-invasively recorded not only in cortex, but also concurrently in the spinal cord, potentially providing a novel window into neuronal activity across the entire human central nervous system.The data was acquired during an experiment in which 36 participants received electrical stimulation of the upper and lower limb (Nierula et. al., 2022, bioRxiv). Electrospinography was recorded from 40 spinal electrodes arranged in two patches over the cervical and lumbar spine, while EEG was simultaneously recorded from 64 scalp channels. To extract HFOs from these surface recordings, canonical correlation analysis (CCA) was applied to find spatial filters that maximise the correlation between single-trial data and the trial-averaged signal. This procedure enhances the extraction of evoked signals of interest in recordings with low signal-to-noise ratio, as is typical for HFOs.First, we aimed to replicate previous findings concerning cortical HFOs evoked by upper and lower limb

stimulation, testing if our recordings were of sufficient quality at the individual participant level, which revealed that HFOs were clearly visible with the somatotopy corresponding to the stimulated nerves. Next, we tested for the existence of HFOs in the cervical and lumbar spinal cord, and detected HFOs at the individual level in most participants (cervical cord: ~90%, lumbar cord: ~50%), located in the frequency range from 400 to 800 Hz and overlying the cervical N13 and lumbar N22 potentials. Group-level results in the spinal cord and cortex were then assessed by averaging across the HFO amplitude envelope of single-participant responses. To allow for grand-averaging, each individual HFO envelope was temporally aligned based on the latency of the underlying low-frequency SEP to account for between-participant variation. Using this procedure, we obtained evidence for group-level HFOs across both cortex and spinal cord. Finally, using time-frequency analyses, we were able to provide evidence for the spatial specificity of spinal cord HFOs. Overall, our results demonstrate that HFOs to both upper and lower limb stimulation can be observed in both cortex and spinal cord, and when individual variation in response-latency is considered, robust group-level HFOs can be obtained.

Disclosures: E. Bailey: None. **B. Nierula:** None. **T. Stephani:** None. **V. Nikulin:** None. **F. Eippert:** None.

Poster

PSTR207. Spinal Sensory Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR207.17/AA17

Topic: D.01. Somatosensation

Title: Recreating Sensory Pathways in Vitro Using Human Keratinocytes, Sensory Neurons, and Dorsal Horn Neurons in Microfluidic Devices

Authors: *M. DAU, P. WALSH, V. TRUONG;
Anatomic Inc., Minneapolis, MN

Abstract: Pain is prevalent in a diverse array of diseases ranging from migraines to cancer. Molecularly, the sensation of pain originates in damaged peripheral tissues such as skin, which is detected by nociceptive sensory neurons, which further relay this information into the dorsal horn of the spinal cord. This peripheral to spinal pain circuit is poorly understood in humans due to a lack of well-characterized model systems, and a better understanding of this circuit could yield breakthroughs in pain drug discovery. Here, we demonstrate the ability to recreate a sensory pathway through the co-culture of primary keratinocytes, hiPSC-derived sensory neurons, and hiPSC-derived dorsal horn neurons using microfluidic technology. These microfluidic devices allow multiple cell types to be maintained in their microenvironments, while separating the somas to enable axonal elongation towards the other cell types. Seeding techniques, maturation medias, and feeding techniques were optimized so cultures could stay healthy through time. At weekly timepoints, immunocytochemistry was used to characterize the maturation of cell types and look at synapse formation. Calcium imaging was also used to look at

signal propagation upon various stimuli. Together, these findings demonstrate the ability to connect multiple cell types together to enable more physiologically relevant in vitro sensory models.

Disclosures: **M. Dau:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. **P. Walsh:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. **V. Truong:** A. Employment/Salary (full or part-time):; Anatomic Incorporated.

Poster

PSTR208. Descending Modulation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR208.01/AA18

Topic: D.02. Somatosensation – Pain

Support: R37 NS098660
F31 DE030677

Title: High-density single-unit recordings from brainstem pain-modulating neurons

Authors: *C. C. DE PRETER[1,2], A. SONNEBORN[3,1], M. M. HEINRICHER[2,1];
[1]Behavioral Neurosci., [2]Neurolog. Surgery, [3]VA Portland Hlth. Care Syst., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The primary output of the brainstem pain-modulating system is the rostral ventromedial medulla (RVM). The RVM facilitates or suppresses nociceptive transmission at the level of the dorsal horn though two cell classes, termed “ON-” and “OFF-” cells. Over the last 30 years, the connectivity, physiology, and function of these neurons in pain-modulation has been extensively characterized using *in-vivo* electrophysiological recordings of single RVM neurons, identified by their response during noxious input: ON-cells exhibit a burst of activity, while OFF-cells exhibit a pause on ongoing activity just prior to nocifensive withdrawal. These evoked responses are attenuated after systemic morphine administration. ON- and OFF-cells fluctuate in activity in unstimulated conditions, with alternating silent and active periods. Between cell classes, ON- and OFF-cells display asynchronous ongoing and noxious evoked activity, while within each class, activity is in phase. Additionally, the ON-cell burst does not precede the pause of the OFF-cell, and pharmacological manipulation of the ON-cell burst does not influence OFF-cell activity. These parallel processes suggest that ON- and OFF-cell firing patterns reflect intrinsic excitability or coordinating input originating from outside the RVM. However, previous studies have relied on opportune recordings of paired single-units at a single local electrode site, or multiple electrodes in RVM at distant sites. Interactions within the RVM itself are almost entirely unknown. Using a 64-channel silicon probe, we simultaneously monitored the activity of numerous (15 to 40) single units in the RVM during noxious stimulation and systemic opioid administration in male and female Sprague-Dawley rats. We confirmed single-unit data that ON-cells are activated and OFF-cells were inhibited by noxious stimulation. In animals that became

analgesic after morphine administration, the ON-cell burst and OFF-cell pause were attenuated as expected. OFF-cells were recruited after morphine administration. Rescue with naloxone also recruited ON-cells that were previously silent. Analysis of spike trains revealed that RVM neurons share common synaptic input and that some RVM neurons are monosynaptically interconnected. Additionally, while many cells could be classified as ON- or OFF-cells, these multichannel recordings revealed some cells that did not fit traditional classification parameters. The present data document within-RVM interactions using two approaches to classifying RVM neurons, and demonstrate the advantages of using multichannel recordings of pain-modulating neurons in brainstem.

Disclosures: C.C. De Preter: None. **A. Sonneborn:** None. **M.M. Heinricher:** None.

Poster

PSTR208. Descending Modulation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR208.02/AA19

Topic: D.02. Somatosensation – Pain

Support: NIH NS045549
NIH GM067795
NIH AR073187

Title: Local synthesis of estradiol in the rostral ventromedial medulla modulates activity-induced muscle pain in males

Authors: *A. PLUMB[1], J. B. LESNAK[2], L. RASMUSSEN[1], K. A. SLUKA[3];
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Abstract: Animal studies show that testosterone reduces pain behavior in multiple models, including in our *activity-induced muscle pain model.* Widespread pain, as experienced by females and males with reduced testosterone in the activity-induced muscle pain model, is accepted to be mediated by changes in the central nervous system; the rostral ventromedial medulla (RVM) is a key nuclei involved in modulating pain. Local synthesis of estrogen from testosterone modulates several aspects of neuronal functioning in males, however, it is unknown how estrogen receptors modulate pain in the RVM. Based on preliminary data, we hypothesized that testosterone is converted to estrogen in the RVM to attenuate muscle pain by altering inhibitory neurotransmission. 8-week-old C57BL6 mice were used for all experiments. The pain model was induced by two injections (pH 5.0 saline) into the left gastrocnemius muscle combined with 6 minutes of fatiguing muscle contraction. Hyperalgesia was tested with withdrawal threshold of the gastrocnemius muscle using force sensitive tweezers. The hypothesis was tested using behavioral pharmacology to manipulate sex hormone receptors in the RVM and qPCR to examine mRNA expression of inhibitory receptors in the RVM. Animals were

randomly assigned experimental group and the experimenter was blinded to drug injection. Power analysis was performed on the primary outcome to determine sample size for the experiments. One-way ANOVA to assess the change in MWT between 24-hr post pain and 30-mins post drug injection with a Tukey multiple comparison when appropriate. Blockade of estrogen receptors ($F_{3,26} = 5.4$, $p<0.01$), aromatase ($F_{3,15} = 10.49$, $p<0.001$), or estrogen receptor-α ($F_{2,15} = 13.78$, $p<0.01$) in the RVM resulted in contralateral hyperalgesia in male mice but had no effect in female mice ($p=0.457$). Removal of testosterone from males prior to induction of the pain model had no effect on $GABA_A$ or μ-opioid receptors. Our data suggests that males convert testosterone in the RVM to estrogen to protect from development of widespread hyperalgesia without initiating new transcription of inhibitory receptors.

Disclosures: A. Plumb: None. **J.B. Lesnak:** None. **L. Rasmussen:** None. **K.A. Sluka:** None.

Poster

PSTR208. Descending Modulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR208.03/AA20

Topic: D.02. Somatosensation – Pain

Title: Pharmacological insights into pain mediation by the kinin receptors

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Abstract: One of the major functions of the kinin receptors is to mediate the sensation of pain. Specifically, they consist of the Bradykinin-1 (B1) and Bradykinin-2 (B2) receptors. These receptors are expressed in different areas of pain modulation in the central and peripheral nervous system including the dorsal root ganglia, the cerebral cortex, the spinal cord, and in nociceptive neurons. While B1 is expressed at very low levels in healthy tissues, B2 is constitutively expressed. The B1 and B2 receptors are activated by four different endogenous peptide agonists that are known to mediate pain through both direct activation and sensitization of nociceptor neurons. As G Protein Coupled Receptor (GPCRs), kinin receptors convert extracellular messages to signaling events through the activation of heterotrimeric G proteins. However, how the kinin receptors determine G protein subtype selectivity is not well understood. In other words, the different peptides may couple to different Gα subunits activating distinct intracellular events and thus cause different physiological outcomes. Previous studies have characterized the activation of Gαq family by the kinin receptors but have not taken into account the variability of Gα subunits in specific cell types. This study uses Bioluminescence resonance Energy Transfer (BRET) based *in vitro* assays that detect direct coupling to 14 different Gα proteins upon receptor activation. Our results show that there is a shift in G protein activation profiles among the different peptides. These results help better understand the intracellular mechanisms through which the kinin system regulates pathological and physiological pain.

Disclosures: **S.V. Darira:** None. **L.P. Sutton:** None.

Poster

PSTR208. Descending Modulation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR208.04

Topic: D.02. Somatosensation – Pain

Support: STI2030-Major Projects 2021ZD0203302
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Title: Spinal mechanisms underlying the laterality control of mechanical allodynia

Authors: *J. HUO, F. DU, D. DONG, G. YIN, Q. MA, K. DUAN, X. LIU, H. HU, L. CHENG; Sch. of life sciences, Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Mechanical allodynia-pain evoked by innocuous tactile stimuli-is a hallmark symptom of chronic inflammatory and neuropathic pain. For some devastating patients, a local injury can lead to full-blown body-wise pain that lasts for long time, whereas for most patients the pain could be short-lasting or confined to the unilateral injury side. Our recent study reported the brain-to-spinal descending neural circuits that control the laterality and duration of mechanical allodynia. The underlying spinal mechanisms, particularly on laterality control, are, however, still remain unsolved. Here we showed that kappa opioid receptors (KORs) in the spinal dorsal horn control the laterality and duration of mechanical allodynia via modulating the excitability of KOR-expressing neurons following peripheral inflammation or nerve injury. Pharmacologically blocking spinal KORs, or conditional knockout of KORs from dorsal horn neurons (n = 6 mice per group; both male and female were included in this study), prevented the induction of hind paw capsaicin-, formalin-, complete Freund's adjuvant (CFA)-, or spared nerve injury (SNI)-induced mechanical allodynia on the contralateral un-injured, but not the ipsilateral injured side (n = 5-6 mice per group), and caused a relapse, or prolonged lasting duration of the ipsilateral mechanical allodynia. Moreover, we observed increased excitability of dorsal horn KOR-expressing neurons in hind paw capsaicin model mice (versus hind paw vehicle control injected mice), and intersectional genetic ablation/chemogenetic silencing (n = 5-6 mice per group) dorsal horn KOR-expressing neurons could prevent/rescue hind paw capsaicin- and SNI (combined with chemical lesion of the lateral parabrachial nucleus)-induced contra-, but not ipsilateral mechanical allodynia. Conversely, chemogenetic activation of KOR-expressing dorsal horn

neurons re-occurred bilateral mechanical allodynia induced by hind paw capsaicin injection (n = 6 mice per group), and opened the gate for the normally gated contralateral mechanical allodynia in SNI model mice (n = 5-6 mice per group). Collectively, our data suggest that dorsal horn KORs could control the laterality and lasting duration of peripheral inflammation and nerve injury-induced mechanical allodynia via modulating the excitability of dorsal horn KOR-expressing neurons. Targeting dorsal horn KORs/KOR-expressing neurons, could therefore, provide preclinical studies and/or clinical trials a mechanism-based strategy to treat mechanical allodynia.

Disclosures: **J. Huo:** None. **F. Du:** None. **D. Dong:** None. **G. Yin:** None. **Q. Ma:** None. **K. Duan:** None. **X. Liu:** None. **H. Hu:** None. **L. Cheng:** None.

Poster

PSTR208. Descending Modulation

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant AR073187
NIH Grant NS045549
NIH Grant GM144636

Title: The role of descending dopaminergic pain modulation in the transition to chronic pain

Authors: ***A. F. SMITH**, K. HAYASHI, A. N. PLUMB, K. A. SLUKA;
Univ. of Iowa, Iowa City, IA

Abstract: Chronic pain is a significant health burden, yet the factors that control the transition to chronic pain are still poorly understood. Following an acute peripheral insult such as surgery, 10-50% of people develop chronic pain, and currently available therapies aiming to prevent this transition have had limited success. Changes in the central nervous system and descending pain modulation pathways which modulate the perception of pain may underlie the transition to chronic pain. Our current understanding of these changes is insufficient to adequately treat this population, therefore further studies into these pathways in the context of the transition to chronic pain is necessary. The descending dopamine pain modulation pathway originates in the A11 nucleus, located in the dorsal posterior hypothalamus, and projects ipsilaterally to all levels of the spinal cord. It has previously been shown that lesioning A11 or blocking dopamine D1 like receptors in the spinal cord can prevent or delay the transition to chronic pain. We hypothesized that A11 neurons become active following peripheral insult to facilitate the transition to chronic pain via D1 like receptors. We used a model of chronic widespread pain that is induced by two spaced muscle insults. This model is ideal for studying the transition to chronic pain because the first insult causes physiological changes that leave the animal vulnerable to transition to chronic pain at the time of the second insult. By applying drugs at the

time of the second insult, we can probe mechanisms of the transition to chronic pain. Adult male and female C57BL6/J mice were used in this study. Pain was induced by 2 20µl pH 4.0 injections 5 days apart in the left gastrocnemius muscle. To assess pain, muscle withdrawal thresholds (MWT) and paw sensitivity (vF) was assessed before and after induction of the model. Behavioral pharmacology techniques were used to inhibit the descending dopamine pain modulation pathway during the second pH 4.0 injection to test if activity in this pathway is required for the transition to chronic pain. We found that blockade of the dopamine pathway delayed the development of chronic widespread pain in our model. Thus, the A11 dopamine pathway appears to mediate the transition to chronic pain.

Disclosures: **A.F. Smith:** None. **K. Hayashi:** None. **A.N. Plumb:** None. **K.A. Sluka:** None.

Poster

PSTR208. Descending Modulation

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Topic: D.02. Somatosensation – Pain

Support: KAKENHI 18K17019
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Title: The activation of insular cortical projections to the trigeminal spinal subnucleus caudalis regulates pain-related behaviors in rats

Authors: *Y. NAKAYA, K. YAMAMOTO, M. KOBAYASHI;
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Abstract: The trigeminal spinal subnucleus caudalis (Sp5C) receives orofacial noxious information and sends this to the higher central nervous system, including the parabrachial nucleus (PBN). The insular cortex (IC) plays a role in processing orofacial nociceptive information and sends corticofugal projections to the Sp5C. However, it is little known about the role of the descending projections from IC to Sp5C neurons. We aimed to investigate the synaptic relationship between the IC to glutamatergic and GABAergic/glycinergic Sp5C neurons of laminae I/II. We used a combination of whole-cell patch clamp recording from slices including the Sp5C in vesicular GABA transporter-Venus transgenic rats and optogenetics. We recorded monosynaptic excitatory postsynaptic currents induced by selective stimulation of IC axon terminals in Sp5C slice preparations from both excitatory glutamatergic and inhibitory GABAergic/glycinergic Sp5C neuron, and we found these amplitudes were comparable. In addition, we explored features of synaptic transmission from GABAergic/glycinergic neurons to

glutamatergic neurons in the Sp5C. We recorded unitary inhibitory postsynaptic currents from inhibitory neurons to excitatory neurons, including neurons projecting to the PBN, exhibited a high failure rate and were suppressed by both bicuculline and strychnine. Moreover, selective stimulation of IC neurons increased the firing rate of Sp5C neurons in vivo using extracellular single unit recording. Finally, we examined whether IC projections modulate pain behaviors in rats. We recorded the head withdrawal thresholds (HWT) to mechanical stimulation and heat stimulation of the whisker pad using rats received AAV-hSyn-hM3D(Gq)-mCherry injection into the IC. Behavior test showed that activation of IC axons in the Sp5C by a chemogenetic approach decreased the thresholds of both mechanical and thermal nociception. These results suggest that IC projection to the Sp5C is likely to facilitate rather than suppress excitatory outputs from the Sp5C.

Disclosures: Y. Nakaya: None. **K. Yamamoto:** None. **M. Kobayashi:** None.

Poster

PSTR208. Descending Modulation

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Program #/Poster #: PSTR208.07/AA23

Topic: D.02. Somatosensation – Pain

Support: NIH-NIA RO1 AG073126

Title: Treadmill exercise increases endogenous pain inhibitory response in young, but not old, Fischer 344 rats, without altering plasma testosterone levels.

Authors: *R. SANTO, J. PAK, Z. YOUPING, S. HUIZHONG, J. DA SILVA, J. RO;
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Abstract: The prevalence of chronic pain significantly increases with age. However, available treatments remain inadequate as there are significant gaps in our understanding of chronic pain mechanisms in older adults. We have previously shown that endogenous pain inhibition, measured as diffuse noxious inhibitory controls (DNIC) responses is reduced in young female and old rats, and that testosterone (TS) plays a critical role in maintaining efficient DNIC. In this study, we aim to determine the effects of a nonpharmacological treatment, i.e., exercise, on DNIC responses and the relationship with TS levels. Old and young male and female Fisher rats were submitted to a treadmill exercise protocol for 6 weeks (week 1: 6m/min and 5 weeks: 12m/min for 30min). When the training was finalized, DNIC responses were measured. Blood samples were collected before and after the exercise to quantify corticosterone (CT) and TS levels. First, we examined whether our treadmill exercise protocol induces stress in rats. The number of shocks (a stress correlate) decreased sharply after the first week of exercise and plasma CT levels did not change between pre and post exercise in any group. We then investigated whether the DNIC responses could be modulated by exercise. We found that our exercise protocol is effective in enhancing DNIC responses in young males and females.

However, the exercise did not affect DNIC responses in old males and females. Exercise increased DNIC responses in young rats compared to old rats (age effect), and in males in comparison to females (sex effect). Exercise did not change TS levels in any group, but there was a trend of increased DNIC responses with high plasma TS levels. Collectively, these results support our hypothesis that exercise can improve DNIC responses. However, this effect was seen only in young rats and more strongly in young males. This exercise protocol did not directly change TS levels and did not induce stress. Further research is necessary to better understand the role of exercise and TS in endogenous pain inhibition, especially for the aged population.

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Poster

PSTR208. Descending Modulation

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Topic: D.02. Somatosensation – Pain

Support: Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung 310030_197888

Title: Descending GABAergic Neurons of the RVM That Mediate Widespread Bilateral Antinociception

Authors: M. SOUSA[1], R. GANLEY[2], K. WERDER[3], M. RANUCCI[1], H. WILDNER[1], *H. ZEILHOFER[1];
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Abstract: The descending projections from higher brain centers to the spinal dorsal horn provide a top-down control of pain sensitivity by balancing both facilitatory and inhibitory signals. Neurons projecting from the rostral ventromedial medulla (RVM) to the spinal dorsal horn are critical elements of the endogenous pain control system. The majority of the previous research in the field has focused on monaminergic pathways, while potential contributions of pathways using fast amino acid neurotransmission have attracted less attention. Here, we describe a GABA/glycinergic pathway that innervates predominantly the superficial dorsal horn.
In this study, male and female mice were used. Anatomical and optogenetic tracing of these neurons from a single unilateral site of the lumbar spinal cord indicated that these neurons give rise to a dense bilateral innervation of the spinal cord along its entire rostrocaudal axis. Chemogenetic activation of these neurons caused a bilateral and wide-spread reduction in heat, cold, and mechanical sensitivity, while their silencing with tetanus toxin induced allodynia and spontaneous pain-like aversive behaviors. Consistent with a continuous role in the prevention of

spontaneous pain, many descending RVM GABAergic neurons were found to be tonically active. This data provides evidence that inhibitory projection neurons of the hindbrain that project their axons to the spinal dorsal horn are critical components controlling physiological pain sensitivity and are capable of powerful suppression of nociception when activated. Overall, these results suggest that the inhibitory RVM projection neurons are required for maintaining normal physiological sensitivity to mechanical stimuli. This pathway may therefore be relevant for widespread conditioned analgesia, while its dysfunction underlies chronic widespread pain syndromes. This highlights the importance of descending projections of the RVM for regulating basal sensitivity to external stimuli.

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Poster

PSTR208. Descending Modulation

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Topic: D.02. Somatosensation – Pain

Support: Dept. of Veterans Affairs RX001776-05

Title: Differential Serotonergic and Noradrenergic Controls of Descending Pain Modulation after Mild Traumatic Brain Injury

Authors: *P. SAHBAIE[1,3], K.-A. IRVINE[2,4], X. SHI[2,4], D. CLARK[2,4];
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Abstract: Introduction: Traumatic brain injury (TBI) is a significant public health concern, with the majority of injuries being mild. Chronic pain has been an often-overlooked consequence of TBI complicating rehabilitation efforts and constituting an ongoing management challenge in many patients. The mechanisms underlying pain after TBI are poorly understood. Here we examined the contribution of spinal monoamine signaling to dysfunctional descending pain modulation after TBI.
Methods: For these studies we used a well-characterized concussive mouse model of mild TBI. Measurements of mechanical allodynia were done using von Frey filaments. Efficacy of diffuse noxious inhibitory control (DNIC) endogenous pain control pathways were assessed using a noxious stimulation-induced analgesia protocol. Effects of atipamezole, dexmedetomidine and ondansetron on DNIC response in TBI and control groups were assessed. Reboxetine, escitalopram or duloxetine were used to determine the relative contribution of serotonin (5-HT) and norepinephrine (NE) signaling in DNIC after TBI. Lumber spinal cord NE and 5-HT levels were measured by enzyme immunoassay (EIA).
Results: We observed that DNIC is strongly reduced in both male and female mice after mild

TBI for at least 12 weeks. In naïve mice, DNIC was mediated through α2 adrenoceptors, but sensitivity to α2 adrenoceptor agonists was reduced after TBI, and reboxetine failed to restore DNIC in these mice. Intrathecal injection of ondansetron showed that loss of DNIC was not due to excess serotonergic signaling through 5-HT3 receptors. On the other hand, the serotonin-norepinephrine reuptake inhibitor, duloxetine and the serotonin selective reuptake inhibitor escitalopram both effectively restored DNIC after TBI.
Conclusions: Enhancing serotonergic signaling as opposed to noradrenergic signaling more commonly used in targeting neuropathic, musculoskeletal and other forms of chronic pain, may be an effective pain treatment strategy after TBI. Clinically available medications duloxetine and escitalopram were both highly efficacious in our mouse TBI model, and do have translational value for making clinical trials justifiable.

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Poster

PSTR208. Descending Modulation

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Topic: D.02. Somatosensation – Pain

Support: Department of Physiology and Medical Science (Daejeon, South Korea)
Chungnam National University (Daejeon, South Korea)
National Research Foundation of Korea (2021R1F1A1062509)

Title: The antinociceptive effect of the orexin A pathway induced by intermittent fasting on formalin-induced acute pain

Authors: *H. SHIN[1], D.-W. KANG[1], J. HWANG[2], H.-W. KIM[1];
[1]Chungnam Natl. Univ., Daejeon, Korea, Republic of; [2]Orthopaedic Surgery, Chungnam Natl. Univ. Hosp., Daejeon, Korea, Republic of

Abstract: Stress may be brought on by a variety of things, including hunger, loudness, and exposure to the cold. The stress response can also produce analgesia by increasing various stress-related hormones. We designed this study to determine whether the programmed or learned stressful factor can produce antinociception without severe stress responses because predictable stress may elicit a minimized response. As a stress factor, we chose fasting by grouping two different conditions such as acute fasting (AF) as a non-predictable stress group and intermittent fasting (IF) as a programmed stress group. Additionally, we examined the blood level of corticosterone (CORT), a key stress hormone, and the orexin A (OXA) neuronal activity of the hunger center, lateral hypothalamus (LH). In the present study, AF group mice were fasted for 6, 12, or 24 hours before formalin test while IF group mice were fasted for 12 hours/12 hours or 24 hours/24 hours fasting/eating sequences. For the acute pain model, we injected formalin solution (1%, 20ul) into the plantar surface of the right hind paw and recorded the pain behavior for 40

minutes and measured the licking time (sec). Except for 6 hours AF, all mouse groups showed antinociception in the formalin test. Except for 6 hours of AF, co-expression of LH OXA and fos-B (a neuronal activation marker) was increased both in AF and IF groups. The CORT level was increased in 12 and 24 hours of AF, but not 6 and 12 hours of IF. In conclusion, 12 hours of IF may produce a significant antinociception on formalin-induced pain without CORT elevation and this result suggests IF may have a high potential as a pain treatment.

Disclosures: H. Shin: None. **D. Kang:** None. **J. Hwang:** None. **H. Kim:** None.

Poster

PSTR208. Descending Modulation

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Topic: D.02. Somatosensation – Pain

Support: National Research Foundation of Korea (2021R1A6A3A01086598)
National Research Foundation of Korea (2021R1F1A1062509)
Department of Physiology and Medical Science (Daejeon, South Korea)
Chungnam National University (Daejeon, South Korea)

Title: Modulation of Brain-Derived Neurotrophic Factor Expression: Assessing the Analgesic Efficacy of Median Nerve Stimulation in Mice with Chemotherapy-Induced Peripheral Neuropathy

Authors: *D.-W. KANG, J.-G. CHOI, H. SHIN, H. SONG, M. LEE, T. KIM, S. LEE, H.-W. KIM;
Chungnam Natl. Univ., Daejeon, Korea, Republic of

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a distressing condition characterized by severe pain caused by chemotherapeutic agents such as docetaxel (DTX). This study aimed to investigate the analgesic effects and underlying neuronal mechanisms of low-frequency median nerve stimulation (LFMNS) on DTX-induced tactile hypersensitivity in male ICR mice. CIPN was induced by intraperitoneal administration of DTX over a span of four doses, with a two-day interval between each dose. LFMNS was applied to the wrist area, and von Frey filaments were used to assess the pain response in both hind paws. Western blot and immunofluorescence staining were conducted on samples from the dorsal root ganglion (DRG) and spinal cord to evaluate the expression of brain-derived neurotrophic factor (BDNF). Repeated LFMNS significantly alleviated abnormal sensory responses induced by DTX and suppressed the enhanced expression of BDNF in DRG neurons and the spinal dorsal area. These findings suggest that LFMNS could be a viable non-pharmaceutical treatment option for patients with CIPN by modulating the expression of peripheral and central BDNF.

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Poster

PSTR208. Descending Modulation

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Topic: D.02. Somatosensation – Pain

Support: NIH EB029354, NS124564, MH114233

Title: Modulating brain rhythms of noxious heat pain in humanized mouse model of sickle cell disease using low-intensity focused ultrasound

Authors: *M. KIM[1], C.-Y. YEH[1], K. YU[1], K. GUPTA[2], B. HE[1];
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Abstract: Pain is a major comorbidity of sickle cell disease (SCD), which impairs quality of life in millions of people worldwide. To promote new non-pharmacological treatment without side effects of opioids administration, we have recently presented experimental evidence that non-invasive low-intensity transcranial focused ultrasound (tFUS) applied to pain processing brain circuits can profoundly change pain-associated behaviors in humanized mouse model of SCD (HbSS-BERK) and wild-type mice. This study aimed to further understand how tFUS modulates neuronal activity in brain circuits for pain. We employed 5 to 10 month-old female HbSS-BERK mice expressing > 99% human sickle hemoglobin S, demonstrating severe features of SCD including constitutive chronic hyperalgesia. tFUS delivered by a 128-element array transducer was applied to the right somatosensory hindlimb cortex (S1HL), followed by noxious heat stimulation (≤ 45°C) to the plantar surface of the contralateral hind paw. Electroencephalography (EEG) recordings from the right S1HL were analyzed, and the effect of tFUS was compared to the negative control where no tFUS was delivered prior to heat stimulation. By evaluating EEG during 1) noxious high temperature paw stimulation (HTPS) and 2) low temperature paw stimulation (LTPS) to the baseline EEG, our results demonstrated noxious pain responses with markedly decreased alpha power and increased gamma power (*p<0.05), and decreased beta power (p=0.31) in 8 sickle mice. Next, the impact of tFUS was assessed by comparing the differences (Δ) between oscillatory power during HTPS and LTPS. Fig. 1a-b shows that tFUS with a lower pulse repetition frequency (PRF) of 40 Hz (N=7) led to a significant increase in the normalized Δalpha and Δbeta power relative to the negative control (N=8) (*p<0.05), which exhibits a distinct contrast to the EEG pattern of pain indicators. The results suggest that tFUS with 40 Hz PRF can effectively modulate brain rhythms of noxious heat pain in sickle mice, and thus analgesics strategies using tFUS may offer significant relief for the suffering of those with SCD.

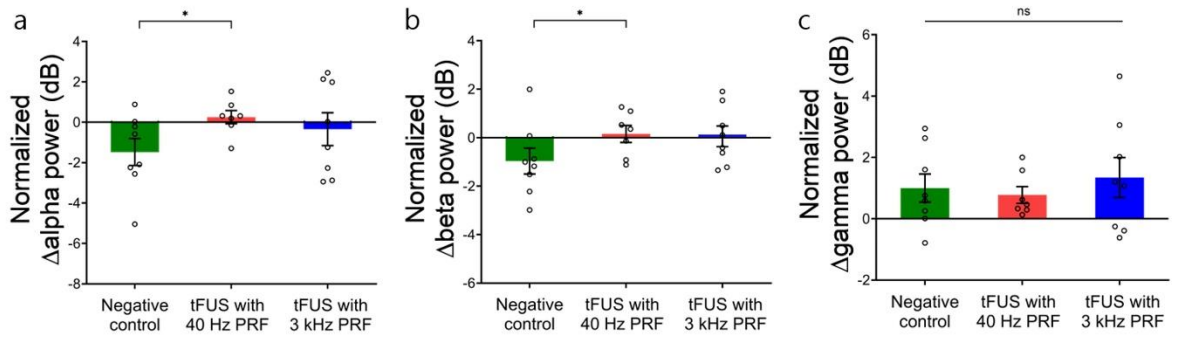

Fig. 1. Quantification of the normalized power differences (Δ) between HTPS and LTPS among three experimental conditions: 1) negative control, 2) tFUS with a low PRF (40 Hz), and 3) tFUS with a high PRF (3 kHz) in female sickle mice. a-b, After tFUS stimulation with PRF of 40 Hz, but not PRF of 3 kHz, to S1HL, the normalized power in Δalpha and Δbeta frequency bands during HTPS were significantly increased compared to the values from the negative control. **c,** The tFUS with a PRF of 40 Hz and 3 kHz generated slight modulatory effects on Δgamma power, but the changes were less pronounced compared with the results from other frequency bands. Statistical analysis was conducted with one-tailed Mann-Whitney test, ns: not significant, $^{*}p<0.05$. Data were presented as mean ± standard error of the mean.

Disclosures: **M. Kim:** None. **C. Yeh:** None. **K. Yu:** None. **K. Gupta:** None. **B. He:** None.

Poster

PSTR208. Descending Modulation

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR208.13/BB1

Topic: D.02. Somatosensation – Pain

Support: UVA Brain Institute
UVA Neurosurgery

Title: Differential correlations between conditioned pain modulation and painful laser-evoked potentials in high versus low pain catastrophizers

Authors: *W. CARTER, D. WANG, D. KUMAR, S. MOOSA, W. ELIAS, C.-C. LIU;
Univ. of Virgina, Charlottesville, VA

Abstract: Conditioned pain modulation (CPM) is an endogenous pain inhibition phenomenon associated with treatment outcomes and chronic pain development. CPM can be induced experimentally in a laboratory environment or clinics using pain inhibits pain protocols. An earlier study show that both the amplitude of painful laser-evoked potentials (LEPs) and pain ratings decreased following CPM induction in healthy subjects. However, the study did not take into account individual difference in pain-related behavioral factors, and thus it remains unclear whether pain-related individual difference plays a role in modulating the reported association between LEPs and CPM. In the present study, we utilized an iced-water based CPM protocol and painful laser stimulation to test the hypothesis that pain catastrophizing plays a role in mediating the relationship between CPM and LEPs. Pain catastrophizing is an important psychological

construct that has been widely acknowledged for its impact on the experience of pain. A Nd:YAP laser stimulator (wavelength 1.34μm, beam diameter 5mm, pulse duration 4ms) was used to deliver painful stimulations. Stimulus-related EEG responses were recorded for evoked potential analysis. Amplitude of LEPs was determined as the largest negative-positive deflections at 200 -400ms post-stimulation (i.e. LEP_N2-P2). A total of 31 subjects (22.2 ± 4.2 yrs, 12 female) were enrolled in the study. Following CPM, our preliminary results showed significant reductions in both laser pain intensity, and amplitude of LEP_N2-P2 and LEP_P2 peak at the midline central Cz channel (paired Wilcoxon signed-rank test, $p = 0.001$). Furthermore, by separating subjects into high- and low-pain catastrophizing groups using median split the pain catastrophizing score (median=16), we found that high pain catastrophizers showed significant correlations between the CPM magnitudes and reductions of amplitude of LEP_N2P2 and LEP_P2 at the midline Cz channel ($r = 0.46$, $p = 0.009$ and $r = 0.48$, $p = 0.006$, respectively). In conclusion, our findings suggest that psychological constructs like pain catastrophizing play a role in mediating the association between endogenous pain inhibition magnitude induced by CPM and pain-related EEG responses evoked by painful laser in healthy subjects.

Disclosures: W. Carter: None. **D. Wang:** None. **D. Kumar:** None. **S. Moosa:** None. **W. Elias:** None. **C. Liu:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.01/BB2

Topic: D.02. Somatosensation – Pain

Support: NIDA R00 DA035865
R01 CA284075
NIAMS R01 AR075241

Title: Inhibition of 12/15-LOX reverses neuropathic pain like behaviors in male and female mice

Authors: *B. BROWN[1], I. CHEN[2], L. MURDAUGH[3], C. MILIANO[2], M. BUCZYNSKI[2], A. GREGUS[2];
[1]Virginia Tech. Neurosci. PhD Program, Blacksburg, VA; [2]Neurosci., [3]TBMH, Virginia Tech., Blacksburg, VA

Abstract: It is estimated that 1 in 3 Americans suffer from chronic pain, with 17.1 million experiencing high impact chronic pain severe enough to limit daily activities. Chronic pain syndromes with neuropathic components are extremely challenging to manage as they are refractory to treatment with nonsteroidal inflammatory drugs (NSAIDs), and many patients report inadequate relief from first-line therapies such as anticonvulsants and antidepressants. While opioids are the mainstay for moderate to severe pain, concerns regarding risks of misuse, tolerance, and dependence with chronic dosing highlight the need for novel non-opioid

analgesics. We demonstrate that spinal 12/15-Lipoxygenases (12/15-LOX) contribute to pain hypersensitivity in males and females following intrathecal (IT) delivery of the Toll-Like Receptor 4 (TLR4) agonist Lipopolysaccharide (LPS), which models the transition from acute to chronic pain in Rheumatoid Arthritis. Systemic delivery at 30mg/kg intraperitoneally (IP) of CNS permeant inhibitors of 15-LOX-1 (ML351) or 12-LOX (ML355) reverses established tactile and cold allodynia in this model. In contrast, pain-like behaviors are not attenuated by NSAIDs administered at analgesic doses shown to inhibit spinal Prostaglandin E2 (PGE2) release. Accumulating evidence implicates TLR4 activation in chemotherapy-induced peripheral neuropathy (CIPN), so we interrogated the role of 12/15-LOX enzymes in paclitaxel-induced pain hypersensitivity. Similarly, we show that systemic administration of ML355 or ML351 reverses both tactile and cold allodynia in mice of both sexes. These results highlight involvement of 12/15-LOX enzymes across multiple models of neuropathic-like pain in males and females. Accordingly, our findings provide preliminary support for the use of selective 12/15-LOX inhibitors for mitigating chronic pain that is unresponsive to treatment with NSAIDs. Future studies will incorporate additional models of chronic neuropathic-like pain, effects of 12/15-LOX inhibition on candidate molecular biomarkers as well as spontaneous behavioral outcome measures to increase clinical translatability.

Disclosures: B. Brown: None. **I. Chen:** None. **L. Murdaugh:** None. **C. Miliano:** None. **M. Buczynski:** None. **A. Gregus:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.02/BB3

Topic: D.02. Somatosensation – Pain

Support: Department of Defense SC210210.

Title: Peripheral blood derived recombinant hiPSCs chromaffin cells releasing serine-histogranin attenuate SCI induced hypersensitivity in rats

Authors: *S. JERGOVA, A. EESWARA, K. PERRUCCI, H. CUKIER, J. SAGEN;
Univ. of Miami, Miami, FL

Abstract: Development of pain following spinal cord injury (SCI) reduces quality of life and impedes cooperation with rehabilitation programs designed to improve prognosis and functional recovery. Highly secretory chromaffin cells have been identified with potential for transplantation due to synthesis of analgesic agents. Employing recent induced pluripotent stem cell (iPSC) technology may allow chromaffin cells to be derived autologously from transplant recipients and eliminate the need for immunosuppression. The analgesic effect of grafted cells may also be potentiated by genetic engineering additional cDNAs of potent analgesic peptides. Our previous studies showed highly beneficial effects of recombinant NMDA antagonist serine-

histogranin (SHG) or combination of SHG with opioid peptide endomorphin 1 (EM1) in the reduction of SCI pain. The aims of the present study are to evaluate consistency of peripheral blood hiPSC derived chromaffin cells for transplantation when obtained from a broad range of donor backgrounds and parameters to engineer hiPSC-derived chromaffin cells to produce additional analgesic peptides SHG and SHG-EM1. Several lines of peripheral blood derived iPSCs from male and female donors of different age groups and demographics were evaluated for efficiency to fully differentiate into chromaffin cells, cell culture purity, viability, and stability over time. We engineered recombinant cells using AAV2/8_ 6SHG and 6SHG_EM constructs previously designed in our lab. Male and female Sprague Dawley rats underwent SCI injury using clip compression. Four weeks post injury, selected male and female cell lines were used for intrathecal grafting to animals of the same sex. Hypersensitivity to tactile, cold and heat stimuli was assessed weekly. Our results showed different rates of differentiation and culture stability between cell lines derived from different ages, with more efficient production of catecholamines observed in the cultures derived from peripheral blood of younger individuals. The presence of SHG and EM was confirmed in all recombinant cultures by FLISA and immunostaining. Animals receiving intrathecal grafts of non-recombinant or recombinant cells showed progressive attenuation of hypersensitivity. The effects were partially blocked by intrathecal injection of SHG-antibody in a subgroup of animals, suggesting active participation of SHG on the observed effects. Our data suggests that peripheral blood-derived hiPSC is an efficient source to generate chromaffin cells for alleviation of chronic pain. Recombinant cells releasing additional analgesic peptides might further increase the potency of this approach.

Disclosures: **S. Jergova:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property.. **A. Eeswara:** None. **K. Perrucci:** None. **H. Cukier:** None. **J. Sagen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property..

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.03/BB4

Topic: D.02. Somatosensation – Pain

Support: NIH HEAL Grant 1R01DE029951

Title: Synthesizing CGRP antagonist nanoparticles for prolonged inhibition of oral cancer pain

Authors: ***P. K. LEWIS**[1], T. H. NGUYEN[2], C. PEACH[3], S. TENG[3], R. POLLARD[1], V. CHOKSHI[4], B. LESLIE[1], D. G. ALBERTSON[3], N. W. BUNNETT[3], B. L. SCHMIDT[5], N. M.

PINKERTON[1];
[1]Dept. of Chem. and Biomolecular Engin., NYU Tandon Sch. of Engin., Brooklyn, NY; [2]Dept. of Oral and Maxillofacial Surgery, [3]Dept. of Mol. Pathobiology, [5]Bluestone Ctr. for Clin. Res., [4]New York Univ. Col. of Dent., New York, NY

Abstract: Oral cancers affect 30,000 Americans per year. These cancers have been known to cause extreme pain, which worsens as the disease progresses. Calcitonin gene-related peptide (CGRP) receptors have been identified as an important target for inhibiting oral cancer pain. Moreover, this class of receptors has recently been shown to be endocytosed after stimulation, where they continue to signal from within endosomes. Current small molecule CGRP antagonists have limited therapeutic efficacy due to rapid clearance from leaky tumor vasculature, enhanced lymphatic drainage, and failure to reach receptors within endosomes.
Here, we present a nanoparticle delivery approach for sustained inhibition of the CGRP pain pathway in oral cancer. We hypothesized that polymeric nanocarriers loaded with the CGRP antagonist olcegepant would traffic into oral cancer cells and prolong pain inhibition by continuously delivering the drug in oral cancer models.
Olcegepant was encapsulated into PEG-PLA nanoparticles via flash nanoprecipitation. Hydrophobic ion pairing with pamoic acid was employed to drive encapsulation of the weakly hydrophobic antagonist into the hydrophobic nanoparticle core. Olcegepant-loaded nanoparticles were administered via tumoral injection to mouse models of oral cancer. Xenograft paw and tongue models were generated by inoculating HSC-3 human squamous cell carcinoma cells in the left hind paw or tongue for 3 weeks ($2x10^5$ cells in 20 µL Matrigel injection). Nociceptive response was measured via von Frey filament and Hargreaves assays.
Inclusion of pamoic acid into the nanoparticle assembly process yielded a seven-fold increase in olcegepant encapsulation efficiency. This approach generated nanocarriers with a diameter of 90 nm and olcegepant loading of 4 wt.%. The nanocarriers exhibited prolonged release of olcegepant (continuous drug release over 24 hours after dispersion into biological media). Olcegepant-loaded nanocarriers were endocytosed by HSC-3 and Schwann cells. Nociception in *in vivo* mouse cancer models was attenuated for 9 hours longer than free olcegepant at the same dose. Olcegepant-loaded nanocarriers yielded 4.8 times greater attenuation of nociception compared to free olcegepant at the same dose.
Nanoparticle encapsulation of olcegepant significantly improved the efficacy and duration of anti-nociception. This study presents a pioneering and versatile method of encapsulating pain-signaling antagonists for treating oral cancer pain.

Disclosures: **P.K. Lewis:** None. **T.H. Nguyen:** None. **C. Peach:** None. **S. Teng:** None. **R. Pollard:** None. **V. Chokshi:** None. **B. Leslie:** None. **D.G. Albertson:** None. **N.W. Bunnett:** None. **B.L. Schmidt:** None. **N.M. Pinkerton:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.04/BB5

Topic: D.02. Somatosensation – Pain

Support: Craig H. Neilsen Foundation SCIRTS 890307

Title: Attenuation of chronic SCI-pain by intrathecal grafting of skin-derived and recombinant hiPSC chromaffin cells releasing conopeptide MVIIA

Authors: *J. SAGEN, L. TIERNEY, V. SHAH, A. EESWARA, K. PERRUCCI, S. JERGOVA; Univ. of Miami, Miami, FL

Abstract: Pain following SCI is a particularly challenging clinical target with a compelling need for identification of new and potent therapeutic strategies. Transplantation of adrenal medullary tissue or chromaffin cells in the lumbar spinal subarachnoid space led to robust reduction of chronic pain in a number of preclinical rat pain models. Chromaffin cells are highly secretory and thus could act as a local cellular "mini-pump", providing a continually renewable local source of pain-reducing neuroactive molecules in the spinal CSF. To further boost antinociceptive potency, grafted cells could be engineered to produce supplementary pain-reducing neuroactive substances. A limitation for their broad clinical use is the ability to obtain sufficient human adrenal medullary tissue from donors for transplantation. This may be overcome with the advent of iPSC (induced pluripotent stem cells) technology. The goal of the present study is to evaluate and optimize parameters for production of human chromaffin cells (hCC) from human fibroblast-derived hiPSCs. In addition, since previous research showed that N-type Ca^{2+} channel blocker, omega conopeptide MVIIA (aka Prialt) is effective in several pain models, the ability to generate MVIIA-producing recombinant hCCs was tested. Several hiPSC lines derived from skin fibroblasts from male and female donors were used to generate hCCs according to the protocol of Abu-Bonsrah et al. (2018), and the efficiency of the protocol parameters to achieve fully differentiated cells was analyzed. Recombinant cells were engineered using AAV2/8 vector encoding MVIIA previously generated by our lab. Male and female Sprague Dawley rats were used to induce SCI injury using clip compression. Four weeks post injury, age-matched cell lines resuspended either in media or in Vitrogel matrix were intrathecally injected to animals of the same sex. Animals were weekly evaluated for the presence of tactile, cold and heat hypersensitivity. The presence of recombinant peptide was evaluated in cell culture, and a pilot group of animals received transplants of recombinant hCC-MVIIA. After transplantation of the best fully differentiated cell lines, attenuation of tactile, cold and heat hypersensitivity was observed in animals with better outcomes when Vitrogel matrix was used for cell suspension. Recombinant chromaffin cells were generated with confirmed production of MVIIA. Grafting of recombinant hCC-MVIIA cells showed further reduction of hypersensitivity in animals. Our data suggests that naïve and recombinant hiPSC-derived chromaffin cells may provide a promising cell transplantation source for management of chronic pain.

Disclosures: **J. Sagen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property.. **L. Tierney:** None. **V. Shah:** None. **A. Eeswara:** None. **K. Perrucci:** None. **S. Jergova:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

funds); The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property..

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.05/BB6

Topic: D.02. Somatosensation – Pain

Support: SIP-20220300 (MDC)

Title: Synergistic antinociceptive interaction between N-palmitoylethanolamide in coadministration with paracetamol or indomethacin

Authors: *M. DECIGA-CAMPOS[1], R. VENTURA-MARTINEZ[2], G. E. ÁNGELES-LÓPEZ[3]; [1]Inst. Politécnico Nacional, Ciudad de México, Mexico; [2]Farmacologia, Fac Med, UNAM, Mexico, Mexico; [3]Facultad de Medicina, Univ. Autónoma de México, Ciudad de México, Mexico

Abstract: N- palmitoylethanolamide (PEA) is an endocannabinoid involved in antinociceptive effects in several nociceptive preclinical tests. PEA is synthesized in the presence of injury; it is present in areas for pain control in both the peripheral and central nervous systems. This study assesses the pharmacological interaction in the nociception of PEA combined with paracetamol and indomethacin in mice. Female mice were injected with 1% formalin on the right paw, and the number of flinches was quantified as nociceptive behavior. The local treatment with PEA, indomethacin, and paracetamol decreased the number of flinches in a dose-dependent manner. The order of antinociceptive potency was PEA (EC_{30}= 6.2 ± 1.3 μg/20 μL, paw), paracetamol (EC_{30}= 6.2 ± 1.3 μg/20 μL, paw), and indomethacin (EC_{30}= 6.2 ± 1.3 μg/20 μL, paw). An isobologram was used to determine whether the combined effects were supra-additive or infra-additive. The PEA-paracetamol combination produced a synergistic effect; their Z_{mix} (6.2 ± 1.3 μg/20 μL, paw) was lower than Z_{add} (26.9 ± 5.2 μg/20 μL, paw). In the other case, PEA-indomethacin presented an antagonism effect when Z_{exp} (55.7 ± 15.1 μg/20 μL, paw) was higher than Z_{add} (31.2 ± 4.3 μg/20 μL, paw). These findings indicate that coadministration with NAID´s could produce synergism or antagonism. Furthermore, it is important to analyze the combinations with possible therapeutic utility.

Disclosures: M. Deciga-Campos: None. **R. Ventura-Martinez:** None. **G.E. Ángeles-López:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.06/BB7

Topic: D.02. Somatosensation – Pain

Title: Dha metabolite pdx, a pro-resolving lipid mediator, prevents and reverses postoperative pain after tibial fracture surgery in mice

Authors: *Y. LI, S. BANG, S. CHANDRA, R.-R. JI;
Dept. of Anesthesiol., Duke Univ., Durham, NC

Abstract: Postoperative pain remains a significant health concern for anesthesiologists, and finding novel non-opioid analgesics is critical for reducing the use of opioid analgesics. Our previous research demonstrated that specialized pro-resolving mediators (SPMs) in the DHA metabolic pathway, such as resolvin D series, maresins, and protectin D1/neuroprotectin D1 (PD1/NPD1), have potent analgesic actions in various animal models of pathological pain. We have also demonstrated that PD1/NPD1 activates GPR37 to resolve inflammation and pain. Protectin DX (PDX, 10(S), 17(S)-DiHDHA) is another DHA-derived analog of PD1/NPD1. However, the role of PDX in postoperative acute and chronic pain and its mechanism of action is currently unclear. This study used a mouse tibial fracture fixation model, and we administered PDX intravenously at different doses (1, 10, 100 ng/mice) to determine its analgesic effects. We measured spontaneous pain using the Grimace test, mechanical pain using the Von Frey test, and cold pain using the acetone test. Our findings indicate that intravenous injection of PDX (100ng/mice) significantly reduced chronic mechanical pain caused by fracture and provided long-lasting analgesia for more than 6 hours. Furthermore, a single intravenous injection of PDX (100 ng/mice) immediately after fracture surgery prevented the development of acute postoperative pain caused by fracture for more than 5 days. Behavioral experiments found that PDX could not reverse mechanical and cold pain after fracture in GPR37 knockout mice. We found that PDX can bind to GPR37 through computer simulation experiments, and in vitro experiments found that PDX can increase the calcium influx in Chok1 cells expressing GPR37, suggesting that PDX may participate in the analgesic effect through GPR37. In conclusion, our study demonstrates that the DHA metabolite PDX effectively reduces acute postoperative pain and the development of chronic pain in a mouse model and may become a new choice for perioperative analgesics. We are currently investigating whether PDX is another ligand of GPR37 for pain control.

Disclosures: **Y. Li:** None. **S. Bang:** None. **S. Chandra:** None. **R. Ji:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.07/BB8

Topic: D.02. Somatosensation – Pain

Support: CDMRP Grant W81XWH-21-2-0011

Title: Reversal of thermal and mechanical hypersensitivity in rat models of inflammatory and peripheral neuropathy pain by (2R,6R)-Hydroxynorketamine

Authors: *M. A. CAMPANILE[1], J. O. PAMPALONE[1], K. R. CASTELL[1], I. LUCKI[2], C. A. BROWNE[1];
[1]Henry M. Jackson Fndn. for the Advancement of Military, Bethesda, MD; [2]Pharmacol., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: (2R,6R)-hydroxynorketamine ((2R,6R)-HNK), has antinociceptive and analgesic effects in several murine models of pain. This study evaluates the effects of (2R,6R)-HNK in a new species(rat) confirming its analgesic activity in inflammatory pain and screening it for the first time as a potential therapeutic for chemotherapy induced peripheral neuropathy (CIPN). Male and female Sprague-Dawley rats were administered λ-Carrageenan (1%, 100µl injected into left hindpaw) to evoke inflammatory pain. (2R,6R)-HNK was administered subcutaneously at 0, 3, 10, 30, or 56mg/kg to evaluate its dose response curve on reversal of mechanical hypersensitivity. Electronic Von Frey animals were tested at baseline and 24 hours post (2R,6R)-HNK treatment. Once the paw was withdrawn, the trial was terminated. Each measurement consisted of 4 trials, 5 minutes apart. CIPN was induced following 5 intraperitoneal injections of oxaliplatin hydrochloride (6mg/kg).For the oxaliplatin study, mechanical hypersensitivity was assessed by Von Frey 1, 4, and 6 weeks following CIPN, with (2R,6R)-HNK (30mg/kg) screened at 4 and 24 h at the 6 week timepoint. The ability of (2R,6R)-HNK to reverse thermal hypersensitivity was screened at 8 weeks post-oxaliplatin on a hot plate set to 52ºC . Sensorimotor gating deficits, Acoustic Startle Response/Prepulse inhibition (ASR/PPI), were measured 1 and 10 weeks following CIPN induction, with HNK screened at 10 weeks.Dose response curves for (2R,6R)-HNK reversal of mechanical hypersensitivity induced by carrageenan, indicated that the most efficacious doses were 30 -56 mg/kg. CIPN produced considerable mechanical hypersensitivity at 1, 4 and 6 weeks and long-lasting hypersensitivity to heat at 8 weeks. Thresholds of (2R,6R)-HNK treated animals were significantly improved relative to saline-treated animals at 4 and 24 h. CIPN attenuated %PPI, this was normalized by (2R,6R)-HNK treatment.
Overall, these data confirm that (2R,6R)-HNK reverses mechanical hypersensitivity associated with inflammation pain, and provides the first demonstration of (2R,6R)-HNK reversal of CIPN induced mechanical and thermal hypersensitivity. DISCLAIMER: The opinions or assertions contained herein are the private views of the authors and are not necessarily those of the Uniformed Services University of the Health Sciences or the Department of Defense, USA. RESEARCH SUPPORT: CDMRP - W81XWH-21-2-0011

Disclosures: **M.A. Campanile:** None. **J.O. Pampalone:** None. **K.R. Castell:** None. **I. Lucki:** None. **C.A. Browne:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.08/BB9

Topic: D.02. Somatosensation – Pain

Title: Quantifying Neural Response to Kilohertz Spinal Cord Stimulation Using Calcium-Sensitive Imaging

Authors: *D. LEE[1], D. WANG[3], K. LEE[4], Z. KAGAN[2], K. BRADLEY[1];
[1]Nevro, Redwood City, CA; [2]Nevro, Redwood city, CA; [3]Nevro Corp., Nevro Corp, Redwood City, CA; [4]Res., Nevro Corp, Redwood city, CA

Abstract: High-frequency (10kHz) spinal cord stimulation (SCS) is an established and expanding neuromodulatory technique used for the treatment of chronic pain. Despite its clinical success, the specific cellular mechanism by which 10kHz SCS activates neurons is not yet fully understood. To overcome the challenges associated with electrical field stimulation artifacts, we employed calcium imaging techniques to quantitatively assess the impact of electrical stimulation.In this study, transverse spinal cord slices of thickness 400um were loaded with calcium-sensitive dyes (Cal520). Subsequently, extracellular electric fields with varying frequencies (1kHz, 3kHz, 5kHz, 8kHz, and 10kHz) andidentical pulse widths (charge-balanced biphasic square pulses of 30us), were applied in ascending frequency order to the slices using a microelectrode in aCSF. After a 10 second no-stimulation baseline, the stimulation was delivered for a duration of 10 seconds for each frequency. The fluorescence (F) changes due to the stimulation were computed by calculating the difference between the overall tissue F at during stimulation to that measured at baseline. To minimize the impact of stimulation sequence, the frequency sequence was applied in order of descending frequency for half of the samples, except for the 1kHz stimulation, which was always applied at the beginning and end of the sequence to serve as a reference for evaluating the repeatability of neural responses.A total of 512 cells from 10 rats were analyzed in this study. The results showed that the mean F change was highest at 10kHz (+14±10%) and 8kHz (+14±8%), with no statistically significant difference between them. The fluorescence changes at the other frequencies demonstrated a direct linear relationship with frequency, such as 1kHz (+7±4%), 3kHz (+8±5%), and 5kHz (+10±7%), and these differences were statistically significant ($p<0.01$) when compared to 10kHz.These findings indicate that superficial spinal cord neurons are activated by extracellular electric fields in the kHz range, and their responses are directly dependent on the frequency of stimulation. This effect may extend to deep dorsal horn neurons, including inhibitory interneurons and projection neurons within the spinal cord, suggesting the potential of high-frequency SCS for pain management and modulation of sensory information.

Disclosures: **D. Lee:** A. Employment/Salary (full or part-time):; Nevro. **D. Wang:** A. Employment/Salary (full or part-time):; Nevro. **K. Lee:** A. Employment/Salary (full or part-time):; Nevro. **Z. Kagan:** A. Employment/Salary (full or part-time):; Nevro. **K. Bradley:** A. Employment/Salary (full or part-time):; Nevro.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.09/BB10

Topic: D.02. Somatosensation – Pain

Title: Morton'S neuroma: a model to study neuropathic pain and test its treatment

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Abstract: Morton's neuroma is a chronic pain condition in forefoot caused by microtraumas of the common plantar nerve resulting in inflammation and fibrous proliferation and is most common in middle-aged women. Its peripheral and localized nature make it an optimal model for testing neuropathic pain symptomatology and treatments. Frequent symptoms include burning, stabbing or shooting pain upon weight-bearing in the ball of the foot that might radiate to the toes. Currently, the standard of care for Morton's neuroma ranges from conservative measures to surgical excision. Approximately one third of patients continue to experience pain after nonsurgical treatments which limits their activities of daily. Our lab aims to address the need for a successful treatment of pain from Morton's neuroma via a multi-layered approach: On an anatomical-molecular level, we are analyzing excised neuromas using histological characterization and next-gen RNASeq. Sequencing, alongside multiplex labeling, will identify induced or overexpressed transcripts that could contribute to Morton's pathology. Immunohistochemistry revealed significant collagen IV deposition in the endo- and perineurium, demyelination of axonal fibers and dislocation of fibrinogen to the endoneurium that indicates vascular damage. On a psychophysical level, we performed phenotyping of the major sensory modalities. We used a comprehensive quantitative sensory testing (QST) battery including stimuli testing for thermosensation, mechanosensation, and deep tissue pressure. Consistent with the clinical presentation, patients with Morton's neuroma had lower pressure pain thresholds and reported higher pressure pain ratings consistent with a sudden onset of pain perception in the ball of their affected foot in comparison to healthy participants. We also observed an extended area of heat hyperalgesia in the foot affected by Morton's neuroma. These findings suggest first, sensitization of nociceptive fibers expressing the heat and inflammatory receptor TRPV1, and second, secondary sensitization of dorsal spinal cord circuits. On a clinical level, we are about to enroll patients with refractory pain from Morton's neuroma into a Phase I Clinical Trial to test a perineural injection of the super-potent TRPV1 agonist resiniferatoxin (RTX). This drug has been successful tested in clinical trials for patients with refractory cancer pain and pain from knee osteoarthritis. Based on results of our preclinical perineural RTX injections and the findings in our sensory testing study, we hypothesize that a perineural injection of RTX will provide effective pain relief in patients with Morton's neuroma.

Disclosures: E.S. Staedtler: None. **S. Satyanarayana:** None. **E. Frangos:** None. **M. Sapio:** None. **M. Backonja:** None. **M. Iadarola:** None. **A.J. Mannes:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR209.10/BB11

Topic: D.02. Somatosensation – Pain

Support: UCLA Training Program on the Translational Neuroscience of Drug Abuse (TNDA) predoctoral T32
The Center for Medicinal Cannabis Research (CMCR)
UCLA Shirley and Stefan Hatos Center for Neuropharmacology

Title: Myrcene, a cannabis terpene, reduces mechanical hypersensitivity in a mouse model of neuropathic chronic pain

Authors: *M. RICE[1], A. RODRIGUES[1], C. WU[1], Z. COOPER[2], C. CAHILL[3];
[2]Jane & Terry Semel Inst. for Neurosci. & Human Behavior, [3]Psychiatry and Biobehavioral Sci., [1]Univ. of California, Los Angeles (UCLA), Los Angeles, CA

Abstract: One fifth of adults suffer from chronic pain in the US. With a need for effective pain medications, many choose to use cannabis to treat their pain. Cannabis contains 550 compounds including terpenes and >100 phytocannabinoids; while delta-9-THC is thought to be the primary constituent that contributes to cannabis-induced analgesia, specific terpenes are hypothesized to contribute to this effect. This study tests whether myrcene, a terpene with one of the highest concentrations in cannabis, reduces mechanical allodynia associated with Chronic Constriction Injury (CCI), a model of chronic neuropathic pain in adult mice; CCI has both face and predictive validity for clinical neuropathic pain. Two weeks after injury, mechanical thresholds were determined in sham (control) and CCI male mice prior to and following 30 minutes after myrcene (10-200mg/kg, i.p.) or vehicle treatment. Each animal served as its own control for within subject analysis, where treatment was stratified between days in a crossover design. Mechanical thresholds were determined using von Frey filaments, using force pressure (4.31, 2 g) applied to the plantar surface of the ipsilateral hind paw. The number of withdrawals out of 10 stimulations was recorded. Statistical analyses were performed using paired or unpaired student t-tests. To determine potential confounding effects of myrcene on locomotion, mice were tested in an open field apparatus 28 days after injury for 30 minutes following administration, calculating total distance moved and average speed. Nerve injury increased the number of responses to the mechanical stimulus in CCI mice ($p<.01$) but not in shams demonstrating the presence of mechanical hypersensitivity. Only high doses of myrcene (100, 200mg/kg) significantly attenuated mechanical hypersensitivity ($p<.05$). Myrcene had no effect on mechanical responses in shams. Myrcene did not alter locomotion compared to vehicle. In conclusion, mice display mechanical hypersensitivity following peripheral nerve injury via a CCI, and myrcene dose-dependently reduced this hypersensitivity as measured by von Frey filaments. Future studies will replicate experiments in a female cohort, assess oral myrcene administration, and probe myrcene's effects on CCI-induced negative affect-like behaviors.

Disclosures: M. Rice: None. **A. Rodrigues:** None. **C. Wu:** None. **Z. Cooper:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Canopy Growth Corp, True Terpenes, Storz & Bickel. **C. Cahill:** None.

Poster

PSTR209. Pain Circuitry and Non-Opioid Treatment Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR209.11/BB12

Topic: D.02. Somatosensation – Pain

Support: Department of Florida of Health COPBC - University of Miami

Title: Attenuation of chronic SCI-pain by intrathecal hiPSC-derived GABAergic cells with chromaffin cell co-grafts or in conjunction with exercise

Authors: *L. TIERNEY, V. SHAH, A. EESWARA, K. PERRUCCI, M. EDWARDS, S. JERGOVA, J. SAGEN;
Univ. of Miami, Miami, FL

Abstract: Treatment of chronic pain following spinal cord injury (SCI) with conventional pharmacotherapy is complicated by off-target side effects of systemic drugs and potential misuse associated with analgesic/opioid drug administration. Our lab explores targeted delivery of analgesic compounds via cell therapy to achieve prolonged attenuation of chronic pain. Previous findings showed improved survival of rat GABAergic neural progenitor transplants (NPCs) when used in combination with neurotrophin-producing chromaffin cells and improved pain-reducing beneficial effects of intraspinal NPCs using a treadmill exercise program. The current study was designed to overcome limitations in translating GABA NPC transplantation strategies for clinical application in chronic SCI pain management by using human induced pluripotent stem cells (hiPSCs) and a relatively non-invasive intrathecal cell administration route. Potential supportive effects of exercise and of hiPSC-derived chromaffin cell co-grafts on hiPSC-derived GABAergic NPC survival and pain reduction were evaluated. We generated GABAergic NPCs and chromaffin cells using hiPSCs derived from human skin fibroblasts and peripheral blood. Four weeks following clip compression SCI, male and female Sprague Dawley rats received GABAergic hiPSCs graft (50,000 and 100,000) or co-grafts of GABAergic and chromaffin hiPSCs (30,000:60,000) in Vitrogel by intrathecal injection into the spinal subarachnoid space. Animals receiving GABAergic graft exhibited dosage dependent reductions in both tactile and cold allodynia, and addition of chromaffin cell co-grafts resulted in comparable effects to the higher dose of NPCs alone. Animals receiving low dose of GABAergic graft (30,000 cells) in conjunction with intensive locomotor training (ILT) using an inclined treadmill showed attenuation of enhanced anti-allodynic responses in comparison to animals undergoing ILT alone. Behavioral results were comparable between male and female animals. ELISA analysis of cytokines in CSF of treated animals showed significant reduction of elevated SCI-induced pro-inflammatory cytokines IL1β and TNFα in CSF of male and female rats receiving NPC transplants in comparison to controls. Additionally, female rats receiving NPC transplants exhibited increased levels of anti-inflammatory cytokine IL10. These findings provide support for the clinical application of cell transplantation in chronic SCI pain management by demonstrating potential to overcome hurdles of human cell sourcing and transplant viability.

Disclosures: **L. Tierney:** None. **V. Shah:** None. **A. Eeswara:** None. **K. Perrucci:** None. **M. Edwards:** None. **S. Jergova:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property. **J. Sagen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property..

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.01/BB13

Topic: D.02. Somatosensation – Pain

Support: 1P50DA044121
F31NS126012

Title: Opioid-exposed patients with chronic back pain exhibit distinct nucleus accumbens functional connectivity

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Abstract: Dopaminergic nucleus accumbens (NAc) circuits are causally implicated in chronic pain and opioid use disorder. Since opioids are used to treat acute and chronic pain, understanding NAc circuit adaptations is imperative, especially as they may relate to previously observed increased negative affect in patients on opioids. This study used resting-state fMRI (RS-fMRI) to compare functional connectivity (FC) with NAc between CBPs and CBPs exposed to opioids.We recruited 70 patients with CBP persisting for at least 12 weeks (CBP) (29 M, 41 F; age (mean ± SD) = 60 ± 12 years) and 70 CBPs exposed to opioids (CBP+OP) (24 M, 46 F; age = 60 ± 11 years), who were scanned on a 3T Siemens Magnetom Prisma with a 64 channel-head/neck coil. RS-fMRI data were preprocessed using fmriprep. After regressing out the 6 motion vectors, signal-averaged overall voxels of the eroded white matter and ventricle region, and global signal of the whole brain, data were filtered (0.008-0.1 Hz, 4th-order Butterworth). We calculated the FC (Fisher's *z*) from each voxel in NAc with each voxel in cortex, subcortex (excluding NAc), and ventral tegmental area (VTA). This produced three FC arrays, each with three dimensions: the target voxel (cortex, subcortex, or VTA), the seed voxel (NAc), and the subject. Linear regression estimated a beta matrix **B** of group differences; each element was the expected group difference in FC between target voxel *i* and NAc voxel *j* after adjusting for age, sex, log(pain_duration), NRS, and head motion. We used permutations with a test statistic $T_j = (\mathbf{B}^T\mathbf{B})_{jj}$ to calculate a single *P*-value for voxel *j* in NAc, representing the extent to which NAc

voxel *j*'s FC differs between groups. Principal component analysis (PCA) revealed these group differences' structure and whether the differences relate to CBP+OP. We projected each CBP+OP subject's FC into this principal component space, enabling us to study how the group differences in FC relate to patients' clinical characteristics (blood level of opioids (ROE), etc.).Compared with CBPs, CBP+OPs exhibited distinct FC from NAc to the cortex, subcortex excluding NAc, and VTA. NAc shell had distinct cortical FC ($-\log_{10}(P\text{-value}) > 3$). In contrast, NAc core had distinct subcortical FC. The first principal component of the cortical beta matrix explained 62% of the variance. FC projections onto the cortical component correlated with blood levels of opioids ($r = 0.33$, P-value = 0.03).This study employed a novel analytical approach to uncover distinct FC patterns of CBPs exposed to opioids compared with CBPs with 3 different brain regions. Observed NAc FC differences likely relate to the increased negative affect associated with opioid use in CBP.

Disclosures: L. Huang: None. **A. Vigotsky:** None. **M. Baliki:** None. **A. Apkarian:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.02/BB14

Topic: D.02. Somatosensation – Pain

Title: Social Modulation of Pain in Drug-Naïve Cohabitants of Mice Administered Chronic Morphine.

Authors: *H. K. DEOL[1], L. J. MARTIN[2];
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Abstract: Opioids, known for their effectiveness, affordability, and long-lasting effects, are commonly prescribed for pain management. In the last decade, opioid prescribing and opioid misuse have dramatically increased, partly due to factors such as pharmaceutical marketing practices and medication formulations. Mice undergoing opioid withdrawal exhibit specific behaviours such as excessive teeth chattering, genital grooming, piloerection, paw tremors and wet dog shakes. Although physical withdrawal symptoms subside after a short time, emotional symptoms such as anxiety & irritability often persist for months due to lasting changes in neuropeptides, receptors, and molecular changes. Such altered emotional symptoms in individuals undergoing withdrawal can impact caregivers in their immediate environment. Family members often suffer greatly due to the unpredictability associated with having a relative with an opioid use disorder. Stressors come in the form of having to deal with the patients' behavioural disturbances while restricting their own social activities. Hence these studies aimed to investigate the effects of drug-dependent individuals' behaviour on the pain sensitivity of their drug naïve cohabitant. All studies were conducted in adult (7-10-week-old) male C57/BL mice. Study 1 (N=8) observed the effects of cohabitation with a cagemate undergoing natural opioid

withdrawal on mechanical pain thresholds. In study 2, corticosterone production was inhibited in drug naïve cohabitant mice (N=8) while their cagemate underwent natural opioid withdrawal. Results revealed hypersensitivity in drug naïve cagemates when coupled with a mouse receiving chronic morphine administration and progressing into a withdrawal phase. Hypersensitivity in drug naïve cohabitants was reversed by the administration of metyrapone, suggesting that hypersensitivity in these mice may be mediated by the chronic stress of living with a morphine-dependent cagement. Emotional and social deficits were also observed in drug naïve cagemates.

Disclosures: H.K. Deol: None. **L.J. Martin:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.03/BB16

Topic: D.02. Somatosensation – Pain

Support: Research productivity fellowship from the National Council for Scientific and Technological Development (CNPq)
Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) Finance Code 001 protocol 40001016038P0

Title: Sex differences in the response to analgesic drugs in a model of post-operative orofacial pain in rats.

Authors: *J. M. ZORTEA, D. F. BAGGIO, F. M. R. LUZ, V. B. P. LEJEUNE, J. G. CHICHORRO;
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Abstract: The success of an invasive dental procedure depends on multiple factors, being one of the most relevant the management of post-operative pain. Pain control is influenced by various factors, and recent evidence suggests that sex may influence analgesics' efficacy. Thus, the aim of this study was to compare the efficacy of three analgesic drugs (ibuprofen 30 and 100 mg/kg, acetaminophen 100 and 300 mg/kg, and codeine 3 and 10 mg/kg), widely used in Brazilian dental clinics, in male and female rats subjected to a model of orofacial post-operative pain. Adults Wistar rats underwent the surgical procedure of intraoral incision or sham surgery. Facial mechanical hyperalgesia was evaluated by applying von Frey filaments (0,04 to 8 g), while heat hyperalgesia was assessed by the approach of a radiant heat source to the orofacial region. Mechanical and heat thresholds were assessed before and on day 3 after surgery, prior to the treatments, and at 1-hour intervals after the treatments up to 4 hours. When a sex difference was detected, ovariectomy was performed, and after 21 days, the animals underwent the same protocol described above. All protocols were approved by CEUA/BIO/UFPR #1464. Data were analyzed by two-way ANOVA with repeated measures followed by Bonferroni post hoc test (n=8-12). In all experiments, male and female rats subjected to incision and treated with vehicle

developed mechanical and heat hyperalgesia on day 3 after surgery, compared to the sham groups. Ibuprofen caused a significant reduction on mechanical hyperalgesia in male and female rats during one hour, while reduced heat hyperalgesia up to 2 hours in males and only at 1 hour in females. Paracetamol reduced facial mechanical hyperalgesia during 3 hours in males and up to 2 hours in females, and reduced heat hyperalgesia up to 3 hours for both sexes. There was no sex difference in the magnitude of antinociception provided by ibuprofen or paracetamol, although the duration of the effect was sometimes greater for males. On the other hand, codeine at 3 mg/kg reduced facial mechanical hyperalgesia only in males, and only the higher dose (10 mg/kg) promoted antinociception for both sexes up to 2 hours. Females subjected to ovariectomy responded to the lower dose of codeine similarly to males, suggesting that sex hormones interfered with the drugs' efficacy. The magnitude of the effect of codeine on heat hyperalgesia was similar in males and females, but the duration of the effect was longer in males (3 hours compared to 1 hour in females). These results indicate that all analgesics evaluated provided shorter duration of effect in females compared to males, and suggest that females are less susceptible to codeine's analgesia.

Disclosures: **J.M. Zortea:** None. **D.F. Baggio:** None. **F.M.R. Luz:** None. **V.B.P. Lejeune:** None. **J.G. Chichorro:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.04/BB17

Topic: D.02. Somatosensation – Pain

Support: CIHR Project Grant (PJT-175256)
Healthy Brains, Healthy Lives Fellowship

Title: Investigation of opioid tapering paradigms using a mouse chronic neuropathic pain model

Authors: *H. DERUE[1], A. RIBEIRO-DA-SILVA[2], J. S. MOGIL[3];
[1]Pharmacol. and Therapeut., [2]Pharmacol. and Therapeut. and Anat. and Cell Biol., McGill Univ., Montreal, QC, Canada; [3]Psychology, McGill Univ. Integrated Program in Neurosci., Montreal, QC, Canada

Abstract: Introduction: An estimated 22% of people are living with chronic pain, which causes significant disability and diminished quality of life and is poorly managed by existing treatments. Among individuals who are prescribed long-term opioids for the management of chronic pain, 21-29% report opioid misuse behaviours and 8-12% of chronic pain patients with long-term opioid prescription develop Opioid Use Disorder (OUD). As a result, it is essential to develop evidence-based regimens that reduce the risk associated with long-term opioid prescription including tapering (incremental decrease) of the dose of opioids for the management of chronic pain. This project aims to develop a novel method for investigating opioid tapering paradigms

within preclinical contexts through the use of the spared nerve injury (SNI) model of chronic neuropathic pain in the mouse.Methods: We will use the SNI model of neuropathic pain on CD-1 mice, and then subject equal numbers of male and female mice to a 1:1 fixed ratio (FR) (FR1; 1 response yields 1 reward dose) oral self-administration of morphine in an operant conditioning box to stimulate opioid addiction. Mice will be separated into two groups: tapering and cold turkey withdrawal. The tapering group will be given access to 0.5 mg/ml concentration of morphine in 0.9% sweetened condensed milk for a period of 1 hour per day 7 days per week throughout the experimental period. For each correct operant response, a single dose of the opioid drug (35 µl/dose) will be dispensed, paired with the illumination of a reward-paired cue light. After a brief acquisition period of two weeks, a rapid dose reduction tapering protocol will be implemented, wherein a full cessation of opioid consumption is reached after three weeks. For two weeks following the complete cessation of opioid consumption, the 1:1FR will be reinstated to examine the reuptake of opioid consumption (relapse behaviour). During the withdrawal period and during the reacquisition period, mice will be assessed for time dependent changes to withdrawal and pain behaviours through Von Frey and withdrawal behaviour assessments.Results: Preliminary data suggests that opioid tapering paradigms are more effective than cold turkey withdrawal in reducing the incidence of relapse behaviours as is found in clinical applications.Conclusion: Future experiments will investigate whether there are opioid tapering paradigms that reduce withdrawal symptoms in distinct sub-populations, with particular attention to sex-specific or incentive salience-specific outcomes.

Disclosures: H. Derue: None. **A. Ribeiro-da-Silva:** None. **J.S. Mogil:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.05

Topic: D.02. Somatosensation – Pain

Support: STI2030-Major Projects (2021ZD0203302)
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Health Commission of Guangdong Province (A2021319)
Shenzhen Innovation Committee of Science and Technology grants (ZDSYS20200811144002008)

Title: Spinal mechanisms underlying opioid-induced mechanical hypersensitivity and tolerance

Authors: *G. YIN, D. DONG, F. DU, K. DUAN, X. LIU, J. HUO, L. CHENG;
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Abstract: Repeatedly administration of opioid drugs is associated with two major side effects: opioid-induced hypersensitivity (OIH) and analgesic tolerance. Among different forms of OIH and tolerance, the opioid receptors and cell types mediating mechanical OIH and analgesic tolerance remain unresolved. According to our recent study, peripheral μ-opioid receptors (MORs) or MOR-expressing neurons are required for the development of morphine-induced thermal, but not mechanical OIH and tolerance, suggesting modality-specific mechanisms underlying OIH and tolerance. Here we reported that the kappa opioid receptors (KORs) in the spinal dorsal horn control morphine-induced mechanical OIH and tolerance (but not thermal tolerance) via modulating the excitability of KOR-expressing neurons. Conditional knockout of KORs from dorsal horn neurons (n = 5-9 mice per group; both male and female were included in this study), or intersectional genetic ablation of dorsal horn KOR-expressing neurons (n = 7-9 mice per group), prevented the development of 5-day morphine-induced mechanical, but not thermal OIH and tolerance. Moreover, we found that 5-day morphine dramatically increased the excitability of dorsal horn KOR-expressing neurons, and chemogenetic silencing (n = 6 mice per group) these neurons could prevent/rescue morphine mechanical OIH and/or tolerance. Conversely, chemogenetic activation of KOR-expressing dorsal horn neurons re-occurred morphine mechanical OIH (n = 6 mice per group), and caused a transition from morphine-sensitive to morphine-resistant state under neuropathic pain condition (n = 5-7 mice per group). Collectively, our data suggest that dorsal horn KORs could control morphine-induced mechanical forms of OIH and tolerance via modulating the excitability of dorsal horn KOR-expressing neurons. Targeting dorsal horn KORs/KOR-expressing neurons, could therefore provide preclinical studies and/or clinical trials a mechanism-based, modality-specific strategy to resolve opioid-induced mechanical forms of OIH and analgesic tolerance.

Disclosures: G. Yin: None. **D. Dong:** None. **F. Du:** None. **K. Duan:** None. **X. Liu:** None. **J. Huo:** None. **L. Cheng:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.06/BB18

Topic: D.02. Somatosensation – Pain

Support: FAPESP grant #22/14342-0 and #23/00298-1

Title: Rna-dependent protein kinase mediates opioid analgesia and tolerance during inflammatory pain.

Authors: P. SANSON[1], A. CARVALHO[2], S. ZANON[2], *G. LUCAS[3];
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Abstract: Aim: RNA-dependent protein kinase (PKR) is a serine/threonine protein kinase activated not only by double-stranded RNA presented by viruses, but also pro-inflammatory mediators, growth factors and cytokines. The aim of this study was to investigate the role played by PKR in the analgesic effect of morphine in a mice model of incisional and burning pain. **Methods:** Male and female C57BL/6 mice, aged 3-4 months old, were used. Inflammatory pain model consisted of plantar incision made through skin, fascia, and muscle of the left hind paw. Burning pain consisted of immersing the left hind paw in water at 60ºC for 8 seconds. Morphine was administered at 1.0, 2.0 or 4.0mg/Kg (s.c.) while PKR inhibitor was given at 250μg/Kg (i.p.). Thermal hyperalgesia and protein expression were analyzed 1 and 72 hours after lesion by behavioral tests and western blot, respectively. Tolerance was induced by daily injections of morphine (4.0mg/Kg, 3x/day for 5 days) or PKR inhibitor 15 min before opioid administration. Data were analyzed by one or two-way ANOVA followed by Tukey HSD post-hoc analysis. **Results:** Both models caused marked thermal hyperalgesia at 1 and 72 hours after lesion. Moreover, morphine induced analgesia in a dose-dependent manner. In the burning pain model, the antinociceptive effect of morphine 1h after lesion was higher in male as compared to female mice ($p < 0.01$). Inhibition of PKR activity 15 minutes before opioid administration reduced significantly, and in a dose dependent manner, the antinociceptive effect of morphine in male and female mice ($p < 0.001$). In addition, a single dose of morphine at doses of 2.0 and 4.0mg/Kg, was able to cause drug tolerance 3 days after the first injection ($p < 0.01$). Chronic treatment with morphine (4.0 mg/Kg, 3x/day, for 4 days) caused significant tolerance after incisional inflammatory lesion whereas PKR inhibition before opioid administration aggravated drug tolerance ($p < 0.01$). **Conclusions:** together, these data show that PKR activity influences the antinociceptive effect mediated by the μ-opioid receptor activity as well as the development of opioid tolerance. These effects could be mediated by a potential interaction between PKR and TRPV1 receptors. Understanding the role played by PKR in the mechanism of opioid analgesia and tolerance should offer valuable insights about chronic pain mechanisms, thus yielding new possibilities to improve the well-being of people whom chronic pain is a serious burden.

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Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.07/BB19

Topic: D.02. Somatosensation – Pain

Title: Stress Exposure induces Morphine Sensitization Involvement of the Dopaminergic System in the Nucleus Accumbens

Authors: *S. MOHAMMADI[1], A. HAGHPARAST[2];
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Abstract: Dopamine is a neurotransmitter that is increased in the nucleus accumbens (NAc) by administration of morphine as a sedative, and repeated exposure to morphine accompanied by stress leads to enhancement of dopamine overflow in the NAc. This study aimed to evaluate the cross-sensitization of stress and morphine, focusing on the role of dopaminergic receptors in the NAc. Two stainless steel guide cannulae were implanted 1mm above the NAc of the adult male Wistar rats weighing 220-250g via stereotaxic surgery. Various doses of SCH23390 as a D1-like dopamine receptor antagonist (0.125,0.25,1 and 4 μg/0.5μl/NAc) and Sulpiride as a D2-like dopamine receptor antagonist (0.25,1 and 4μg/0.5μl/NAc) were microinjected into the NAc. Five minutes after microinjection, three hours of restraint stress (RS) as psychological stress, or six min forced swim stress (FSS) was applied as physical stress. Ten minutes after exposure to stress, an ineffective dose of morphine (1mg/kg) was injected subcutaneously. The procedure was repeated for three consecutive days as a sensitization period followed by a 5-day drug and/or stress-free period. On the ninth day, morphine sensitization was verified by evaluating the antinociceptive response of an ineffective dose of morphine to the tail-flick test. The results revealed that although co-administration of morphine (1mg/kg) and stress in three consecutive days led to morphine sensitization, intra-accumbal microinjection of SCH23390 and Sulpiride disrupted morphine cross-sensitization with stress, either RS or FSS. Our findings suggest an undeniable role for dopamine receptors within the NAc, in morphine sensitization induced by morphine-stress co-administration.

Disclosures: S. Mohammadi: None. **A. Haghparast:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

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Program #/Poster #: PSTR210.08/BB20

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01-NS-105987

Title: Co-expressed delta-, mu-, and kappa-opioid receptors modulate voltage-gated Ca^{2+} channels in gastric-projecting vagal afferent neurons

Authors: *H. GOUDSWARD[1], V. RUIZ-VELASCO[2], G. M. HOLMES[1];
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Abstract: Opioid analgesics are frequently associated with gastrointestinal (GI) side-effects including constipation, nausea, dysphagia, and reduced gastric motility. These effects are mediated by mu-, delta-, and kappa-opioid receptors, which generally modulate neuronal excitability by activating inward rectifying K^+ channels and inhibiting voltage-gated Ca^{2+} channels. Though it has been shown that stimulation of opioid receptors expressed in enteric motor neurons contributes to opioid-induced constipation, it remains unclear whether activation

of opioid receptors in gastric-projecting vagal afferent neurons contributes to the upper GI side effects associated with opioid use. In the present study, whole-cell patch-clamp recordings were performed in acutely dissociated gastric vagal afferent neurons from male Wistar rats (age ≥8 weeks) to investigate opioid receptor-mediated modulation of Ca^{2+} currents. Our results demonstrate marked inhibition (expressed as percent) of Ca^{2+} currents in isolated gastric vagal afferent neurons following exposure to oxycodone (66.1 ± 4.2%, n=7, p<0.0001), deltorphin II (46.7 ± 5.8%, n=8, p<0.0001), and U-50488 (30.4 ± 6.5% n=7, p=0.00351) compared to baseline. Furthermore, we showed that all three opioid receptors inhibit Ca^{2+} currents in a voltage-dependent manner and couple to pertussis toxin-sensitive Gαi/o proteins. These results demonstrate that mu-, delta-, and kappa-opioid receptors are not only co-expressed in gastric vagal afferent neurons but also utilize the same signaling pathway, suggesting analgesics targeting any opioid receptor subtype would likely have the same effect on gastric vagal circuits.

Disclosures: H. Goudsward: None. **V. Ruiz-Velasco:** None. **G.M. Holmes:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.09/BB21

Topic: D.02. Somatosensation – Pain

Support: National Science and Technology Innovation 2030 Major Program Grant 2021ZD0204404
National Natural Science Foundation of China Grant 31825013, 82271244, 61890952
Shanghai Municipal Science and Technology Major Project Grant 2018SHZDZX05

Title: Single neuron projectome-guided analysis revealed the neural circuit mechanism underlying endogenous opioid analgesia

Authors: *Y. DOU[1], Y. LIU[1], W.-Q. DING[1], Q. LI[1], H. ZHOU[1], L. LI[1], M.-T. ZHAO[2], Z.-Y.-Q. LI[3], J. YUAN[2], W.-Y. ZOU[3], A. LI[2], Y.-G. SUN[1];
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Abstract: Single neuron projectome-guided analysis revealed the neuralcircuit mechanism underlying endogenous opioid analgesia Yan-Nong Dou[1*], Yuan Liu[1], Wen-Qun Ding[1,2], Qing Li[1], Hua Zhou[1], Ling Li[1,2],Meng-Ting Zhao[3], Zheng-Yi-Qi Li[4], Jing Yuan[3,5], Wang-Yuan Zou[4], Anan Li[3,5],Yan-Gang Sun[1,6*1]Institute of Neuroscience, State Key Laboratory of Neuroscience,

CAS Centerfor Excellence in Brain Science & Intelligence Technology, Chinese Academyof Sciences, Shanghai 200031, China.[2]University of Chinese Academy of Sciences, Beijing 100049, China.[3]Britton Chance Center for Biomedical Photonics, Wuhan National Laboratoryfor Optoelectronics, MoE Key Laboratory for Biomedical Photonics, HuazhongUniversity of Science and Technology, Wuhan 430074, China.[4]Department of Anesthesiology, Xiangya Hospital, Central South University,Changsha 410008, China.[5]HUST-Suzhou Institute for Brainsmatics, Suzhou 215123, China.[6]Shanghai Center for Brain Science and Brain-Inspired Intelligence Technology,Shanghai 201210, China.**Abstract**Chronic pain is a highly prevalent health problem, leading to a profound impacton the society. Endogenous opioid system is crucial in pain modulation, arethereby of great interest for developing new approaches for pain control.However, the neural circuit mechanism underlying endogenous opioidanalgesia remains unknown. Here, we demonstrate that mu-opioid receptors(MORs) expressed in the central amygdala (CeA) GABAergic neurons arecritical for endogenous analgesia in mouse chronic pain model. Neuronalactivity of MOR-expressing neurons in CeA was increased during inflammatorypain and bi-directional pharmacogenetic modulation of these neurons showedthat suppressing their excitability largely relieved chronic pain. Single-neuronconnectome analysis together with electrophysiological recording revealed thatbranches of single MOR-expressing CeA neurons projected to parabrachialnucleus (PBN) inhibitory neurons, allowing disinhibition of PBN excitatoryneurons known to gate ascending spinal pain pathway, whereas branches ofthese neurons innervating two other subcortical structures had no effect onanalgesia. These findings reveal a major neural circuit underlying endogenousanalgesia, which will shed light on designing innovative strategies for painmanagement, and exemplify the advantage of single-neuron projectomeanalysis for dissecting distinct functions of projections from the same neuron todifferent targeted brain areas.**Keyword**Endogenous opioid analgesia, central amygdala, mu-opioid receptors, single-neuron projectome, chronic pain

Disclosures: **Y. Dou:** None. **Y. Liu:** None. **W. Ding:** None. **Q. Li:** None. **H. Zhou:** None. **L. Li:** None. **M. Zhao:** None. **Z. Li:** None. **J. Yuan:** None. **W. Zou:** None. **A. Li:** None. **Y. Sun:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.10/BB22

Topic: D.02. Somatosensation – Pain

Support: NIAAA Y1AA-3009
NIDA DA048353

Title: Preclinical brain PET study: mu opioid receptor occupancy of mitragynine

Authors: ***M. L. FREANEY**[1], J. SEO[1], W. KIM[1], S. MUKHOPADHYAY[4], T. D. PEARSON[1], A. SHARMA[4], J.-S. LIOW[2], A. HAMPSON[3], C. R. MCCURDY[4], S. KIM[1], N. VOLKOW[3,1];

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Abstract: Background: Kratom, derived from the Southeast Asian tree *Mitragyna speciosa*, has gained popularity for self-medication of pain, depression, and opioid withdrawal. The most abundant alkaloid in kratom products, mitragynine (MTG), and its metabolite, 7-hydroxymitragynine (7-OH-MTG), are partial agonists at mu opioid receptors (MORs) *in vitro* and are suggested to be responsible for many of the pharmacological effects of kratom. Herein, we measured the MOR engagement of MTG and 7-OH-MTG in the rat brain using positron emission tomography (PET).

Methods: Dynamic PET studies were performed in anesthetized male Wistar rats (308.6 ± 44.8 g) using a MOR radiotracer, [^{11}C]carfentanil ([^{11}C]CFN) and monitoring vitals. To assess MOR occupancy, rats received an IV injection of vehicle (2.5% DMSO, n=5) or MTG (0.1 mg/kg, n=3; 5 mg/kg, n=3) 5 min prior to [^{11}C]CFN injection. Influence of metabolites at MORs was studied by oral (PO) administration of MTG through a gavage (100 mg/kg) at 30 (n=3) or 90 (n=3) minutes before [^{11}C]CFN injection. Rats received an IV injection of 7-OH-MTG (2 mg/kg, n=5) 5 minutes prior to [^{11}C]CFN injection to estimate MOR occupancy. For data analysis, [^{11}C]CFN uptake was extracted as standardized uptake value to generate time-activity information. [^{11}C]CFN binding potential (BP_{ND}) in the thalamus was calculated from the simplified reference tissue model using the cerebellum as a reference region.

Results: BP_{ND} of [^{11}C]CFN was not decreased by pretreatment with PO or IV MTG compared to vehicle (**Table 1**), even though bradycardia was observed. [^{11}C]CFN specific binding was 16% lower after pretreatment with IV 7-OH-MTG compared to baseline, though this did not reach statistical significance (p=0.16).

Conclusion: In contrast to published *in vitro* MOR binding data of MTG, MOR occupancy at pharmacologically relevant doses was negligible in rats. The more potent metabolite 7-OH-MTG also showed minimal MOR occupancy indicative of low reward potential. As a prospective therapeutic for analgesia, these results show promise for MTG due to low potential for dependence formation.

Treatment	Route	Dose (mg/kg)	Pretreatment (min)	# of subjects	BP_{ND}*
Vehicle	IV	—	5	5	2.35 ± 0.46
MTG	IV	0.1	5	3	2.28 ± 0.39
		5	5	3	2.80 ± 0.45
	PO	100	30	3	2.85 ± 0.17
			90	3	2.56 ± 0.41
7-OH-MTG	IV	2	5	5	1.97 ± 0.29

Table 1. MOR engagement of mitragynine (MTG) and its metabolite, 7-hydroxymitragynine (7-OH-MTG). *No statistical significance was found between vehicle and any treatment group using two-tailed Student's t-test.

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Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR210.11/BB23

Topic: D.02. Somatosensation – Pain

Support: CIHR grant PJT-162103

Title: The role of delta opioid receptors in primary afferents in an animal model of inflammatory pain

Authors: *H. MOHAMED[1], V. BLAIS[1], L. GENDRON[1,2];
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Abstract: Despite opioids are the most used analgesics for the management of chronic pain, their prolonged use is limited by their adverse effects. In contrast, to the commonly-used mu opioid receptor agonists, delta opioid receptor (DOP) agonists have been shown to cause fewer or no adverse effects which make them promising analgesics. DOP receptors are widely distributed throughout the central nervous system, particularly in the pain pathway. Most interestingly, DOP are found to be highly expressed in primary afferents which are responsible for transmitting signals from the periphery to the spinal cord. The distribution pattern of DOP differs among species. In humans, DOP is mainly found in primary afferents and on their central terminals located in the superficial laminae of the spinal cord. In rodents, however, they are expressed on both dorsal root ganglia (DRG) and spinal neurons. These observations suggest that most studies aiming at studying the role of DOP in pain and analgesia in rodents might not fully predict what could be seen in humans. Indeed, it is uncertain to what extent DOP expressed in primary afferents is involved in regulating pain and DOP-mediated analgesia. We utilized a genetically modified mouse strain (Avil-Cre/ERT2 (cre/-): STOP::Flag-DOP (flox/flox)) that was developed in our laboratory and allowing to induce the expression of DOP only in primary afferents, similar to what has been observed in human spinal cord. In this study, we aimed to assess the specific role of DOP in primary afferents in the CFA model of inflammation using 2 pain behavioral tests; Hargreaves test and von Frey filaments. In addition, the effect of the selective DOP agonist; deltorphin II in conditional knock-in mice (cKI) mice was compared with mice normally expressing DOP (Flag-DOP mice) and mice where DOP is not expressed (STOP::Flag-DOP). The potential sex difference was also evaluated. In these cKI mice, we observed that the intrathecal injection of 3 µg of deltorphin II resulted in a significantly higher antihyperalgesic effect compared to their control congeners. Most interestingly, this effect was found to be intermediary when compared to Flag-DOP mice and STOP::Flag-DOP mice.

Moreover, 1 µg of deltorphin II intrathecally produced a significantly higher antiallodynic effect in DOP knockin mice as compared to DOP knockout mice. Our findings indicate that DOP expressed in DRG neurons are sufficient to produce a significant analgesic effect and therefore have the potential to be targeted in human to alleviate pain.

Disclosures: H. Mohamed: None. **V. Blais:** None. **L. Gendron:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

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Topic: D.02. Somatosensation – Pain

Support: NIH NIDA grant DA042888
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Title: Establishing exon 7-associated full-length 7TM C-terminal splice variants of the mu opioid receptor gene, Oprm1, as novel therapeutic targets for diminishing adverse effects of clinically used mu opioids without altering analgesia in pain management

Authors: S. LIU[1], R. CHIEN[1], A. F. MALIK[1], T. ZHANG[2], V. P. LE ROUZIC[2], J. XU[1], G. C. ROSSI[3], A. MARTINEZ-RIVERA[4], A. M. RAJADHYAKSHA[5], R. QUADROS[6], C. B. GURUMURTHY[6], *Y. PAN[1,2];
[1]Rutgers New Jersey Med. Sch., Newark, NJ; [2]Neurol., Mem. Sloan-Kettering Cancer Ctr., New York, NY; [3]Long Island Univ., Greenvale, NY; [4]Weill Cornell Med., New York, NY; [5]Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY; [6]Univ. of Nebraska, Omaha, NE

Abstract: The single-copy gene (*OPRM1*) encoding the mu opioid receptor (MOR) undergoes extensive alternative splicing, generating multiple splice variants. One set of *OPRM1* variants, exon 7-associated full-length 7 transmembrane (TM) C-terminal splice variants (E7 variants), contain a unique intracellular C-terminal tail with 30 amino acids encoded by E7 that are conserved from rodents to humans. E7 variants are abundantly expressed in the central nervous system with distinct distributions. Increasing evidence has indicated that E7 variants contribute to several mu opioid-induced adverse side-effects, such as tolerance, reward and respiratory depression. Truncating E7-enocded C-terminal tails in mice (mE7M-B6) attenuated morphine tolerance and reward without effect on analgesia. An antisense oligonucleotide (ASO) targeting E7 variants also reduced morphine tolerance. These observations suggest that *Oprm1* E7 variants mediate several adverse effects associated with clinically used mu opioids and targeting E7 variants can diminish mu opioid-induced adverse effects but maintain mu opioid analgesic potency via other *Oprm1* 7TM variants. The current studies further establish the role of E7 variants in mediating mu opioid-induced tolerance, reward, and respiratory depression in naïve mice and mE7M-B6 by using the ASO and a newly developed rabbit monoclonal antibody

(RabmAb) that target E7 variants. Intracerebroventricular administration of either the ASO or RabmAb significantly attenuated morphine tolerance and reward measured by conditioned place preference (CPP) in mice. Additionally, a new mouse model (mMOR-1O-KI) in which only a single E7 variant, mMOR-1O, is expressed showed enhanced morphine tolerance and reward (CPP), complementing the results from mE7M-B6 mice. Furthermore, fentanyl-induced respiratory depression was significantly reduced in mE7M-B6 mice determined by whole body plethysmography (WBP). Together, these studies indicate that E7 variants represent novel therapeutic targets for mitigating adverse effects of clinically used mu opioids without altering analgesia in pain management.

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Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: D.02. Somatosensation – Pain

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Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions (2021SHIBS0002)
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Health Commission of Guangdong Province (A2021319)
Shenzhen Innovation Committee of Science and Technology grants (ZDSYS20200811144002008)

Title: Identification of a hypothalamus-spinal opioidergic pathway controlling morphine-induced mechancial hypersensitivity and analgesic tolerance

Authors: K. DUAN, G. YIN, D. DONG, F. DU, ***L. CHENG**;
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Abstract: Among the different forms of opioid-induced hypersensitivity (OIH) and analgesic tolerance, the mechanisms underlying mechanical OIH and tolerance remain unresolved. Here we report that the “hypothalamic dynorphin (Dyn)-spinal κ opioid receptor (KOR)” inhibitory system controls morphine-induced mechanical OIH and analgesic tolerance. We observed that

repeated administration of morphine caused long-term depression of the hypothalamus-to-spinal projecting prodynorphin (HSP^{Pdyn}) neurons (5-day morphine- versus saline-treated mice; saline group, n = 20 cells from four mice; morphine group, n = 33 cells from four mice). Spinal chemogenetic activation of the axon terminals of HSP^{Pdyn} neurons (n = 5-8 mice per group), or spinal administration of Dyn peptide (n = 6 mice per group), rescued the established morphine mechanical OIH and analgesic tolerance. Conversely, spinal silencing/retro-ablation of HSP^{Pdyn} neurons (n = 7-8 mice per group), deletion of the Dyn peptide from HSP^{Pdyn} neurons (n = 5-6 mice per group), or pharmacological blocking of spinal KORs (n = 6 mice per group), all uncovered de novo morphine-resistant mechanical allodynia under neuropathic pain and caused a relapse, or long-lasting persistent mechanical OIH in naive mice. Finally, we found that spinal chemogenetic activation of the axon terminals of HSP^{Pdyn} neurons during repeated morphine treatment, completely prevented the development of morphine mechanical OIH and tolerance (n = 5 mice per group). Targeting the above "hypothalamic Dyn-spinal KOR" axis could, therefore, provide preclinical studies and/or clinical trials a mechanism-based, modality-specific strategy to resolve opioid-induced mechanical forms of OIH and analgesic tolerance.

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Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

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Topic: D.02. Somatosensation – Pain

Support: CIHR Grant PJT-162103

Title: Investigation of the emotional component in the analgesic response associated with the delta opioid receptor.

Authors: *C. BELMAS[1], C. M. CAHILL[2], L. GENDRON[3,4];
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Abstract: Availability of safe and potent treatments for patients suffering from chronic pain remains a major challenge. Such treatments should be optimal on an individual scale while minimizing the occurrence of aggravating consequences to their physical and psychological well-being. It is widely established that opioid receptors remain good targets for treating chronic pain. It is now recognized that treating chronic pain may request to not only focus on sensory aspect of pain but also on its motivational and emotional components. In order to develop effective treatments for chronic pain, it is therefore essential to better understand the role of the opioid receptors on these components of pain. In this project, we aimed to dissociate the sensory

component of pain from the motive-affective pathway and to independently evaluate their specific roles in the analgesic effects mediated by the delta opioid receptor (DOP).
In this study, we use a mouse neuropathic pain model induced by a chronic constriction of the sciatic nerve (CCI model). Using this model, together with a newly developed conditional-DOP knockin mouse strain, we will specifically determine the contribution of DOP in primary afferents as well as in brain centers involved in the motivational and the emotional components of pain. Firstly, we evaluated whether the DOP agonist SNC80 can produce analgesia as well as a conditioned response in the conditioned-place preference (CPP) paradigm. We observed that 14 days after sciatic nerve constriction, CCI mice developed hypersensitivity in the von Frey test. No significant difference was observed in control mice (Sham). In the CPP test, we found that CCI mice developed a preference for the compartment associated with the administration of SNC80 compared to the saline-paired compartment. This effect was not observed in naïve mice nor in sham mice. The specificity of the effect was confirmed by performing the same experiments in DOP knockout mice. As expected, the latter did not behaviorally associate the analgesic effect of SNC80 with the drug-paired compartment. We are currently studying mice expressing DOP solely in primary afferents to isolate the sensory component from the affective components of pain.

Disclosures: **C. Belmas:** None. **C.M. Cahill:** None. **L. Gendron:** None.

Poster

PSTR210. Physiological and Behavioral Impacts of Opioids

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: D.02. Somatosensation – Pain

Support: NIH R37 DA039997

Title: Allosteric Modulation of the μ-Opioid Receptor by Distinct Drug Classes Enhances Opioid Activity in vivo and in vitro

Authors: *B. M. CLEMENTS[1], R. KANDASAMY[3], J. R. TRAYNOR[2];
[1]Pharmacol., [2]Pharmacology, Medicinal Chem., Univ. of Michigan, Ann Arbor, MI;
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Abstract: Opioid therapy remains our most powerful tool for the treatment of pain. However, adverse effects limit its use, including tolerance, abuse, and overdose. Therefore, development of adjuvants to reduce these negative side effects while enhancing analgesia is critical for the continued use of opioids. Positive allosteric modulators (PAMs) interact with agonist-occupied receptors at a location distinct from the orthosteric binding site to increase the affinity and/or activity of the agonist. Utilization of these PAMs at the opioid receptors could lead to increased analgesia after opioid administration while potentially limiting unwanted effects. Two classes of PAMs are known to act at the μ-opioid receptor: the thiazolidines (e.g. BMS-986122) and the

xanthene-diones (e.g. BMS-986187). These structural distinctions suggest different allosteric sites for each class. Therefore, our objective is to determine the extent to which these two classes of PAMs can enhance opioid analgesia *in vivo* and the extent of interaction and degree of synergy *in vitro*.
To determine the enhancement of opioid analgesia, methadone (10 mg/kg, i.p.) or saline was administered to CD-1 mice alongside one of each class of PAM: BMS-986187 or BMS-986122 (10 mg/kg, i.p.). Antinociception was tested using the hot plate assay and/or tail withdrawal assay. Both classes of PAM administered with methadone increased latency to respond over methadone alone, with no response induced by the PAMs themselves. These results show that although these PAMs are structurally distinct, both enhance methadone analgesia to similar extents. However, it is still unclear how these two compounds deliver this enhancement at the μ-opioid receptor.
To test the enhancement of opioid receptor activation by the PAMs *in vitro*, G protein activation and beta-arrestin recruitment downstream of the μ-opioid receptor were tested using the GTPγ^{35}S and β-galactosidase enzyme complementation assays, respectively. PAMs were tested individually and together at various concentrations of DAMGO in CHO cells expressing the human μ-opioid receptor. Individually, PAMs increased activation of the receptor by DAMGO in both assays. A combination of PAMs increased activation, but in an additive, not synergistic, manner. These results suggest that the two classes of PAMs act via a shared mechanism and/or shared binding site. Future studies will examine potential binding sites on the μ-opioid receptor, and how this *in vitro* additive response translates to more complex systems *in vivo*. Supported by R37 DA039997.

Disclosures: B.M. Clements: None. **R. Kandasamy:** None. **J.R. Traynor:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.01/CC3

Topic: D.03. Somatosensation – Touch

Support: NIH Grant NS129982
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Title: Responsiveness of putative inhibitory and excitatory neurons in area 3b hand representation of owl monkeys and squirrel monkeys to stimuli within and beyond the excitatory receptive field

Authors: *J. L. REED, H. X. QI, J. H. KAAS;
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Abstract: Our long-term goal is to determine the sources of complex receptive field (RF) organization of neurons in the hand representation of cortical area 3b. Previous studies reveal

primarily suppressive surrounds when a site inside the RF is stimulated alongside a site outside the RF, including the opposite hand. Recordings were from layer 3 neurons, which receive primary input from layer 4, as well as longer intrinsic connections likely responsible for inhibitory surrounds related to the opposite hand. First, we evaluate regular spiking units (RSU), putative excitatory neurons, and fast spiking units (FSU), putative inhibitory neurons, in response to surround stimulation. Inhibitory neurons are expected to be excited by surround stimuli within the same hand as well as on the opposite hand. Second, we ask what happens when some of the driving sensory input is lost due to spinal cord dorsal column lesion. Changes at the targets of the lesion would affect both excitatory and inhibitory neurons. We used multielectrode arrays to record from layer 3 of area 3b in squirrel monkeys (*Saimiri boliviensis*, *S. sciureus*) and owl monkeys (*Aotus trivirgatus*) with partial recoveries from dorsal column lesion and those without lesions. Adult monkeys were subjects in control (n = 5, 2 female) and lesion (n = 7, 4 female) groups. Spike signals were acquired while computer-controlled indentation stimuli were delivered to selected hand sites, such that response to single-site stimulation was compared to dual stimuli in the RF center and surround. Spike signals were processed to estimate waveform duration. Inhibition of firing relative to baseline rarely occurred, while suppression of firing relative to a single stimulus in the RF was common. FSU recordings had more inhibition (69/930) than RSU (11/725) recordings (ANOVA $F = 26.754$, $p < 0.001$). When sites on one or both hands were stimulated simultaneously or asynchronously, both FSU and RSU types showed response magnitude suppression, no change, or facilitation. Facilitation was rare, and in a subset of control recordings, 10 times as many FSU (30/382) as RSU (3/382) recordings showed significant facilitation. Similar proportions of FSU and RSU recordings did not change firing rate to a stimulus outside the RF (38%, 37%, respectively). Greater proportions of RSU recordings were suppressed (60%, 65/108) than FSU (50%, 138/274) in control cases. We expected that only inhibitory neurons would be facilitated in response to simultaneous or asynchronous stimulation of the two hands, but we found 3 exceptions to date. Further characterizations are planned using temporary inactivation to identify the sources of the receptive field surrounds in area 3b.

Disclosures: **J.L. Reed:** None. **H.X. Qi:** None. **J.H. Kaas:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.02/CC4

Topic: D.03. Somatosensation – Touch

Support: NIH T32 NS115705-01

Title: Vibrotactile tuning of inhibitory neuron subtypes in the mouse forepaw primary somatosensory cortex

Authors: *M. T. DUHAIN[1], Y. YUN[2], K. H. WANG[1], M. GOMEZ-RAMIREZ[2];
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Abstract: The hands and forepaws are unique organs which allow some mammals to both perceive and manipulate objects in their environment. Crucial to this process of haptic perception is the representation of individual tactile features such as vibration frequency. Vibrotactile signals from the paws are represented in somatotopically aligned regions of the forelimb primary somatosensory cortex (fS1) through the elevated firing rate of frequency-selective neural ensembles. The response properties of these neurons are dependent on both the frequency and amplitude components of the vibrotactile stimuli. Inhibitory GABAergic neurons are known to respond to vibrotactile stimuli, have broader frequency tuning curves than excitatory neurons, and have strong pairwise correlations with other frequency tuned neurons. However, the subtype identity of these inhibitory frequency-selective neurons in fS1, and their unique contributions to population-level frequency representation remain relatively unknown. Here, we performed in-vivo two photon calcium imaging of fS1 in awake-behaving mice to determine the role of two major interneuron subtypes, parvalbumin- (PV) and somatostatin-expressing (SOM) cells, in population coding of tactile vibration. We virally expressed the green fluorescent calcium sensor (GCaMP8m) in neurons of fS1 in transgenic mice that expressed tdTomato in PV cells or SOM cells (separate cohorts). We found that the majority of PV and SOM neurons in fS1 respond to at least one frequency tested, with either significant increases or decreases from baseline fluorescence. We then quantified the tuning parameters (peak fluorescence and curve width), tuning stability (1-8 weeks), and investigated how pairwise correlations change as a function of tuning similarity and cell-cell distance. We additionally tested learning-dependent changes in fS1 frequency representation with a reward association paradigm in fluid restricted animals that paired rewards to specific frequency ranges. Overall, our work provides evidence for how somatosensory circuits represent tactile features within genetically specific inhibitory networks and how these networks are shaped overtime through learning.

Disclosures: M.T. Duhain: None. **Y. Yun:** None. **K.H. Wang:** None. **M. Gomez-Ramirez:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.03/CC5

Topic: D.03. Somatosensation – Touch

Support: JHU/APL Internal Support

Title: Creating complex tactile sensations in the phantom hand using noninvasive stimulation

Authors: *L. OSBORN, C. MORAN, M. HIMMTANN, R. VENKATASUBRAMANIAN, R. ARMIGER;
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Abstract: Touch feedback to the missing limb after amputation can help improve function for prosthesis users. Both invasive and noninvasive techniques have been demonstrated for exciting peripheral nerve pathways to elicit sensations of pressure, vibration, texture, tingling, pain, and most recently temperature. The goal of this study was to quantify the regions of phantom hand activation and the perceived modality of sensation as a result of noninvasive stimulation to targeted regions of the residual limb. In this study, five participants with upper limb amputation underwent noninvasive stimulation of the residual limb to map tactile sensations emanating from the phantom hand. We provided targeted electrical, mechanical, or thermal stimulation to activate underlying peripheral nerves in the residual limb that, when activated, resulted in touch sensations in the missing hand. Electrical stimulation was delivered using targeted transcutaneous electrical nerve stimulation (tTENS) with amplitudes of up to 400 mA and frequency of up to 4 Hz. Mechanical stimulation was delivered using a 1 cm plastic probe. Thermal stimulation was delivered using a thermoelectric cooling device with a target cold-side temperature between 11-16 ^{o}C. Participants reported the perceived location and quality of the stimulation. All experiments were approved by the Johns Hopkins Medicine Institutional Review Boards and all participants provided written informed consent. We found that sensations of pressure and temperature could be induced in the phantom hand of all participants using noninvasive stimulation. Targeted stimulation sites were found that generated tactile sensations only in the phantom hand and not in the residual limb. Interestingly, regions of thermal sensation in the phantom hand did not necessarily overlap with mechanical or tTENS regions. Thermal stimulation sites on the residual limb also did not always overlap with mechanical or tTENS sites. This non-overlapping modality-specific phenomenon was observed in four of the five participants. When measured over a period of three years in one participant, stimulation sites remained stable in that noninvasive stimulation resulted in activation of similar regions of the phantom hand. This work has implications for enabling future prosthetic limbs to provide more complex sensations of touch and further enhance sensorimotor function after amputation.

Disclosures: L. Osborn: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US11227988B1, US11532778B2, US18/071,789. **C. Moran:** None. **M. Himmtann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US11227988B1, US11532778B2, US18/071,789. **R. Venkatasubramanian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US11227988B1, US11532778B2, US18/071,789. **R. Armiger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US11227988B1, US11532778B2, US18/071,789.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.04/CC6

Topic: D.03. Somatosensation – Touch

Support: Alchemist Brain to X (B2X) Project funded by Ministry of Trade, Industry and Energy (20012355, NTIS: 1415181023)

Title: Two distinct functional roles of high-gamma activity in primary somatosensory cortex

Authors: *S. RYUN, J. KIM, C. CHUNG;
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Abstract: High-gamma activity is well-known and robust neurophysiological activity recorded from electrocorticography (ECoG). In vision study, researchers suggested that high and low components of gamma activities have different origins in the primary visual cortex. However, it is unclear whether such segregation can also be observed in the primary somatosensory cortex (S1). Furthermore, the functional roles of S1 high-gamma activities are still controversial. Here, we investigate their roles by measuring neuronal activity during vibrotactile and texture stimuli. Eight patients with drug-resistant epilepsy was included in this study. We delivered complex vibrotactile stimuli (flutter + vibration) to the index finger during ECoG recording. For three patients, they performed texture stimulation task. In this task, the normal force applied to the index finger had two conditions: a light (approximately 20 g wt) condition and a heavy (90 g wt) condition. We found that high-gamma activity for complex vibrotactile stimuli in S1 was dependent on the ratio of low to high mechanical frequencies, and the pattern of high-gamma activity was a mixture of the neuronal activities under low and high mechanical frequency conditions. In texture stimulation, we found that the low component of the high-gamma activity (50 to 70 Hz) was dependent on the strength (normal force) of mechanical stimulus. Additionally, we found that texture types can be classified using the power levels of high component of high-gamma activity (70 to 150 Hz). In this study, we suggest that S1 performs some degree of independent processing for low and high frequency stimuli (flutter and vibration). Additionally, we suggest that 70 to 150 Hz high-gamma activity represents the detail of surface geometry interacting with the fingerprint, while 50 to 70 Hz high-gamma activity represents the intensity of tactile sensation.

Disclosures: S. Ryun: None. **J. Kim:** None. **C. Chung:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.05/CC7

Topic: D.03. Somatosensation – Touch

Title: Multimodal sensory methods of spatial navigation in naked mole rats

Authors: *P. SHENG[1], R. SCHWARK[2], I. ABDUS-SABOOR[3];
[1]Biol. Sci., [2]Columbia Univ., Columbia Univ., New York, NY; [3]Columbia Univ., Univ. of Pennsylvania, NEW YORK, NY

Abstract: Touch is an important sense in humans; through touch we perceive both positive and negative stimuli, as well as gain information about the environment. Here, we present the naked mole-rat as an excellent model for touch-based environmental navigation. Naked mole-rats are a eusocial rodent species that diverged from the laboratory mouse more than 30 million years ago. Naked mole-rats live in intricate underground colonies that can extend to up to three kilometers in length and compose of over 300 animals; despite being totally blind, they are able to navigate these complex, interconnected spaces. Although mole-rats have evolved a complex somatosensory cortex and communicate through intricate vocalizations, comparatively little is known about how these sensory stimuli are synthesized to contribute to spatial navigation. Furthermore, there is a gap in our understanding of how to consistently generate motivated behavior in naked mole-rats, as the eusocial nature of the species makes has it made them difficult to directly train. Here, we describe experiments utilizing two chamber place preference and sensory-discrimination based spatial navigation to demonstrate methods to generate motivated behaviors in individual mole-rats and methods to determine how non-visual cues are used to navigate their environments. Several different stimuli were tested to determine place preference in mole-rats, including temperature, food, home cage bedding, and familiar/unfamiliar mole-rats, quantifying the proportion of time animals spent in the test chamber relative to the adjacent empty chamber. Animals tested show robust preference towards both familiar (0.88 ± 0.15) and unfamiliar animals (0.82±0.15). Animals showed moderate preference towards home cage bedding (0.73±0.14) and high temperature environments (0.79±0.16). This led us to choose familiar mole-rat as a target to test mole-rat behavior in a texture-dependent spatial memory paradigm, in which the mole-rats were required to use environmental textural cues to navigate a maze. Our results showed that mole-rats provided with textural cues were able to successfully navigate the behavioral paradigm more successfully than mole-rats without cues (0.69 ± 0.18 vs 0.46 ± 0.13). Overall, these preliminary data demonstrate the importance of touch for mole-rat spatial navigation and provide a guideline for designing methods to generate motivated behavior in future experiments. Next, it will be important to further elucidate the peripheral and central neural mechanisms that mole-rats use to navigate a world without sight.

Disclosures: **P. Sheng:** None. **R. Schwark:** None. **I. Abdus-Saboor:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.06/CC8

Topic: D.03. Somatosensation – Touch

Title: Neural interactions of motion and proprioception in touch

Authors: *H. AHUJA[1], M. GOMEZ-RAMIREZ[2];
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Abstract: Interpreting tactile information is critical for handling objects and operating tools. During goal-directed actions, such as grasping a glass that is sliding out of one's hand (motion from the palm toward the fingertips, relative to the hand) or pulling a leash to the right when a dog is running to the left relative to the body, the brain calculates the resulting tactile motion direction from the tactile motion on the hand and the posture of the arm. Understanding how a change in posture alters our perception of tactile motion is central to understanding how the brain generates signals that enable goal-directed actions. However, the theoretical framework and neural mechanisms underlying these computations remain to be elucidated. Previous studies have shown that the brain derives tactile motion representations by integrating object cues impinging on the skin (e.g., velocity, force, direction), a mechanism known as the full vector average model. This model was derived from studies in which the hand was placed in the same posture. However, our recent work in humans showed that the perception of tactile motion on the hand is modulated by the proprioceptive state of the arm. Here, we extend our work by investigating the neural interaction of posture and tactile motion in an anesthetized monkey preparation. We recorded from the primary somatosensory cortex (S1) while the monkey was presented with tactile stimuli of different motion directions on the hand. Tactile stimuli were presented with the hand in a pronated or supinated position. Preliminary analyses showed motion tuning curves in S1, as shown in previous studies. However, we did not observe that proprioception modulated motion tuning functions. These preliminary results suggest that proprioceptive information is integrated with tactile motion information in downstream brain regions, or that integration is controlled by a mechanism that was inhibited by the anesthetized preparation of our experiment.

Disclosures: H. Ahuja: None. **M. Gomez-Ramirez:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR211.07/CC9

Topic: D.03. Somatosensation – Touch

Support: NIH Grant NS130475-01

Title: Studying object recognition with a computer-controlled haptic display

Authors: *R. L. MILLER, D. L. SHEINBERG;
Brown Univ., Providence, RI

Abstract: While considerable progress has been made in our understanding of how visual information about shape can access higher brain structures involved in recognizing objects and

directing action, understanding of the circuits by which this information is derived by touch is not as advanced. This is an important question, because we still do not know how objects are represented in the brain, and in particular, how a unified representation of these objects can be derived from multiple senses. Better tools are needed for probing how haptically-presented shapes are represented in the brain. In contrast to the presentation of visual stimuli, presenting haptic stimuli is quite challenging particularly because physical objects need to be constructed and presented to a research subject. For this reason, most haptic object recognition research has utilized a relatively small set of shapes which are repeatedly presented, often with manual help of an assistant. To understand how the brain builds up a de novo representation of shapes, we need the ability to present a large number of novel objects. We have built a device, which we're terming a "haptic display", composed of a large number of pneumatic pistons. Similar to pixels on a visual display, these pistons are independently-controlled and the haptic display can be quickly (~1 s) reconfigured on a trial-by-trial basis to present an effectively limitless number of novel haptic shape stimuli. Additionally, the device is MRI-compatible and safe for use with both human and non-human primates. Tests have been completed to demonstrate the discriminability of unique shapes by human participants. This provides a key starting point for the understanding of how the brain builds a representation of truly novel shapes.

Disclosures: R.L. Miller: None. **D.L. Sheinberg:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Program #/Poster #: PSTR211.08/CC10

Topic: D.03. Somatosensation – Touch

Support: NIH-R21NS130475
National Science Foundation Graduate Research Fellowship

Title: A link between grasp planning and visual-haptic shape recognition in the non-human primate

Authors: *A. WAITE, R. MILLER, D. SHEINBERG;
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Abstract: Real world objects are inherently multimodal and as such are represented by multiple sense modalities. Although routes for object recognition can occur through the different senses, how the brain integrates this information is not well understood. To explore how object features are represented and compared across the visual and haptic domains, we developed a visual-haptic object recognition paradigm where non-human primates (NHPs) match object shapes presented either visually or physically. In the current study, an NHP was trained to select which of two physical 3D shapes presented out of sight matched a sample shape presented visually. In principle, this task could be solved by creating independent shape codes, one visual and one

haptic, and comparing these. An alternative approach would be to use information from one modality to guide acquisition from the other to gather evidence for or against a match. We found evidence in support of the second strategy by analyzing the monkey's grasp behavior during the reach period, before contacting either physical object. Using markerless pose estimation, we characterized the dynamic grasp configuration from streamed video data, and found that grasp pose could be used as a predictor of the visual stimulus to be matched. Because these data were taken from the time period before either object was touched, shape information, obtained by vision, appears to have been rapidly converted into the haptic domain, prior to any physical haptic exploration. Furthermore, we found that dynamic adjustments of this grasp pose and our ability to discriminate between the visual stimulus using the grasp data correlated with the NHP's performance in the behavioral matching task. Together, these data suggest that the stimulus representation from the visual domain is integrated, recognized, and translated into the haptic domain, directly influencing subsequent haptic experience. Future studies will investigate the dynamic adjustments of grasp planning during visual-haptic recognition and the neural interactions between these modalities during natural behavior.

Disclosures: **A. Waite:** None. **R. Miller:** None. **D. Sheinberg:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Program #/Poster #: PSTR211.09/CC11

Topic: D.03. Somatosensation – Touch

Support: Johns Hopkins University Applied Physics Laboratory Internal Funding

Title: Expectation of artificial tactile feedback modulates somatosensory cortex response

Authors: L. OSBORN[1], *B. CHRISTIE[2], A. CREGO[1], D. P. MCMULLEN[3], B. WESTER[1], P. A. CELNIK[4], M. FIFER[1], F. TENORE[1];
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Abstract: Providing sensory feedback to prosthesis users or individuals using a brain-computer interface could help improve function; however, it is unclear how integration of artificial tactile stimulation by a user affects neural activity in a task-relevant scenario. The goal of this study was to explore evoked neural activity as an indicator of stimulation utility.
A human participant with a C5/C6 spinal cord injury had microelectrode arrays implanted in the motor and somatosensory cortices of both hemispheres of the brain. We provided either vibrotactile stimulation to the hand or intracortical microstimulation (ICMS) to the primary somatosensory cortex and characterized the cortical responses evoked by stimulation. In the experiment, a virtual sphere made contact with the 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.
We observed that the somatosensory cortex generated evoked responses, as measured from the spike band power (300-1000 Hz), even in the absence of tactile stimulation after the conditioning period. The average neural signal amplitude, measured from a 100 ms period after the stimulation onset, did not significantly change over the 360 trials, indicating long-term conditioning was not occurring. Rather, the somatosensory cortex response was affected in that the average z-score of the neural amplitude during skin vibration dropout trials was larger (median: 0.62) than in the visual baseline condition (median: 0.09); however, the evoked activity was still not as large as when skin vibration was delivered (median: 1.45). We observed a similar evoked response during ICMS delivery, although at a delayed point in time due to electrical noise artifact, during stimulation dropout trials.
We used the Rescorla-Wagner model to evaluate potential learning effects between the tactile stimulation (conditioned stimulus) and the virtual hand being touched (unconditioned stimulus). However, neither the associative strength nor the change in strength between tactile and visual input explained the neural signal amplitude, suggesting that this conditioning model was not representative of the observed effects and alternative models would need to be explored in future research.
This work has implications for understanding the role of artificial sensory feedback in the nervous system, in that the neural response could provide an objective marker on the utility of artificial sensations.

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Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Topic: D.03. Somatosensation – Touch

Support: DoD SCIRP SC180308
VAMR 5I01RX002654

Title: Mechanical indentation activates human cortical grasp network along with primary somatosensory cortex

Authors: *B. HUTCHISON[1,3], J. KRALL[1,3], A. KETTING-OLIVIER[1,3], P. BHAT[1,3], W. D. MEMBERG[1,3], R. F. KIRSCH[1,3,2], J. P. MILLER[4,3,1,2], E. L. GRACZYK[1,3,2], A. B. AJIBOYE[1,3,2];
[1]Biomed. Engin., [2]Sch. of Med., Case Western Reserve Univ., Cleveland, OH; [3]FES Ctr. of Excellence, Rehab. R&D Service, Louis Stokes Cleveland Dept. of Veterans Affairs Med. Ctr.,

Cleveland, OH; [4]Dept. of Neurosurgery, Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: Touch is essential for interacting with objects—indicating when contact occurs and for evaluating the force applied. Prior work in non-human primates (NHP) indicates the primary somatosensory cortex (S1) and primary motor cortex (M1) modulate to tactile stimuli. Additional work in NHP suggests that grasping information is strongly represented in the inferior frontal gyrus (IFG) and anterior intraparietal area (AIP), which along with M1, traditionally comprise the human grasp network. The encoding of tactile information in IFG and AIP has yet to be elucidated and is the focus of this research.
A participant with AIS-B C3/C4 spinal cord injury (SCI) enrolled in the Reconnecting the Hand and Arm to the Brain (ReHAB) clinical trial, which has the overall goal of restoring motor and somatosensory function through functional electrical stimulation and a brain-machine interface. As part of the clinical trial, the participant was implanted with six 64-channel microelectrode arrays in cortical regions involved in grasping: S1, M1, AIP, and IFG. Tactile indentation was applied to the index, middle, ring, and pinky fingertips, with non-stimulation trials randomly interspersed, using a tactile linear actuator with a trapezoidal indentation profile. Indentation depth was 2.5 mm, onset and offset rates were 40 mm/sec, duration was 1 second, and diameter was 5 mm. Cortical response in S1, M1, AIP, and IFG were recorded. The mean spike band power (250-5000 Hz) in a 100 ms window after stimulation onset was the neural feature outcome.
Indentation location modulated neural activity on 74/128 electrodes in S1, 27/64 electrodes in AIP, and 22/64 electrodes in IFG (ANOVA, $p<0.05$). Using a cross-validated linear discriminant analysis (LDA) model, indentation location could be discriminated using the mean spike band power signal from modulating electrodes in S1 (54% performance, chance = 20%), AIP (31%), IFG (49%), and all regions combined (58%).
Surprisingly, AIP and IFG showed significant modulation to indentation location. Including neural activity from AIP and IFG with S1 slightly improved the performance of an LDA model classifying the stimulation location. These preliminary results indicate tactile information is encoded in AIP and IFG, expanding our understanding of the human cortical grasp network. The insights about how the brain represents tactile information in the grasp network can be used to help restore of somatosensory and motor function to people with SCI.

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Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Program #/Poster #: PSTR211.11/CC13

Topic: D.03. Somatosensation – Touch

Support: We appreciate the support of the WPI-IRCN Human fMRI Core, the University of Tokyo Institutes for Advanced Studies.

Title: Neural representations of subjective tactile feelings for surface textures of common materials

Authors: *S. KUROKI[1], T. YOKOSAKA[1], T. MATSUDA[2], T. HORIKAWA[1];
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Abstract: Tactile exploration of surface textures helps us understand material properties and obtain subjective tactile feelings. While psychological research has extensively studied texture perception concerning typical tactile attributes like “hardness” and “roughness” using a wide range of surface textures, studies investigating the neural mechanisms underlying human tactile texture perception, particularly with neuroimaging techniques, have primarily focused on artificial textures such as embossed dot patterns and gratings or limited sets of naturalistic textures. Consequently, the neural representations of tactile texture perception associated with subjective tactile feelings in natural environments remain unexplored. To fill this gap, we investigated the relationship between brain activity and subjective tactile experiences using functional magnetic resonance imaging (fMRI) and a rich set of 27 tactile textures consisting of common materials, which were annotated with ratings of six tactile attributes. We measured fMRI activity while subjects repeatedly stroked the surface of the texture stimuli with their right hand. We then performed classification decoding analysis on all pairs of touched stimuli to search for brain areas showing content-specific patterns of tactile stimuli. The analysis showed that mean classification accuracies averaged across pairs were significantly higher in multiple brain areas, including the primary and secondary somatosensory cortices (S1 and S2), the motor cortex, the temporal-parietal-occipital junction (TPOJ), and the insular cortex, indicating that diverse brain areas are involved in tactile texture perception. We further explore the relationship between the content specificity and subjective ratings in each brain area by computing correlations between the differences in tactile ratings and fMRI pattern correlations for each stimulus pair. The results showed that distinct ratings correlated with fMRI pattern correlations in different brain areas. For example, “roughness” and “bumpiness” showed a high correlation in S1, whereas “dryness” showed a strong correlation in S2. However, TPOJ showed no significant correlation with any tactile ratings, indicating that other factors contribute to content-specific activity patterns in this area. The present study demonstrates that differences in a rich set of tactile textures can be detected through fMRI responses elicited by touch and that distinct brain areas represent different aspects of subjective tactile feelings. These findings provide a foundation for further investigations into the neural underpinnings of human tactile perception.

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Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Program #/Poster #: PSTR211.12

Topic: D.03. Somatosensation – Touch

Support: MSCA IF 101024523

Title: Encoding of familiar and unexpected tactile stimuli in the barrel cortex of the awake mouse

Authors: *M. PANNIELLO[1], M. BRONDI[2], T. FELLIN[3];
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Abstract: The brain builds models of the world based on sensory regularities, allowing us to react whenever sensory expectations are not met by experience. In this context, neocortical neurons show decreasing activity in response to repetitive sensory stimuli (sensory adaptation), and enhanced activity when unexpected inputs arrive (deviant detection). These mechanisms have been thoroughly studied in the somatosensory system of anaesthetized rodents, but if and how they emerge in the awake somatosensory cortex is less clear. We used a novel sensory stimulation approach to present sequences of familiar or unexpected stimuli to the whiskers of the awake mouse, while carrying out two-photon calcium imaging of neuronal activity in layer 2/3 and 4 of barrel cortex. We found a broad range of sensitivity toward the sensory feature tested, as well as response adaptation followed by enhanced responses whenever repetitive stimuli were followed by unexpected ones. We plan to apply information theory to study how the sensory information content of individual neurons is modulated by unexpected stimuli, as well as how population dynamics change with the level of stimulus familiarity. Overall, our data will provide novel insights into the cortical mechanisms at the basis of the predictive coding framework in the mouse somatosensory system.

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Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Topic: D.03. Somatosensation – Touch

Support: NIH F32 NRSA FHD103481A

Title: Effects of natural vs man-made environmental stimuli on the development of stimulus tuning in the rat somatosensory cortex

Authors: *C. S. BRESEE[1], C. R. PINEDA[2], F. F. GOMEZ[2], L. A. KRUBITZER[2];
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Abstract: The environment an organism develops in highly impacts cortical organization, neural response properties, and sensory mediated behavior. However, most experiments investigating these processes involve sensory deprivation, or enrichment using relatively small enclosures. These experiments also commonly use man-made objects, which have been shown to differ significantly from natural objects in terms of complexity of scene statistics. Here we test the effect of rearing rats in a semi-natural environment on the tuning properties of adult S1 cortical neurons. First, rats are born and reared in large highly enriched, semi-natural conditions which contain multiple natural objects and climbing surfaces (field pens, FP). Second, differences in available textural stimuli between natural and lab environments are visually quantified using gray-level nonuniformity statistics. Finally, we record single-unit neural responses from adult somatosensory cortex while applying stimuli spanning the range of all textures present in the environment. We predict that neurons in S1 of enriched rats will respond more selectively than those of lab-reared animals. Neural responses in S1 to aperiodic textured stimuli are complex for both FP and lab rats. We found neurons tuned to both stimulus onset and offset, as well as neurons that show a preference for specific textures. More data is necessary to draw conclusions regarding finer or broader stimulus tuning in FP vs lab rats, and experiments are ongoing.

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Poster

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Topic: D.03. Somatosensation – Touch

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DAPA-ADD-915027201

Title: Parietal lobe interconnectivity indicates tactile working memory

Authors: *D. LEE[1], J. KIM[2], C. CHUNG[3];
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Abstract: Tactile working memory refers to the ability to retain and process tactile information. In the human brain, it's generally understood that tactile working memory involves the connectivity of several brain regions, including the somatosensory cortex and the prefrontal cortex. Recently the parietal lobe also is reported to play a significant role in tactile working memory. However, little is known about the interconnectivity of the parietal lobe in tactile working memory, although the parietal lobe integrates sensory information and plans movements. To elucidate the interconnectivity of the parietal lobe in tactile working memory, we utilized a vibrotactile discrimination task with electrocorticography (ECoG). We recruited 13

patients with medically intractable epilepsy to perform two tasks. In the discrimination task, subjects were asked to compare two sequentially presented vibrotactile stimuli and identify which stimulus had a higher frequency. For the detection task, subjects were not required to compare two sequential vibrotactile stimuli; rather, they were asked to respond when they perceived the second stimulus. Given the ECoG signal's ability to measure connectivity and causality between areas, we employed partial directed coherence (PDC) to assess brain connectivity during the tactile information retention period. Our observations revealed that the connectivity between the prefrontal cortex and the somatosensory cortex did not significantly increase during the tactile information retention period. However, the connectivity between the inferior parietal lobule, superior parietal lobule, and the somatosensory cortex did significantly increase. This finding suggests that when a person maintains tactile working memory, the tactile stimulus is analyzed through inter-transmission within the parietal lobe before being transmitted to other brain regions for decision-making.

Disclosures: D. Lee: None. **J. Kim:** None. **C. Chung:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.15/CC16

Topic: D.03. Somatosensation – Touch

Support: FONDATION RECHERCHE MEDICALE FRANCAISE
Fondation FondaMental
Prix Marcel Dassault
INSERM
GIS-Autisme

Title: Perceptual and neocortical alterations in a mouse model of autism during a translational tactile decision-making task

Authors: *O. SEMELIDOU, A. CORNIER, T. GAUVRIT, M. GINGER, A. FRICK; INSERM U1215, BORDEAUX CEDEX, France

Abstract: Altered sensory experience is one of the core features of autism spectrum disorder (ASD), a neurodevelopmental disorder characterized by alterations in social communication and repetitive behaviors. Sensory alterations affect approximately 90% of autistic individuals, exert a strong negative influence on day-to-day life and contribute to the development of other core symptoms and medical conditions that co-occur with autism. Touch is particularly crucial during development, with a strong role in defining the self, exploring the environment, and building the foundations of social interactions. Tactile alterations in autistic individuals include differences in stimulus perception (perceptual sensitivity), reactions to perceive stimuli (affective reactivity to sensory input), and sensory-related neural excitability. Better understanding and synthetizing

these features is crucial in characterizing altered tactile experience in autism and can provide insight into the development of other core features. In this study, we aimed to **characterize tactile alterations in Fmr1$^{-/y}$ mice, a genetic mouse model of autism**. To study perception **at the behavioral and neuronal level**, we developed a novel perceptual decision-making task based on vibrotactile stimuli that can be can be combined with measurements of neuronal activity and translated in human studies. Our findings show slower acquisition of the task for Fmr1$^{-/y}$ mice and recapitulate the alterations observed in autistic individuals, with higher detection thresholds leading to perceptual hyposensitivity of vibrotactile stimuli of differing intensities in the flutter range. Notably, increased inter-individual variability in the detection thresholds was observed in the responses of Fmr1$^{-/y}$ mice, as was previously reported in autistic individuals. Parallel *in vivo* calcium imaging recordings of neuronal activity of excitatory and inhibitory neurons in the somatosensory cortex revealed that different patterns of activity in the Fmr1$^{-/y}$ mice underlie perceptual alterations. Aiming to examine changes in affective reactivity to sensory input, a parallel approach was adopted, also showing hypo-reactivity in Fmr1$^{-/y}$ mice. These results help us expand our knowledge of tactile alterations in autism and contribute in our goal to develop objective biomarkers that can be used to test mechanism-based treatments in preclinical models and can also be translated in human studies.

Disclosures: O. Semelidou: None. **A. Cornier:** None. **T. Gauvrit:** None. **M. Ginger:** None. **A. Frick:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.16/CC17

Topic: D.03. Somatosensation – Touch

Support: SFARI
Marcel Dassault
Fondation FondaMental
INSERM

Title: Neocortical noise drives atypical sensory response variability in autism

Authors: *T. GAUVRIT[1], A. A. BHASKARAN[2], Y. VYAS[1], G. BONY[1], M. GINGER[1], A. FRICK[1];
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social impairments and repetitive behaviors. In addition, sensory symptoms are almost universally expressed in autistic individuals, but have only recently been included as a core diagnostic criterion in the DSM-5. Since atypical sensory responses have a strong

neurobiological basis, they can be measured using imaging or electrophysiological approaches - both in humans and mice. Clinical studies have shown that sensory responses are characterized by an increased variability or noise in the neuronal responses, but a mechanistic understanding of these features is still lacking. Here, we probed the role of neuronal noise and response variability for atypical sensory information processing, and their potential use as translational biomarkers and targetable mechanism, in the *Fmr1* knockout (*Fmr1*KO) mouse model of autism and fragile X syndrome. To address these questions, we recorded, *in vivo*, the responses of individual layer 2/3 pyramidal neurons of the somatosensory cortex to tactile stimulation of the hind paw in anesthetized mice. In addition, we measured the spontaneous activity and intrinsic properties of these neurons, enabling us to link alterations in neuronal excitability, noise, and response variability at the neuronal level. Our results reveal overall enhanced tactile stimulus evoked responses in *Fmr1*KO mice compared to their WT littermates, as well as an increased trial-by-trial variability of these responses to repetitions of the same stimulus. In addition, *Fmr1*KO mice presented greater baseline fluctuations of the membrane potential together with a higher power of periodic components (oscillations) and altered up-down-state dynamics. Importantly, we uncovered a strong correlation between these noise parameters, increased variability and atypical sensory information processing. These findings point to the baseline resting state of the network as a crucial contributor to sensory response variability. We provide a novel preclinical framework for understanding the sources of endogenous noise and its contribution to core symptoms in autism, and for testing the functional consequences for mechanism-based manipulation of this noise. We are currently expanding this work by investigating the impact of the imbalance states and neuronal noise on sensory information processing at the neocortical circuit level.

Disclosures: **T. Gauvrit:** None. **A.A. Bhaskaran:** None. **Y. Vyas:** None. **G. Bony:** None. **M. Ginger:** None. **A. Frick:** None.

Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR211.17/CC18

Topic: D.03. Somatosensation – Touch

Support: MSCA-IF grant 101059348

Title: Attenuation of self-generated somatosensory responses: A magnetoencephalography investigation

Authors: *X. JOB[1], L. M. ANDERSEN[2], M. VINDING[3], N. ČEMELJIć[1], D. LUNDQVIST[1], K. KILTENI[4];
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Abstract: Current motor control theories propose that the brain predicts and attenuates the somatosensory consequences of actions, referred to as somatosensory attenuation. Predicting the sensory consequences of our own actions requires temporally dynamic neural mechanisms that modulate the perception of our own touch with millisecond level precision. However, little is known about how these mechanisms operate in the brain, given that few studies have used neuroimaging methods with high temporal resolution in combination with well-matched control conditions. In this study, we employed magnetoencephalography (MEG) to investigate the neural correlates of somatosensory attenuation. MEG data were recorded from twenty-four participants during a 'self-touch' condition: participants pressed with their right index finger on a lever placed directly above, but not in contact with, their left index finger which triggered a tactile stimulus on their left index finger. In the 'external touch' condition, identical tactile stimuli were delivered to the left index finger automatically. A third 'spatially misaligned' condition was identical to the self-touch condition, except that the hands were spatially separated by 25 cm, thus controlling for simultaneous touch on the active hand, temporal predictability, attentional demands, and dual-task requirements - all being potential confounds when comparing external to self-touch conditions. Finally, participants performed a behavioural force discrimination task that assessed the perceived intensity of touch in the above-mentioned conditions. The behavioural results showed a significant reduction in the perceived intensity of self-touch compared to external and spatially misaligned touch. Somatosensory responses as early as 40ms post-stimulus were reduced in the self-touch condition compared to the external and spatially misaligned conditions, while later responses (~100 ms) did not significantly differ from the spatially misaligned condition. This suggests that self-touch is attenuated at early processing stages in the primary somatosensory cortex, while later modulations may reflect processes that operate to suppress responses in addition to predictive attenuation. Furthermore, pre-stimulus changes in the power of alpha, beta and gamma oscillations were observed between the self-touch and the external and spatially misaligned conditions, indicating that the prediction of self-touch may also be underpinned by changes in the pre-stimulus power of neural oscillations. These results provide novel insights into the temporal dynamics of the neural mechanisms underlying somatosensory attenuation.

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Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Topic: D.03. Somatosensation – Touch

Support: NIH R01NS121073
NIH UG3MH126868
Hock E. Tan and K. Lisa Yang Center for Autism Research

Title: Circuit specific fMRI of sensory network function in rodents and primates

Authors: *Y. JIANG[1], Y. KE[2], S. CHUNG[2], B. STALLINGS[2], H. DE VALMONT[3], A. Y. DONG[2], A. JASANOFF[2];
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Abstract: The brain consists of a complex network of interconnected regions that work together to process information and generate behavior. Studying how these regions communicate reveals how information flows and transforms during perception, cognition, and behavior. We studied interregional communication in the brain by applying a genetically encoded probe called NOSTIC in anesthetized rats and awake marmosets. NOSTIC transduces intracellular calcium activity into hemodynamic responses from neural circuit elements that express the probe; the hemodynamic responses can be detected at a brain-wide scale by functional neuroimaging techniques. We used a retrogradely transported viral vector to selectively target NOSTIC expression to neurons that project to viral injection sites. We then performed functional magnetic resonance imaging (fMRI) in the presence and absence of a NOSTIC-specific inhibitor to measure information flow over the labeled projections in the rodent somatosensory system and the primate visual system, allowing patterns of input to be characterized and related to population activity throughout the brain. We discovered that sensory projections display tuning properties distinct from their source and target regions, indicating the importance of local processing in transforming information prior to generating output. We also identified distinct stimulus-dependent response properties among feedforward and feedback projections relating different nodes in the rodent and primate sensory hierarchies. Finally, we determined the extent to which popular linear models could account for population activity in sensory brain regions in terms of their inputs. These studies reveal fundamental aspects of integrated network function in mammalian brains and can inform the interpretation of systems-level neurophysiological data from animals and humans alike.

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Poster

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Topic: D.03. Somatosensation – Touch

Support: DoD SCIRP SC180308
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DARPA INI HR00111990044
T32EB004314

Title: Direct comparison of perceived sensations evoked by intracortical microstimulation and peripheral nerve stimulation

Authors: B. HUTCHISON[1,3], ***B. SPILKER**[1,3], P. BHAT[1,3], W. D. MEMBERG[1,3], A. KETTING-OLIVIER[1,3], R. KIRSCH[1,3,2], J. MILLER[4,2,3], B. AJIBOYE[1,2,3], E. L. GRACZYK[1,2,3]; [1]Biomed. Engin., [2]Sch. of Med., Case Western Reserve Univ., Cleveland, OH; [3]FES Ctr. of Excellence, Rehab. R&D Service, Louis Stokes Cleveland Dept. of Veterans Affairs Med. Ctr., Cleveland, OH; [4]Neurol., Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH

Abstract: Both peripheral nerve stimulation (PNS) and intracortical microstimulation (ICMS) can restore tactile sensation to people living with physical disabilities, such as spinal cord injury (SCI) or amputation. However, it is unclear what the relative benefits and limitations are for these stimulation approaches, as previous analyses of each modality have been dependent on separate subjective user experiences. In this study, we directly compared the perceived sensations evoked by PNS and ICMS in the same person for the first time. This study was conducted with a participant with AIS-B C3/C4 SCI enrolled in the Reconnecting the Hand and Arm to the Brain (ReHAB) clinical trial. The goal of ReHAB is to restore motor and somatosensory function to people with high-cervical SCI resulting in tetraplegia. The participant was implanted with six microelectrode arrays in sensorimotor cortices of the brain, including two 64-channel recording and stimulating arrays in primary somatosensory cortex (S1). The participant was also implanted with multi-contact composite flat interface nerve electrodes (C-FINE) around eight upper extremity peripheral nerves that deliver stimulation to activate arm movement or sensory percepts. The detection threshold and maximum comfortable magnitude were determined for each electrode by varying stimulation pulse amplitude or pulse width for ICMS and PNS, respectively. Stimulation magnitudes at 30%, 60%, and 90% of the dynamic range for each modality were presented for one second each in a randomized order. The perceived location, intensity, and naturalness were recorded using psychophysical methods. The perceived intensity increased with stimulation magnitude for both PNS and ICMS (ANOVA with post hoc comparisons, $p<0.05$ for all). The average perceived intensity for PNS was higher than for ICMS (2-sample t-test, $p <0.001$). The projected field area also increased with stimulation magnitude for PNS (ANOVA with post hoc comparison, $p<0.05$). The average projected field area was higher for PNS than ICMS (2-sample t-test, $p<0.05$). The perceived naturalness was lower for PNS than ICMS (2-sample t-test, $p<0.001$). The perceived naturalness significantly decreased with perceived intensity for all PNS and ICMS electrodes tested (linear regression, $p<0.001$). This study provides the opportunity to understand how different levels of the nervous system respond to stimulation and the benefits of each stimulation approach in modulating evoked sensation. These insights will guide the development of future sensory restoration approaches to best meet patient needs and improve rehabilitation efforts for people with a variety of somatosensory deficits.

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Poster

PSTR211. Tactile Processing: Cortical Mechanisms

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Topic: D.03. Somatosensation – Touch

Support: DoD SCIRP SC180308
VAMR I01 RX003699
DARPA INI HR00111990044
T32 EB004314

Title: Quantifying the S1 cortical response and perceptual response to peripheral nerve stimulation in humans

Authors: *P. BHAT[1,3], B. HUTCHISON[1,3], W. MEMBERG[1,3], A. KETTING-OLIVIER[1,3], R. F. KIRSCH[1,2,3], J. MILLER[4,2,3], B. AJIBOYE[1,2,3], E. L. GRACZYK[1,3,2];
[1]Biomed. Engin., [2]Sch. of Med., Case Western Reserve Univ., Cleveland, OH; [3]FES Ctr. of Excellence, Rehab. R&D Service, Louis Stokes Cleveland Dept. of Veterans Affairs Med. Ctr., Cleveland, OH; [4]Neurol., Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH

Abstract: Individuals with tetraplegia or limb loss may experience diminished to no sensory feedback, which impairs their ability to perform dexterous movements and feel socially connected with others. Peripheral nerve stimulation (PNS) can restore the sense of discriminative touch by electrically activating afferent fibers. However, current PNS techniques often evoke paresthesia - a tingling, sometimes unpleasant sensation. While advanced stimulation paradigms are being developed to enhance sensation naturalness, the relationship between PNS patterns and the response in the primary somatosensory cortex (S1) remains unclear. In this study, we examined how sensory percepts evoked by PNS are represented in S1 in an individual with tetraplegia who is implanted with both cortical and peripheral interfaces. Stimulation was delivered to a participant with sensory-incomplete, AIS-B C3/C4 spinal cord injury through individual contacts of 16-channel Composite Flat Interface Nerve Electrodes (C-FINEs) placed around the median and ulnar nerves. We modulated the pulse width (PW) of PNS, which affects the number of afferent fibers recruited. The sensory dynamic range was first found by identifying the PWs corresponding to sensory detection threshold and maximum comfortable sensation. PNS trials were then pseudorandomly presented over a total of ten conditions: control (no stimulation), six equally spaced PW values that spanned the sensory dynamic range, and three equally spaced PW values below threshold. Each stimulus lasted for two seconds. After each stimulus, the participant was asked to report the perceived intensity of the resulting sensation. Additionally, multi-unit action potentials were recorded from two 64-channel microelectrode arrays (MEAs) implanted in S1. The normalized firing rate was calculated to quantify neural modulation. For all four C-FINE contacts tested, perceived intensity increased with higher PW values from threshold (ANOVA with post hoc analysis, $p<0.01$; Pearson's correlation coefficient, $r>0.7$). Individual MEA channels captured bursting at the beginning of perceivable stimuli that was significantly above baseline (2 sample t-test, $p<0.05$) and lasted for a period of up to 300 ms. Population analysis across MEA channels demonstrated a positive correlation between multi-channel MEA activation during this period and perceived intensity. These results

are initial steps that will aid in the generation of more functional and reliable PNS patterns for sensory restoration. Future work will compare the cortical responses to PNS and mechanical touch to evaluate the similarities and differences between their cortical representations.

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Poster

PSTR211. Tactile Processing: Cortical Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR211.21

Topic: E.02. Cerebellum

Support: DFG SCHW577/21-1
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Title: Disentangling two movement-related neuronal predictive systems: Sensory gating vs State estimation

Authors: K. GUPTA[1,2], **R. ROY CHOWDHURY**[1,2,3], S. CHAKRABARTI[1], C. SCHWARZ[1]; [1]Werner Reichardt Ctr. for Integrative Neuroscience, Tübingen, Tübingen, Germany; [2]Grad. Training Ctr. (GTC), Univ. of Tübingen, Tübingen, Germany; [3]Intl. Max Planck Res. Sch. for Mental Function and Dysfunction, Tübingen, Germany

Abstract: The theory of state estimation is the modern instantiation of a classic sensorimotor theory called the reafference principle (von Holst and Mittelstaedt, 1950). It estimates the kinematic state of the body or its limbs based on the optimal integration of the internal model's predictive signals (based on e.g. efference copy or other contextual signals) and external sensory signals. It generates the so-called sensory prediction error that can be used to update movement and perception, as well as to train the internal model. In mammals, the primary somatosensory cortex has been suggested to be the site of state estimation. Here we established an experimental paradigm in behaviorally trained head-fixed mice to measure the effect of state estimation in whisker-related somatosensory cortex (S1) and to show that it is NOT identical to the well-known sensory gating signal (Chakrabarti and Schwarz, 2018, Nat. Comm.) The experimental setup includes the chronic implantation of a micro-electrode in the facial nucleus (FN) to measure the motor command, and multi-electrode devices in S1 to record sensory signals. The reafferent loop is opened by surgically disrupting two branches of distal the facial motor nerve, which innervate intrinsic whisker muscles. We precisely triggered experimentally controlled, artificial sensory consequences of the motor act, following the motor command by deflecting the whisker using a Piezo actuator. In a different closed-loop approach, we electrically stimulated the trigeminal nucleus to mimic the sensory consequence following a defined whisker movement. We compared predicted and unpredicted sensory responses in S1. Our preliminary results obtained from 12 mice in open-loop and 3 mice in closed-loop configuration demonstrate

the existence of state estimation signals, and that they are different from the classical sensory gating signals.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR212.01/DD2

Topic: D.06. Vision

Support: Wellcome Trust 090843/F/09/Z
Wellcome Trust 214333/Z/18/Z
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Title: Layer 6 cortico-cortical neurons are the major source of interhemispheric communication between the monocular primary visual cortices

Authors: *S. WEILER, M. VELEZ-FORT, T. W. MARGRIE;
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Abstract: Callosal projection neurons (CPNs) mediate information processing for interhemispheric integration. To date, CPNs have been described as bridging different sensory cortical layers via projections to and from Layers 2/3 and 5. Functionally, CPNs have been shown to recruit cell-type specific circuits, serving bilateral tactile integration in the primary somatosensory or sound localization in the primary auditory cortices. Using brain-wide viral retrograde and anterograde transsynaptic tracing followed by serial 2-photon tomography and automated 3D detection of labelled cells, we find that callosal input to the primary visual cortex (VISp) of mice is dominated by a subset of layer 6 (L6) cortico-cortical (CC) neurons that does not include L6 cortico-thalamic (CT) projecting cells. Functionally, *in vivo* 3-photon calcium imaging in the monocular VISp area shows that L6 CPNs are less visually responsive and poorly tuned to drifting grating stimulation compared to L6 CT cells. Additionally, *in vivo* translaminar Neuropixels recordings and optogenetic activation of L6 CPNs in the monocular VISp area predominantly drive either short-latency increases or decreases in the firing rate of individual neurons in the infragranular layers of the contralateral monocular VISp region. Optogenetic activation of L6 CPNs during presentations of drifting gratings was also found to produce mostly an additive effect on L6 responses while predominantly suppressing L5 neurons. To begin to understand the microcircuitry underlying these *in vivo* observations, we performed whole-cell recordings from VISp L6 in acute brain slices while optogenetically stimulating callosal inputs. We find that CPNs evoke excitatory and inhibitory postsynaptic currents (EPSCs, IPSCs) in all major L6 cell types and in contrast to previous reports that CPN stimulation evokes

monosynaptic EPSCs of similar amplitude in all target cell types except for VIP-expressing interneurons. Within each L6 principal cell type, monosynaptic excitatory and disynaptic inhibitory inputs were found to be similarly weighted and dominated by inhibition, in contrast to PV- and SST- expressing interneurons. Finally, fast-spiking interneurons, regardless of their molecular identity (SST/PV), were shown to mediate callosal-driven feedforward inhibition. These data show that L6 CPNs are a major source of VISp input and influence both local cortical and subsequent thalamic processing via its integration with L6 microcircuits. Furthermore, convergence of eye-specific visual field information indicates that the 'classically-defined' monocular region of mouse VISp performs binocular integration.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Distinct functional roles and connectivity rules for lower and higher order intracortical and pulvinar thalamocortical pathways in mouse visual cortex

Authors: *X. HAN[1], **V. BONIN**[2];
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Abstract: Functional specialization of cortical areas is a hallmark of the mammalian neocortex. The visual cortex in particular features dozens of specialized higher visual areas (HVAs) characterized by specific visual functions and organized into segregated visual processing streams. Such an organization must depend on specialized connectivity between areas, yet the underlying wiring rules remain unclear.We combined circuit labeling with in vivo imaging to characterize the joint anatomical and functional organization of input and output pathways to and from three HVAs (AL, PM, and A) in the mouse, probing the visual tuning (spatiotemporal frequency) of lower and higher order L2/3 and L5 intracortical pathways, and higher-order thalamocortical pathways. Retrograde tracing of neurons projecting to HVAs revealed, at the mesoscopic level, a correlation between the areas' tuning and the density of intracortical inputs. At the cellular level, visual information conveyed by L2/3 intracortical pathways (i.e. tuning of retrogradely labeled L2/3 neurons), are biased towards the tuning of the target HVAs, showing high target-specificity. Importantly, the degree of specificity varies across pathways and matches the functional heterogeneity in the target areas. While AL and PM integrate diverse inputs from diverse sources, area A receives homogeneous and stereotyped information. However, target

specificity is not a universal rule of intracortical pathways. Unlike L2/3 pathways, L5 pathways, labeled by AAV-mediated GCaMP8 expression in Rbp4+ neurons in AL or PM and functionally characterized by axonal imaging, are found to broadcast source-specific information with no regard to the functional biases of the recipient areas. Finally, after characterizing all major cortical input pathways to AL and PM, we found intracortical pathways lack the specificity accountable for the highly specific HVA representations. Hence we investigated another important route of visual information along the higher-order thalamocortical pathways via the lateral posterior nucleus (LP). Characterizing the visual tuning of LP axonal projections in six different HVAs, we found LP pathways send highly distinct information that is functionally aligned to the targets, suggesting a prime role of higher-order thalamocortical pathways in driving specialized HVA representations.Altogether, this study features to date the most comprehensive functional study of cortical and thalamic inputs to HVAs, revealing novel, distinct wiring rules of interareal communication pathways that compose the visual processing network and underlying specialized HVA representations and visual functions.

Disclosures: X. Han: None. **V. Bonin:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Comparing motor and visual cortex layer-5 microcircuits using 2-photon optogenetics

Authors: S. ALAGESWARAN[1,2], H. RENAULT[1,2], C. Y. C. CHOU[1,2], A. SUVRATHAN[3,2], *P. J. SJOSTROM[4,2,3];
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Abstract: The neocortex is classically divided into six layers with functionally distinct roles. For example, excitatory pyramidal cells (PCs) in layer 5 (L5) provide key output from primary visual (V1) and motor (M1) cortices. Although V1 and M1 share many cytoarchitectural similarities,

V1 mediates sensory input and M1 provides motor output. We were therefore interested in V1 and M1 microcircuit differences. However, the current state-of-the-art technique for probing synaptic connectivity – multiple patch clamp – is technically challenging and yields only a few connections per day. We therefore developed optomapping, a high-throughput connectivity mapping method that combines 2-photon optogenetics with patch-clamp electrophysiology. We injected AAV9-CAG-DIO-STChroME-P2A-mRuby in neonatal Emx1-Cre mice to drive expression of the soma-targeted opsin, ChroME, in cortical PCs. In P18-P25 acute slices, we elicited action potentials in ChroME-expressing PCs with 1040-nm Ti:Sa laser spiral-scans. This method allowed us to activate neurons with single-cell spatial and millisecond temporal resolution. We whole-cell recorded from PCs in L5 of V1 and M1 while sequentially spiral-scanning hundreds of surrounding PCs to search for presynaptically connected PCs across all cortical layers. In both M1 and V1, there were more intralaminar than interlaminar connections onto L5 PCs (M1: 8.6% ± 2% vs. 2.4% ± 2%, $p < 0.01$; V1: 14% ± 2% vs. 7.1% ± 2%, $p < 0.001$). Intralaminar connectivity was higher for L5 PCs in V1 than in M1 (14% ± 2% vs. 8.6% ± 2%, $p < 0.05$). In M1, we found greater synaptic strengths for intralaminar inputs onto L5 PCs than for interlaminar inputs (1.1 ± 0.03 mV vs. 0.19 ± 0.01 mV, $p < 0.01$). In contrast, for V1 L5 PCs, intralaminar and interlaminar synapses were of indistinguishable strength (0.31 ± 0.004 mV vs. 0.42 ± 0.008 mV, $p = 0.23$). Overall, inputs onto M1 L5 PCs were stronger than those onto V1 L5 PCs (M1: 1.1 ± 0.03 mV; V1: 0.31 ± 0.004 mV, $p < 0.01$). Radial connectivity did not differ between V1 and M1 L5 PCs ($p = 0.87$). We additionally found that in both V1 and M1, synaptic strengths recorded in L5 PCs were log-normally distributed, consistent with the existence of Hebbian assemblies. In conclusion, although L5 V1 and M1 microcircuits differ in terms of intralaminar pathways, these microcircuits are overall quite similar. This is a striking finding since V1 mediates sensory input whereas M1 provides motor output.

Disclosures: **S. Alageswaran:** None. **H. Renault:** None. **C.Y.C. Chou:** None. **A. Suvrathan:** None. **P.J. Sjostrom:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR212.04/DD5

Topic: D.06. Vision

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Title: Comparing motor and visual cortex layer-2/3 microcircuits using 2-photon optogenetics

Authors: *H. RENAULT[1,2], S. ALAGESWARAN[1,2], A. SUVRATHAN[2,3], P. J. SJÖSTRÖM[2,4,3];
[1]Integrated Program in Neurosci., [2]Ctr. for Res. in Neurosci., [3]Neurol. & Neurosurg., [4]Med., McGill Univ., Montreal, QC, Canada

Abstract: Neuronal connectivity circuits critically determine information processing in the brain. For example, connections between pyramidal cells (PCs) in layer (L) 2/3 of primary visual cortex (V1) determine their functional preference. Therefore, mapping microcircuit connectivity is imperative to understanding how the neocortex processes and integrates incoming and outgoing signals. However, current state-of-the-art methods such as multiple patch clamp are inefficient for large-scale microcircuit mapping. Therefore, we created optomapping, a high-throughput, two-photon optogenetic mapping technique that tests hundreds of connections across all cortical layers in a single experiment. Here, we compare V1 — a key neocortical input area — to primary motor cortex (M1) — a key neocortical output area.
We injected AAV9-EF1a-DIO-STChroME-P2A-mRuby into V1 and M1 of postnatal day(P)1-2 $Emx1^{Cre}$ mice to activate PCs. In acute slices from P20-P27 injected mice, we used two-photon spiral scans at 1040 nm to activate candidate presynaptic PCs in a cortical column, while looking for responses in postsynaptic patch-clamped PCs.
We found higher L5 → L2/3 PC connectivity in V1 than in M1 (7.9% ± 2% vs. 0.87% ± 0.5%, $p < 0.01$), and these connections were also stronger (0.34 ± 0.007 mV vs. 0.21 ± 0.002 mV, $p < 0.05$). However, when comparing across all layers in V1, no laminar differences in synaptic strength were detected ($p = 0.23$). L2/3 of M1 had more intralaminar than interlaminar connections (8.9% ± 2% vs. 0.87% ± 0.5%, $p < 0.05$), whereas L2/3 of V1 had higher interlaminar connectivity from L4 than intralaminar from L2/3 (24% ± 5% vs. 7% ± 1%, $p < 0.001$) consistent with the textbook V1 canonical circuit. Similarly, V1 trended towards higher connectivity farther from the patched cell (exponential decay constant λ: 200 ± 60 μm vs. 55 ± 30 μm, $p = 0.059$), likely a reflection of the L4 to L2/3 pathway in V1. Additionally, synaptic strengths in L2/3 of both M1 and V1 were log-normally distributed.
Taken together, although we find some distinct differences, our findings chiefly highlight how L2/3 microcircuits of M1 and V1 are similar, even though V1 mediates sensory input and M1 provides motor output.

Disclosures: H. Renault: None. **S. Alageswaran:** None. **A. Suvrathan:** None. **P.J. Sjöström:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Program #/Poster #: PSTR212.05/DD6

Topic: D.06. Vision

Support: ERC Starting Grant #678250
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Title: Synaptic properties of top-down inputs contacting Layer 1 Interneurons in the primary visual cortex

Authors: *M. MARTINEZ, D. DHANASOBHON, N. REBOLA;
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Abstract: In the visual system, the processing of visual information is strongly influenced by the behavioral relevance of the stimulus, the animal's internal brain state, as well as the presence of other sensory modalities. These contextual signals are conveyed to primary sensory cortices by multiple pathways including thalamic and intra-cortical feedback projections that strongly innervate layer 1 (L1). Thus, by integrating bottom-up sensory input with top-down information, L1 is a key hub structure underlying contextual encoding of sensory information. Yet, the functional architecture of L1 circuits is still poorly defined. Through an optogenetic approach, we dissect the synaptic and connectivity properties of long-range, top-down projections carrying contextual signals to L1 microcircuits in the primary visual cortex. We expressed the light-sensitive opsin channelrhodopsin2 (ChR2) in different associative visual areas (V2L and V2M) and lateral posterior (LP) nucleus of thalamus and recorded from L1 interneurons (L1-INs) while stimulating ChR2-positive axons. At the cellular level, we found that both V2L and V2M inputs preferentially target NDNF-positive interneurons. Surprisingly, functional synaptic properties were quite heterogeneous across inputs. We found significant variations in the NMDA/AMPA ratio based on the origin of stimulated glutamatergic fibers. The NMDA/AMPA ratio was higher when the stimulated glutamatergic fibers originated from V2M, intermediate for LP and smallest for V2L. Such a difference in synaptic properties between inputs from high-order visual areas was not observed while recording from L2/3 PNs indicating selectivity towards L1-INs. In addition, differences in synaptic properties between V2M and V2L were absent in animals that were dark-reared from birth suggesting establishment of synaptic function into L1-INs require sensory experience. Furthermore, through current clamp recordings, we discovered that NMDA-mediated currents play a crucial role in the temporal integration of glutamatergic EPSPs in L1-INs. Preliminary results have also unveiled a non-linear interaction between AMPA and NMDA receptors, specifically in the V2M input onto L1-INs. We are presently investigating the functional relevance of such atypical synaptic properties for V1 function. In conclusion, our study provides both anatomical and functional evidence of L1 microcircuits with pathway-specific integration properties in V1. These findings highlight the diverse effects of multiple long-range inputs contacting L1-INs in V1.

Disclosures: M. Martinez: None. **D. Dhanasobhon:** None. **N. Rebola:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR212.06/DD7

Topic: D.06. Vision

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Title: Summation and temporal dynamics of parvalbumin and somatostatin expressing interneurons during binocular processing

Authors: *M. BHAGAVATHI PERUMAL, S. GHARAEI, E. ARABZADEH, G. J. STUART; The Eccles Inst. of Neurosci., Australian Natl. Univ., Canberra, Australia

Abstract: Neuronal circuits in the primary visual cortex integrate inputs from our two eyes to generate a single binocular percept. Previous work indicates that integration of binocular inputs in layer 2/3 pyramidal neurons in the binocular visual cortex (V1B) occurs via sublinear summation of monocular responses. Sublinear binocular integration in pyramidal neurons helps to preserve orientation selectivity and requires recruitment of inhibition. Here we investigate the role of specific inhibitory interneuron subtypes in this process. We recorded the activity of two major subtypes of inhibitory interneurons - parvalbumin (PV) and somatostatin (SST) expressing interneurons - in layer 2/3 of adult mouse V1B in vivo using Neuronexus silicon arrays. PV and SST neurons were identified by optical tagging following targeted expression of ChR2 and excitation with a blue light (470 nm). We then measured monocular and binocular responses to sinusoidal drifting gratings at different orientations presented to each eye separately, or together, using a haploscope. We found that both PV(n=38) and SST(n=39) neurons responded to contralateral (C) and ipsilateral (I) eye stimulation, indicating that both subtypes of inhibitory interneurons are binocular. We calculated the ocular dominance index (ODI) defined as the ratio of (C-I)/(C+I) spiking. On average, the ODI for PV neurons was significantly smaller than that for SST neurons, indicating that PV neurons are more binocular than SST neurons (ODI PV =0.2± 0.03 vs. SST=0.6±0.05, mean ±SEM; t-test, $P<0.001$). During binocular simulation the peak spiking response of both PV and SST neurons was smaller than the linear sum of the responses to activation of each eye alone, indicating sublinear summation occurs in both PV and SST neurons during binocular integration. We quantified the extent of sublinear summation as the ratio of the response to binocular stimulation relative to the linear sum of monocular responses. This revealed significantly more pronounced sublinear summation in PV neurons compared to SST neurons (PV ratio=0.6±0.01 vs. SST ratio = 0.8±0.03, mean±SEM; t-test, $P<0.001$), suggesting greater inhibition of PV than SST neurons during binocular processing. Consistent with this idea, while SST and PV had similar onset latencies (10% of the peak firing rate), SST neurons reached their peak firing rate significantly earlier than PV neurons and showed less accommodation (t-test, $P<0.001$) during binocular stimulation. These findings reveal differences in summation and temporal dynamics of PV and SST neurons, suggesting that these two inhibitory cell types play different roles in binocular processing.

Disclosures: M. Bhagavathi Perumal: None. **S. Gharaei:** None. **E. Arabzadeh:** None. **G.J. Stuart:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Like-to-like connectivity between pyramidal cells and somatostatin interneurons in the visual cortex drives image segmentation

Authors: *W. HENDRICKS[1], M. SADAHIRO[1], D. MOSSING[1], J. VEIT[2], H. ADESNIK[1]; [1]Univ. of California, Berkeley, Berkeley, CA; [2]Physiol. I, Univ. of Freiburg, Freiburg, Germany

Abstract: Visual cortical circuits perform complex computations to segment a visual scene into identifiable objects. Orientation-dependent surround suppression in the primary visual cortex (V1) is a candidate computation underlying segmentation; however, the circuit mechanisms that mediate it are still not well known. Stimuli extending outside of a neuron's classical receptive field suppresses activity, but this 'surround suppression' is highly orientation dependent. Surround suppression in pyramidal cells (PCs) is strongest when the center and surround of a stimulus match (are iso-oriented) but suppression is relieved when they are cross-oriented. In contrast to pyramidal cells, somatostatin-expressing (SST) inhibitory interneurons show minimal surround suppression and are driven only for iso-oriented stimuli. Here we test two candidate models for orientation-dependent surround suppression. The first model proposes that vasointestinal-peptide expressing (VIP) interneurons inhibit SSTs and could therefore 'switch-off' SST cells for cross-oriented stimuli, relieving surround suppression. Alternatively, 'like-to-like' functional connectivity within PC to SST circuits could 'switch-on' surround suppression for iso-oriented gratings. We find optogenetic inhibition of VIP cells only mildly alters orientation-dependent surround suppression in pyramidal cells, leaving a large unexplained component to surround suppression. Instead, by mapping PC to SST connections in vivo using 2p holographic optogenetics, we find SSTs are functionally connected with co-tuned pyramidal cells, supporting a 'switch-on' model for orientation-dependent surround suppression. Additionally, in vivo whole-cell recordings from SSTs reveals strong excitatory input encoding the surround likely shapes their iso-oriented grating preference. Finally, photo-stimulating ensembles of surround-driven pyramidal cells strongly drives SSTs and suppresses visual responses in center-aligned pyramidal cells. Together, these experiments strongly support a SST-driven 'switch-on' circuit mechanism for the orientation-specific nature of surround suppression and provide greater insight into cortical circuit mechanisms enabling visual perception.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Topic: D.06. Vision

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Title: Impact of V1 parvalbumin interneurons on visual discrimination depends on strength and timing of activation, and task difficulty

Authors: *L. KUKOVSKA, J. POORT;
Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Parvalbumin-expressing (PV) cells are the most common inhibitory interneurons in the visual cortex. They are densely connected with excitatory cells and play important roles in balancing cortical circuit activity and learning. Strong activation of PV cells is used to silence the primary visual cortex (V1) to help establish the role of V1 in visual processing and behaviour. In parallel, moderate PV cell activation is used to understand the contribution of PV cells to local V1 circuit computations. It has been suggested that moderate PV cell activation improves visual discrimination but evidence is mixed. Varying the timing of activation and difficulty of discrimination have also been shown to influence behaviour. However, the mechanisms are not yet well understood.

Here, we investigate how manipulating these three major factors (the strength and timing of activation, and task difficulty), affect performance of mice in a go/no-go orientation discrimination task. To activate PV cells, we optogenetically stimulated Channelrhodopsin-2 in transgenic PV-cre::Ai32 mice through a cranial window chronically implanted over V1, with levels varying between 0-0.43mW/mm^2. We manipulated the timing of activation by introducing four time-windows targeting the entire stimulus response, early activity (0-120ms, corresponding to the initial feedforward sweep of information), or late activity only (from 120 or 180ms after stimulus onset onwards, corresponding to the feedback sweep of information arriving in V1). Finally, we increased task difficulty by reducing the angle difference between the go and no-go orientations from 90° to 15°.

We found that activating PV cells throughout the entire stimulus impaired performance at all laser levels (while wild-type controls retained high performance). Likewise, performance was reduced when moderate PV cell activation lasted only during the late V1 activity, indicating that activity beyond the initial 120ms is in fact crucial for high accuracy discrimination. We next restricted the stimulation to the earliest V1 activity and found that performance was improved in easy but not difficult discrimination. Changes in performance in each condition were not explained by mice becoming more conservative or liberal in their responses, thus ruling out a simple decision criterion shift.

Overall, our data demonstrates that persistent V1 activity is critical for maintaining high perceptual discrimination. Moreover, precisely timed PV cell activation produces a bi-directional modulation in V1, with earliest stages of cortical processing (<120ms) benefitting from moderately increased inhibition to improve easy discriminations.

Disclosures: **L. Kukovska:** None. **J. Poort:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Program #/Poster #: PSTR212.09/DD10

Topic: D.06. Vision

Support: NIH Grant EY025102

Title: Excitatory and inhibitory interactions sharpen disparity tuning and improve stereo matching

Authors: ***J. M. SAMONDS**[1], M. SEVERSON[2], C. BARR[3], N. M. PRIEBE[3];
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Abstract: Neurons in the mouse visual cortex are tuned for binocular disparities in correlated random dot stereograms, but still respond to anti-correlated stereograms (a-RDS). The disparity energy model predicts that individual neurons respond to a-RDS and do not completely solve the stereo correspondence problem. Responses in the primary visual cortex and higher visual areas in mammals suggest that cortical circuits suppress false matches and/or enhance globally consistent matches. We examined two-photon calcium responses of excitatory and inhibitory neurons, as well as the interactions between neurons, in mouse visual cortex to see how they contribute to disparity tuning and suppressing the responses to a-RDS. We find that excitatory neurons with significant disparity tuning have suppressed responses compared to neurons without significant disparity tuning. This suppression was progressively stronger at less preferred disparities, suggesting that it contributes to significant disparity tuning. For a-RDS, the suppression was uniform across disparities resulting in weaker selectivity compared to correlated stereograms. The correlated variability (r-ee-noise) among excitatory cells was positive for neurons with similar disparity tuning (r-ee-signal &gt 0) and negative for neurons with different disparity tuning (r-ee-signal &lt 0) producing an overall correlation of r = 0.27 for r-ee-noise versus r-ee-signal. The suppression was significantly weaker during a-RDS stimulation. During a-RDS stimulation, r-ee-signal and the relationship with r-ee-noise moves closer to zero, which likely explains why there are less effective suppressive interactions among neurons. Pavalbumin-expressing (PV+) inhibitory neurons had significantly weaker disparity tuning compared to PV- or excitatory neurons that was consistent with local non-specific integration of excitatory neuron inputs. These inhibitory neurons responded to all disparities, while excitatory or PV- neurons have no response to the least preferred disparity. The interactions between mostly excitatory (PV-) and inhibitory (PV+) neurons (r-ei-noise) had a very similar relationship with r-ei-signal as we observed between excitatory neuron pairs suggesting that these interneurons provide the suppressive sharpening of disparity and weaker tuning for a-RDS observed in excitatory neurons. Overall, we find that neuronal interactions and inhibitory interneuron integration play an

important role in disparity tuning and suppressing responses to a-RDS. This demonstrates their importance in solving the stereo correspondence problem.

Disclosures: **J.M. Samonds:** None. **M. Severson:** None. **C. Barr:** None. **N.M. Priebe:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Distinct inhibitory circuits modulate the response of visual cortex to stimulus familiarity and reward association

Authors: *A. J. HINOJOSA, S. E. DOMINIAK, Y. KOSIACHKIN, L. LAGNADO;
Univ. of Sussex, Brighton, United Kingdom

Abstract: The continuous stream of visual information that the environment provides must be filtered to select relevant stimuli based on previous experience. One example of such simple form of learning is habituation: repeatedly presenting a stimulus with no salience results in behavioural responses being suppressed, while associating a reward to it enhances responses. Simultaneously, behavioural responses also vary as a new stimulus simply becomes familiar independently of its saliency. Primary visual cortex (V1) encodes both saliency and familiarity as changes in neural activity occurring over days, but sensory circuits also undergo short-term plasticity changes in a process called adaptation. In V1, a subset of pyramidal cells (PCs) adapt to a high-contrast stimulus within seconds by reducing their gain (depression) while other subset increase their gain (sensitize), and these are controlled by distinct inhibitory interneurons. However, whether these same circuits exert the longer-term gain changes of habituation and stimulus familiarity is unknown. To answer this question, we presented awake mice with a 10 s stimulus (drifting grating: 20°, 100% contrast, 1 Hz) repeatedly during 6 sessions (2 days apart) while monitoring calcium signals using multiphoton microscopy. A subset of these mice was rewarded with condensed milk at the end of each stimulus presentation. To test the role of interneuron inhibition, we simultaneously modulated neuronal activity with inhibitory or excitatory opsins in one of three main types: parvalbumin (PV), somatostatin (SST) or VIP interneurons. With habituation, one quarter of PCs ceased responding significantly to the stimulus and the activity of the responsive cells shifted from depression to sensitization. Associating a reward with the stimulus prevented the decrease in responsivity but maintained the shift towards sensitization, suggesting that reward association is related to cell responsivity while stimulus familiarity is linked to the change in adaptation. Optogenetically silencing PVs showed

that their inhibition onto PCs weakens 10-fold during habituation while, on the contrary, activating SSTs showed that the effect they exert on PC adaptive properties doubles. When the stimulus was rewarded, SST cells weakened their inhibition onto PCs whilst PV inhibition remained unchanged compared to habituation. We propose that SST interneurons contribute more to the reward association response while PV interneurons play a dominant role in modulating the response to familiarity. Our study identifies some basic circuit mechanisms involved in simple forms of learning and their link to short-term adaptation.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Exploring individuality of inhibitory circuits in visual perception using deep learning analysis

Authors: *X. HOU[1], E. KITAYAMA[1], K. SAKIMURA[2], S. SUGIYAMA[1];
[1]Dept. Med.Dent. Sci., Niigata Univ., Niigata, Japan; [2]Brain Res. Ins Niigata Univ., Niigata, Japan

Abstract: Freely moving animals interact with environment across a range of situations and choose the best action to take. When they faced with a complex visual discrimination task, some may lead to indecision, whereas others may lead to instead take a direct route without hesitation. However, the role of local circuits within the primary sensory cortex in choosing their action with sensory discrimination is not well understood. The cell-type specific properties and connectivity of cortical inhibitory neurons underlie their ability to spatiotemporally shape information processing. Although activation of a distinct interneuron subtype facilitated visual responses and improved visual discrimination, it is still unclear how the local circuits associate animal behaviors with mind such as hesitation. We demonstrated the dendritic transdifferentiation from a specific interneuron subtype to another one improved visual discrimination of behaving animals. These mice were successfully trained to select the correct

choice (go or no-go) in response to one grating image (target or non-target) and performance for the correct choice reached over 80 %. Importantly, in the complex task like two-choice discrimination in which target and non-target images were presented together on both sides of the display, these mice significantly improved their performance compared to control. Tracking mice trajectories revealed how the control mice seemed to come closer to the display and look around for decision-making, whereas the mice with transdifferentiated subtype would instead take a direct route. As a result, both distance and response time to reach the correct choice were significantly shorter in dendritic transdifferentiation phenotype than in control. Furthermore, we tracked their movements with the deep learning pose estimation algorithm DeepLabCut in order to compare and extract behavior patterns for decision making. Performing calcium imaging during behaviors may allow us to link a distinct behavior to cortical connectivity of inhibitory neurons. Thus, machine learning approach offers a deeper understanding of a link between interneuron subtypes and sensory processing, and of a heterogeneity of intracortical circuits to provide functional individuality.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Support: NIH Grant R01MH126684
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Title: Using NeuVue to Trace Inputs to Spiny Interneurons in the MICrONS Cubic Millimeter Connectomic Dataset from the Mouse Visual Cortex

Authors: *P. K. RIVLIN, J. K. MATELSKY, D. XENES, H. GOODEN, V. ROSE, B. A. WESTER;
Applied Physics Lab., Johns Hopkins Univ., Laurel, MD

Abstract: Despite continuing advances in image segmentation capabilities, modern volumetric electron microscopy (EM) connectome datasets still require significant proofreading to correct merge and split errors and ensure higher fidelity scientific analysis. To facilitate large-scale proofreading of the MICrONS Minnie dataset, a cubic millimeter volume from mouse visual cortex containing over 200,000 cells (MICrONS Consortium et al. 2021), we previously developed *NeuVue* (Xenes et al. 2022), a web-based proofreading application with priority-queue and visualization tools, powered by popular open-source connectomics tools: Neuroglancer, PyChunkedGraph (PCG), and Connectomics Annotation Versioning Engine (CAVE). Here we demonstrate *NeuVue's* capabilities for *ad hoc* proofreading by tracing inputs to spiny interneurons in the Minnie volume to enable a detailed analysis of connectivity. Though

inhibitory interneurons are typically classified as aspiny, a subset of interneurons with spines on dendrites and soma have been described in the neocortex (Kawaguchi et al. 2006; Hwang et al. 2020). Given their location, synaptic inputs to somatic spines may have a major impact on the neuron's probability of firing an action potential. To better understand the role of these inputs, we first trace the origin of input axons onto a subset of spiny interneurons and programmatically enumerate underlying motifs of this type across the MICrONS volume. Our interest in spiny interneurons was prompted, in part, by discovering MC-7787, a putative spiny L5 Martinotti cell (MC), during a screening of neurons recovered from our large-scale IARPA MICrONS proofreading effort (Xenes et al. 2022). When compared to MC types recently described in Gamlin et al. 2023, MC-7787 appears to belong to the MET-8 type. Our analysis therefore includes MC-7787 and 3 MET-8 MCs. As a first step, we used *NeuVue's* synapse viewer to survey MC somatic spines. Like that observed for L23 spiny interneurons in the mouse neocortex (Hwang et al. 2020), the number of somatic spines (6 to 29) and number of synapses per spine (up to 9) varied across MCs. Interestingly, preliminary tracing of MC-7787 inputs revealed that L23 and L4 pyramidal (PY) axons form two types of convergent motifs onto MC somatic spines. The first motif consists of two PY axons from the same column (with respect to MC-7787) converging onto a single spine. The second, more striking motif is composed of two PY, one from the same and one from a neighboring column, converging onto a single spine. To determine if synaptic convergence is a common feature of spiny interneurons, we will extend this analysis to MET-8 MCs and survey the Minnie volume for convergent motifs.

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Poster

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Topic: D.06. Vision

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Title: Enhanced cortical gradient of interneuron distribution in macaque monkeys compare to mice

Authors: M. GLATIGNY[1], F. GEFFROY[1], ***T. VAN KERKOERLE**[2];
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Abstract: A defining architectural principle of the primate neocortex is that is contains a hierarchy of distinct cortical areas, with bottom-up or feedforward connections running from lower to higher areas, and top-down or feedback connections running in the opposite direction. In contracts, it is still debated to what degree the rodent brain also contains a cortical hierarchy.

A direct comparison between species remains challenging as the connectivity patterns within the cortex are highly complex. Recently, it has been suggested that feedforward and feedback processing involve circuits of specific types of interneurons. While parvalbumin interneurons are thought to be more involved in fast feedforward inhibition, VIP or calretinin interneurons are thought to selectively involved in slower disinhibition by feedback input. Mapping the cortical density of these two types of interneurons could therefore provide a way to quantify the degree in which a cortical area is involved in feedforward versus feedback processing.
We measured the distribution of parvalbumin and calretinin across a large part of the visual hierarchy in macaque monkeys and mice using post-mortem antibody staining. We found a steep gradient in macaque monkeys, where parvalbumin gradually decreases and calretinin gradually increases across the hierarchy. With parvalbumin being the dominant interneuron in early visual areas, and calretinin being the dominant interneuron in the frontal cortex. In contrast, a gradient was nearly absent in mice, except for a similar decrease in parvalbumin and in increase of calretinin in the frontal cortex relative to sensory areas, although parvalbumin remained the dominant interneurons across the mouse cortex. The laminar distribution confirmed the functional role of these two interneurons, with parvalbumin interneurons being densest in feedforward input layer 4, while calretinin being relatively densest in feedback input layer 1. Taken together, in macaque monkeys, there is a deep cortical hierarchy, where early visual areas seem to be dominated by feedforward processing, while frontal cortex becomes dominated by feedback processing. In contrast, this hierarchy seems nearly absent in mice, and feedforward processing remains dominant across the cortex.

Disclosures: **M. Glatigny:** None. **F. Geffroy:** None. **T. van Kerkoerle:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR212.14/DD14

Topic: D.06. Vision

Support: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 154113120 – SFB 889, project C04

Title: Noradrenaline receptor expression in macaque striate and extrastriate cortex

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Abstract: Visual attention is a selection mechanism which enhances the processing of behaviourally relevant stimuli. It has been hypothesised that the underlying mechanism deploys

neuromodulators, which target different receptor types, expressed by diverse populations of neurons. One of the most common neuromodulators involved in higher cognitive functions is noradrenaline. While the attentional modulation of firing rates has been extensively studied, there is little to no previous work that has examined the layer and cell-type specific distribution of neurotransmitter receptors in visual cortex, especially in macaques. In this project, the expression of three of the most common noradrenaline receptor subtypes (α2A, β1 and β2) on two inhibitory interneuron subtypes (parvalbumin, PV; calretinin, CR) in rhesus macaques was examined in the visual cortical areas V1, MT and MST - where neuronal responses are modulated by attention. Brain sections of 2 adult, male rhesus macaques (age: 22 and 20 years, transcardially perfused with PFA) were used. A standard dual-immunofluorescence staining was performed on every tenth serial coronal section (40 μm) per region of interest, including antigen retrieval with 10mM sodium citrate buffer and quenching of Lipofuscin. Overview scans were taken with a ZEISS widefield microscope witha 10x objective, controlled by ZEN blue. Detailed images were then taken with a ZEISS microscope with the Airyscan function (63x/1.40 oil lense). We observed that in macaque striate and extrastriate cortex all three noradrenaline receptor subtypes were abundantly expressed in all layers, besides the expected low expression of all receptor classes in layer I (due to the infrequency of neuronal somata compared to other layers). We found that α2A noradrenergic receptors and β2 noradrenergic receptors were more frequently expressed than β1 adrenergic receptors in all three areas. The experiments revealed a higher density of CR-neurons in layers II-III compared to layers IV-VI in V1, MT and MST. Regarding the PV expression, we found it evenly expressed across layers II-VI in all three areas, although in less density in MT and MST, compared to V1. In V1 we found, that PV-immunoreactive neurons are expressed more abundantly, while CR-immunoreactive neurons appear in less density. The detailed Airyscan images revealed that α2A, β1 and β2 show a strong signal close to the cell membrane and also partly within the cell bodies. In summary, this project provides support for V1, MT and MST, as a model of noradrenergic modulation of inhibition in macaque visual cortex, providing insights into the molecular basis of the neuropharmacology of attention in rhesus macaques.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR212.15/DD15

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: BBRF Young Investigator Award
Future Leaders in Canadian Brain Research Program
FRQS Salary Award
Start up grant (#324009)

Title: Spatially resolved expression of noradrenergic receptor transcripts in cortical neurons

Authors: *E. TANGUAY[1], P. DE KONINCK[2], V. BRETON-PROVENCHER[2];
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Abstract: The noradrenergic system plays a critical role in learning by influencing cortical activity through a dense network of noradrenergic axons and their interactions with diverse receptors. However, the mechanisms by which noradrenaline modulates the distinct cellular components of cortical circuits remain poorly characterized. Here, our objective is to map the expression of each subtype of noradrenergic receptors - α-1(a,b,d), α-2(a,b,c), and β-(1,2,3) - onto the subclasses of cortical interneurons. To achieve this, we analyzed the RNA expression of noradrenergic receptor subtypes using a transcriptomic cell-type atlas of the mouse and human motor cortex. Our findings revealed robust expression of α-1a, α-1b, α-2a, α-2c, and β-1 receptor transcripts in the cortex. In addition, α-1 receptor transcripts showed peak expression in inhibitory neurons, while glutamatergic neurons primarily expressed β receptor transcripts. To investigate the spatial distribution of these expression patterns within cortical microcircuits, we used fluorescence *in situ* hybridization to label the RNA of noradrenergic receptors alongside the main subclasses of GABAergic interneurons: Vip-, Sst-, Pval-, and Ndnf-expressing cells. To automate region annotation and fluorescence quantification, we developed a pipeline to detect somas and register their position to a reference atlas. Our preliminary results showed a difference in the expression of α-1 receptor transcripts across cortical layers in Vip+ and Sst+ cells, suggesting that the influence of noradrenaline release is layer-specific for these subclasses of interneurons. We are currently extending those analyses to include other noradrenergic receptors and explore regional differences in noradrenergic receptor expression. Future experiments will evaluate how the expression of noradrenergic receptors affects interneuron activity during the learning of a sensorimotor task. Thus far, our results suggest that noradrenaline modulates cortical circuits by differentially targeting GABAergic cortical interneurons based on their subclass and position within the cortex. These findings represent an important step toward our goal of understanding the mechanisms through which noradrenaline shapes cortical circuits at various stages of learning.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR212.16/DD16

Topic: D.06. Vision

Support: NIH R01EY033835

Title: Human Primary Visual Cortex Displays Earlier Development of Parvalbumin Interneurons than Higher-Level Face- and Place-Selective Visual Cortex

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Abstract: Visual functions, such as detecting edges or perceiving faces, develop at different rates, and are crucial for us to successfully perceive and interact with our environment. Parvalbumin-positive (PV) interneurons are key regulators of cortical networks, yet little is known about their development in human visual cortex. As an example, while myelination is essential to the proper function of PV interneurons, how developmental myelination of PV interneurons occurs across distinct areas of human visual cortex is not clear. Here, we examine the microstructural development of three functionally-distinct human visual areas—primary visual cortex (V1) and high-level face- and place-selective cortex—across the first decade of life (34 gestational weeks to 9 years of age). Using immunohistochemical analyses of parvalbumin (anti-parvalbumin) and myelination (anti-MBP), we quantify the density of parvalbumin interneurons and the distribution of myelin sheaths across cortical layers and ages in our visual areas of interest. We find that, by 34 gestational weeks, PV interneurons have emerged in primary visual cortex (V1), but not in face- and place-selective cortex. Further, while overall neuronal density decreases across infancy (0-3 months of age) in all three visual areas, the density of PV interneurons increases with age during this time. V1 shows an overall higher density of PV interneurons throughout infancy, and, at 9 years of age, the density of PV interneurons remains higher in V1 than in face- and place-selective cortex. Parvalbumin density is non-uniform across cortical depths: layer 4C of V1 and layer 4 of place- and face-selective cortex show a higher density of PV interneurons relative to other cortical layers, suggesting that the input layer 4 has higher PV density. Finally, myelination of PV interneurons occurs earlier in V1 than in higher-level visual areas. By 3 months of age, the axons of PV interneurons in layer 4C of V1 are already myelinated, while axons in layer 4 of face- and place-selective areas do not yet possess myelin sheaths. Together, these results suggest that functionally-distinct visual areas show different trajectories of PV interneuron emergence and myelination that may coincide with their functional development.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Excitatory/inhibitory ratio disruption modulates synchronized oscillation and neuronal interaction directions in a cortical microcircuit with PV, SOM, and VIP inhibitory interneuron classes

Authors: *N. WAGATSUMA[1], S. NOBUKAWA[2,3], T. KURIKAWA[4];
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Abstract: Autism spectrum disorder (ASD) and Schizophrenia (SZ) are a group of complex and heterogeneous mental disorders involving multiple neural system dysfunctions. Atypical visual perception and the impaired temporal coordination of neuronal activities in people with these disorders is hypothesized to stem from an Excitatory/Inhibitory (EI) imbalance in the brain (Rubenstein and Merzenich, Genes Brain Behav., 2003). However, the detailed neuronal microcircuit and mechanism for underlying dysfunctions in people with ASD and SZ remains largely unclear. To investigate the mechanism of dysfunctions in ASD and SZ, we developed a computational microcircuit model with biologically plausible visual cortical layers 2/3 that combined excitatory pyramidal (Pyr) neurons with three inhibitory interneuron classes (parvalbumin [PV], somatostatin [SOM], and vasoactive intestinal polypeptide [VIP]) (Pfeffer et al., Nature Neurosci., 2013; Lee et al., Cell Reports, 2018; Wagatsuma et al., Cerebral Cortex, 2022). We then performed model simulations with different E/I imbalances by changing different numbers of each specific subtype of interneuron to become Pyr neurons (or vice versa). Simulations using our model indicated that when the E/I balance was disrupted by decreasing the PV population, activity changes in the Pyr population followed those of the PV population, which enhanced neuronal firing at beta and gamma frequencies. Conversely, Pyr neuronal population activity was the precursor of PV interneuron activity when the E/I imbalance was induced by decreasing the SOM population; this preferentially impaired gamma frequency activity. In addition, these enhancement and suppression of the gamma frequency induced by E/I imbalance were in agreement with experimental results of magnetoencephalography (MEG) study for patients with SZ and ASD, respectively (Grent-‘t-Jong et al., eLife, 2018; Sun et al., The Journal of Neuroscience, 2012). Our results provide important insights into the atypical structure of neuronal networks in ASD and SZ arising from E/I imbalances.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Topic: D.06. Vision

Support: ERC Starting Grant No. 101041669

Title: An implicit generative model of neuronal morphologies

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Abstract: The brain is unrivaled in its diversity of cell types and their function. Understanding the diversity and complexity of neuronal morphologies is important for understanding how the brain works. In particular, we need quantitative, unbiased methods to capture the structural and morphological features of neurons. However, currently only few generative models of cortical neuronal morphology exist, especially when it comes to precise 3D shape beyond the dendritic and axonal skeleton graph. With the advent of large-scale structural datasets such as MICrONS, using machine learning methods to model precise 3D morphologies becomes feasible. In this work, we propose MorphOcc, a generative model that allows us to (1) learn low-dimensional latent representations of complex neuronal morphologies and (2) generate realistic and diverse neuronal morphologies. We train our model on the MICrONS Minnie dataset, modeling the dendritic morphology of neurons from mouse primary visual cortex. The model is able to represent and reconstruct the morphological structures of hundreds of neurons in this dataset. The learned latent space captures morphological features well and enables cell type classification into known cell types. By interpolating between samples in embedding space, we can generate new instances of neurons without supervision. MorphOcc has the potential to improve our understanding of neurons in the brain by facilitating large-scale analysis and providing an efficient generative model for neuronal morphologies.

Disclosures: L. Hansel: A. Employment/Salary (full or part-time):; Institute of Computer Science and Campus Institute Data Science, University of Göttingen, Germany. **M.A. Weis:** A. Employment/Salary (full or part-time):; Institute of Computer Science and Campus Institute Data Science, University of Göttingen, Germany. **T. Lüddecke:** A. Employment/Salary (full or part-time):; Institute of Computer Science and Campus Institute Data Science, University of Göttingen, Germany. **A.S. Ecker:** A. Employment/Salary (full or part-time):; Institute of Computer Science and Campus Institute Data Science, University of Göttingen, Germany.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: D.06. Vision

Support: Wellcome Trust 104285/B/14/Z
Francis Crick Institute FC001153
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Title: Quantitative analysis of rabies virus-based synaptic connectivity tracing

Authors: A. TRAN-VAN-MINH[1], Z. YE[3,2], ***E. RANCZ**[4,2];
[1]The Francis Crick Inst., [2]The Francis Crick Inst., London, United Kingdom; [3]Univ. of Washington, Seattle, Univ. of Washington, Seattle, Seattle, WA; [4]Mediterranean Inst. of Neurobio., Marseille, France

Abstract: Monosynaptically restricted rabies viruses have been used for more than a decade for synaptic connectivity tracing. However, the verisimilitude of quantitative conclusions drawn from these experiments is largely unknown. The primary reason is the simple metrics commonly used, disregarding the effect of starter cell numbers. Here we present an experimental dataset with a broad range of starter cell numbers and explore their relationship with the number of input cells across the brain using descriptive statistics and modelling. We show that starter cell numbers strongly affect input fraction and convergence index measures, making quantitative comparisons unreliable. Furthermore, we suggest a principled way to analyse rabies-derived connectivity data using the starter vs input cell relationship we describe and validate across independent datasets.

Disclosures: A. Tran-Van-Minh: None. **Z. Ye:** None. **E. Rancz:** None.

Poster

PSTR212. Visual Cortex: Circuits and Cell Types

Location: WCC Halls A-C

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Topic: D.06. Vision

Support: Wellcome Trust & Royal Society 211258/Z/18/Z

Title: Visual discrimination learning changes neuronal activity in primary visual cortex and posterior parietal cortex

Authors: *J. MCCLURE, Jr, M. VARADI, J. POORT;
Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Primary visual cortex (V1) contains precise representations of visual features. Association areas such as the posterior parietal cortex (PPC) are thought to play a key role in translating visual representations into decisions and motor output. Previous studies found that V1 and PPC are both associated with discriminating visual features in a go/no-go discrimination task, where mice had to respond to a rewarded and withhold from responding to a non-rewarded

visual stimulus. Optogenetic silencing of V1 and PPC impairs visual discrimination performance. V1 neurons show increased stimulus selectivity after learning, while PPC neurons are primarily selective for the rewarded stimulus when mice are engaged in a visual discrimination task. However, the pattern of response changes in V1 and PPC, at two opposite levels of the visual cortical hierarchy, have not yet been established. We therefore investigated to what extent V1 and PPC responses to two orthogonal visual orientations changed during visual discrimination learning. We applied two-photon calcium microscopy to image the activity of the same layer 2/3 neuronal populations in V1 and PPC as head-fixed mice learned to discriminate two visual orientations, where one orientation (go stimulus) was rewarded. Mice learned to reliably discriminate between the go/no-go orthogonal stimuli in the visual discrimination task (behaviour d-prime after learning > 2) within 4 weeks. To quantify the amount of information in neural responses, we calculated the neural selectivity of responses before and after learning by dividing the difference in the response to the two stimuli by the pooled standard deviation. Both PPC and V1 neurons displayed an increased selectivity after learning. While the increase in PPC was mainly driven by go-preferring neurons, both go- and no-go-preferring neurons showed increased selectivity in V1. Interestingly, V1, but not PPC, neurons reduced their overall stimulus response across learning. The distinct characteristics of V1 and PPC activity changes in the go/no-go discrimination task indicate that the increased stimulus discriminability in both areas is driven by different mechanisms. Our results show that learning a visually-guided discrimination task increased information about relevant sensory features through distinct patterns of changes in the bottom and top levels of the visual cortical hierarchy. Investigation of these changes will increase our understanding of how visual areas at different hierarchical levels filter relevant sensory input to guide decision-making.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Pan-cortical 2-photon mesoscopic imaging and neurobehavioral alignment in awake, behaving mice

Authors: *E. D. VICKERS[1], S. RECANATESI[2], L. MAZZUCATO[1], D. A. MCCORMICK[1]; [1]Inst. of Neurosci., Univ. of Oregon, Eugene, OR; [2]Dept. of Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: The flow of neural activity across cortex during active sensory discrimination is constrained by task-specific cognitive demands. During spontaneous behavior, the brain is free to sample widely from a vast repertoire of activation motifs. Understanding how these patterns of local and global activity are selected by task demands in a sensory modality-dependent manner will require in-depth study of densely sampled spontaneous activity at single neuron resolution across large regions of cortex.
In a significant advance toward this goal, we developed procedures to record mesoscale 2-photon Ca^{2+} imaging data from two novel *in vivo* preparations that allow simultaneous access to nearly all of the dorsal or lateral cortex. We aligned neural activity with behavioral primitives and high-level behavioral motifs to reveal the existence of large populations of neurons that either increased or decreased their activity with changes in movement and / or arousal. This led to the discovery of widespread, spatially heterogeneous neural ensembles encoding diverse aspects of spontaneous behavior. Preliminary analyses suggest that dynamic activation of these neural communities may align in a principled manner with high-level behavioral motif transitions.
We characterized global neural activity state using factorial Hidden Markov Modeling (f-HMM), revealing complex interactions between behavioral states, movements and population dynamics exhibiting varying degrees of regional and/or global synchrony in different epochs. Finally, we found evidence that directed functional connectivity between cortical areas is modulated by changes in global brain states and movement. Taken together, these findings have the potential to redefine the way we think about the dual influence of arousal and movement on global patterns of cortical activity and information processing.

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Poster

PSTR212. Visual Cortex: Circuits and Cell Types

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Title: Retinotopic organization of feedback projections in primate early visual cortex: implications for active vision

Authors: *M. WANG[1,2,3], Y. HOU[4], L. MAGROU[4], J. A. AUTIO[5], P. MISERY[4], T. COALSON[6], E. REID[6], Y. XU[1], C. LAMY[4], A. FALCHIER[7], Q. ZHANG[1], M.-M. POO[1,2,8,3], C. DEHAY[4], M. F. GLASSER[6,9], T. HAYASHI[5,10], K. KNOBLAUCH[4,11], D. VAN ESSEN[6], Z. SHEN[1], H. KENNEDY[4,1];
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Abstract: Feedback connections play a major role in many theories of brain functions. Previous studies of feedback connections to early visual areas mainly concentrated on the representation of the central visual fields. Here, injections of tracers in macaque at different retinotopic subdivisions of areas V1 and V2 show that the projection patterns to the peripheral representations show significant differences to those to the central representations. The main findings include: (1) preferential projections to subdivisions of different eccentricities: there are 15 cortical areas projecting to peripheral subdivisions but not the central regions; (2) preferential projections to subdivisions for upper and lower visual fields at a given eccentricity: far peripheral lower visual field subdivisions receive stronger projections from the dorsal visual pathway, whereas far peripheral upper visual field, central visual field, and paracentral lower visual field subdivisions all receive stronger projections from the ventral visual pathway. A functional connectivity analysis in human suggests a similar anatomical organization as that observed in macaque. The current study provides the mesoscale connectivity data for understanding the modulation of early visual cortex by higher level cortical regions, which could play an important role in active vision.

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Poster

PSTR213. Visual Responses During Behavior II

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Topic: D.06. Vision

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Title: Retinal optic flow in freely moving non-human primates

Authors: *B. CAZIOT[1], A. KAMINIARZ[2], F. BREMMER[3];
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Abstract: Self-motion through an environment induces retinal optic flow (Lappe et al., 1999). Whether and how self-motion information is recovered from this optic flow pattern has been the topic of intense investigation but remains poorly understood. Furthermore, studies have so far largely focused on special cases of self-motion, such as linear motion with gaze fixed in head-restrained observers. The actual optic flow of naturally behaving observers is far more complex and depends on the three-dimensional structure of the environment, motion of the head and body, and motion of the eyes. We aimed at estimating the statistics of optic flow in naturally behaving non-human primates (macaca mulatta). Monkeys were traveling freely down a ~3m long corridor. We designed a wireless eye-tracker to record eye videos (800x600 at 90Hz) and "point-of-view" head-centered videos (1920x1080 at 30Hz). Validation in humans showed that this system reliably tracked eye-position with less than 1deg of accuracy in freely-moving observers. We also monitored head-position with an Optitrack motion-capture system fusing inertial measurements with positional tracking of active LEDs on the monkey's head at 120Hz, allowing continuous tracking with less than 1mm of positional accuracy. We computed the statistics of eye-movements, head-movements and their interactions. Monkeys exhibited a bimodal distribution of eye-speeds with a majority of slow pursuit eye-movements interspaced by higher-velocity saccadic eye-movements. The distribution of intersaccadic intervals was a typical heavy-tailed distribution with a median of approximately 200ms. The distribution of head-speeds was also bimodal with an alternation of static phases and forward-movement phases, in broad agreement with previous reports (Carriot et al., 2017). Saccadic eye-movements were correlated with head-movements. We then computed head-centered optic flow, eye-centered optic flow, and the position of the focus of expansion within them. We found that eye-position correlated with

the position of the focus of expansion in head-centered optic flow, and was near the fovea in eye-centered optic flow, also in broad agreement with prior studies in humans (Matthis et al., 2022). Overall, our results deepen our understanding of gaze dynamics and optic flow in freely-moving primates.

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Poster

PSTR213. Visual Responses During Behavior II

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Program #/Poster #: PSTR213.02/DD24

Topic: D.06. Vision

Support: DBT/Wellcome Trust India Alliance #IA/S/17/1/503081

Title: Studying the neural basis of real-world vision and cognition in freely moving monkeys

Authors: *S. ARUN, D. BHADRA, S. BHARADWAJ, T. CHERIAN, J. DAS, G. JACOB, J. JOBY, S. MUNDA, S. SAHA, S. SIMON;
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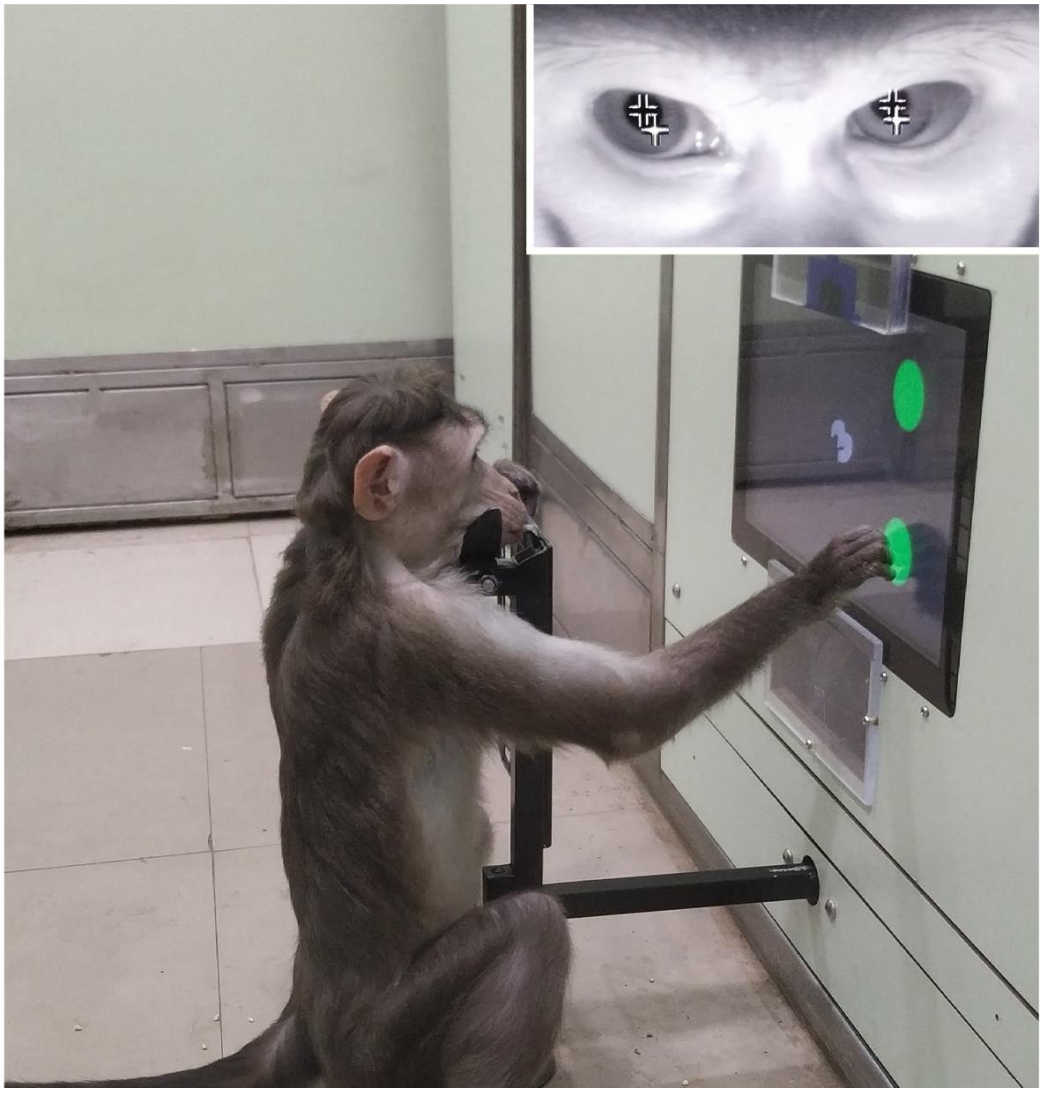
Abstract: Monkeys are widely used to study the neural basis of vision and cognition. Real-world vision involves extensive exploration of objects as well as our surroundings, but most studies are conducted in highly artificial settings where images are shown on a monitor with animals restrained to obtain stable eye signals and neural recordings. Here, we implanted two monkeys with 256 electrodes into high-level visual and motor areas (IT, PMv, vlPFC) to simultaneously record brain activity wirelessly while the animals engaged in a variety of real-world natural behaviors as well as touchscreen-based tasks. We validate and demonstrate the utility of this approach through the following observations: (1) We demonstrate characteristic neural responses to visual images in IT, and to eye and hand movements in PMv and vLPFC; (2) We demonstrate that object identity can be decoded from neural activity while monkeys interact with real objects; (3) We demonstrate that hand movements can be decoded from neural activity while monkeys engage in natural behaviors; (4) We demonstrate common neural signatures during sleep; and (5) We demonstrate simultaneous brain recordings from both monkeys engaged in social interactions. We propose that such wireless recordings in naturalistic environments can reveal the neural basis of real-world vision and cognition.



Disclosures: **S. Arun:** None. **D. Bhadra:** None. **S. Bharadwaj:** None. **T. Cherian:** None. **J. Das:** None. **G. Jacob:** None. **J. Joby:** None. **S. Munda:** None. **S. Saha:** None. **S. Simon:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR213.03/DD25

Topic: D.06. Vision

Support: JSPS KAKENHI Grant Number 20K06920
JSPS KAKENHI Grant Number 15K06709

Title: Single-unit activity in cortical area medial superior temporal (MST) area associated with short-latency ocular following responses (OFRs): Evidence for temporal impulse response function of the visual system

Authors: *A. TAKEMURA[1], K. MIURA[2];
[1]AIST, Tsukuba, Japan; [2]Dept. of Pathology of Mental Diseases, Natl. Inst. of Mental Hlth., Kyoto Univ., Kodaira, Tokyo, Japan

Abstract: Two frame animations presented with inter-stimulus intervals (ISIs) induce reversed motion percepts, suggesting that the characteristics of the temporal filters embedded in the visual system are biphasic. Recently, an experimental paradigm was developed to identify the kernel of the temporal filters was reported that two-frame apparent motion stimuli induced short-latency ocular following responses (OFRs) on humans (Ohnishi et al. 2016). Furthermore, we found that the OFRs of monkeys were qualitatively consistent with the findings on humans (Takemura and Miura, 2017). In the present study, to further understand the neuronal representations underlying the early visual motion processing, single neuron activities were recorded in the medial superior temporal (MST) area of monkeys (*Macaca fuscata*). We applied the above-mentioned experimental paradigm on monkeys to infer temporal resolutions of the visual system from quantitatively identified the kernels of the filters based on the OFRs. The OFRs were elicited by two-frame apparent motion stimuli of a vertical sinusoidal grating (spatial frequency, 0.25 cycles/°; Mickelson contrast, 32%) on two monkeys. Eye movements were recorded by the electro-magnetic induction technique. Each trial was started by presenting a small fixation spot on a gray uniform background. After the left eye was positioned within ±1.5º of the fixation target for a brief, randomized period, a grating appeared as a background of the fixation spot. In the first experiment, a 90° step of the grating was presented with various ISIs (0-640 ms). In the second experiment, a 90° step of the grating was applied with various durations of the initial image frame (Motion onset delay: MODs; 10-640 ms).We found that most of the MST neurons increased their firing rate before the OFRs and their neuronal responses were larger when the stimulus stepped in the preferred than anti-preferred direction, as was observed in the previous OFRs studies. The first experiment showed the presence of inverted neuronal and ocular responses (population averages) to 90° steps of gratings presented with ISIs, as was observed with motion percepts. And the second experiment showed the decreases of neuronal and ocular responses (population averages) to longer exposure to the initial image, suggesting that longer exposure to the stationary image prior visual motion reduces the driving signals of OFRs, as was observed with “post-saccadic enhancement” of OFRs. Since the neuronal responses preceded the OFRs, these results suggest a causal link between neuronal responses in the MST and OFRs to two-frame movies.

Disclosures: **A. Takemura:** None. **K. Miura:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR213.04/DD26

Topic: D.06. Vision

Support: National Science and Technology Innovation 2030 Major Program (2022ZD0204802)
National Natural Science Foundation of China (31930053)

Title: Time Course of Orientation Ensemble Representation in Human Brain

Authors: *X. GONG[1], T. HE[2], Q. WANG[1], J. LU[1], F. FANG[1];
[1]Peking Univ., Beijing, China; [2]Beijing Language and Culture Univ., Beijing, China

Abstract: Natural scenes are filled with groups of similar items. To exploit this feature, humans employ ensemble coding to extract the summary statistical information of the environment, thereby enhancing the efficiency of information processing. However, the neural mechanism underlying the representation of ensemble information in the brain remains elusive. In particular, it remains unclear whether ensemble representation is the mere summation of representations of individual items or it engages other specific processes. Here, we utilized a set of orientation ensembles wherein none of individual item orientations was the same as the ensemble orientation. We recorded human magnetoencephalography signals when participants performed an ensemble orientation discrimination task. Time-resolved multivariate pattern analysis (MVPA) and the inverted encoding model (IEM) were employed to unravel the neural mechanisms of the ensemble representation and track its time course. First, our findings revealed successful decoding of the ensemble orientation, with a high correlation between decoding accuracy and behavioral accuracy. In addition, using IEM, we demonstrated that the representation of ensemble orientation was different from the sum of the representations of individual item orientations, suggesting that ensemble coding would further modulate orientation representation in the brain. Using source reconstruction, we identified V2 and V3 as potential neural substrates for the representation of ensemble orientation. Taken together, our findings revealed the emergence of the ensemble representation in the human visual cortex.

Disclosures: X. Gong: None. **T. He:** None. **Q. Wang:** None. **J. Lu:** None. **F. Fang:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

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Program #/Poster #: PSTR213.05/DD27

Topic: D.06. Vision

Support: The startup fund for junior researchers from King Mongkut's University of Technology Thonburi (KMUTT) to S.I.
The Frontier Research Unit Grant for Neuroscience Center for Research and Innovation to Learning Institute at KMUTT
The Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B) (fiscal year 2022-2023)

to CC and SI
The research grant from the Research & Innovation for Sustainability Center, Magnolia Quality Development Corporation Limited, Thailand to SI

Title: Dissociable neural mechanisms underlying selective attention, perceptual difficulty, and global cognitive factors

Authors: *P. SAWETSUTTIPAN[1], P. PHUNCHONGHARN[1], K. OUNJAI[1], S. PONGSUWAN[2], S. INTRACHOOTO[2], N. RUNGRATSAMEETAWEEMANA[3], S. ITTHIPURIPAT[1];
[1]King Mongkut's Univ. of Technol. Thonburi, Bangkok, Thailand; [2]Happiness Sci. Hub, Res. & Innovation for Sustainability Ctr., Bangkok, Thailand; [3]Neurosciences Grad. Program, Univ. of California, San Diego, CA

Abstract: Selective attention and perceptual demand play a critical role in shaping the efficiency and speed of early sensory and decision processes. Directing attention enhances processing of the attended stimulus, while increased perceptual difficulty can amplify the effects of selective attention on visual cortical processing. However, when a perceptual task becomes excessively challenging, the effects of selective attention may diminish. Although recent studies have systematically investigated the effects of selective attention and perceptual difficulty, the potentially distinct neural dynamics that underlie these processes and their relationship with global cognitive factors such as stress and arousal remain poorly understood. To address this gap, our study aimed to uncover the distinct neural processes associated with selective attention and perceptual difficulty, and examine how they differ from global cognitive factors. Using EEG, we recorded brain activity while participants performed a visual discrimination task. We manipulated selective attention with spatial cues and perceptual difficulty by adjusting contrast increments of visual stimuli. Additionally, we examined the effects of general task difficulty on medium-difficulty trials interleaved between easy and difficult blocks. Our findings revealed that subjective stress and arousal levels were significantly higher during difficult blocks compared to easy blocks. Consistent with past studies, we observed that attentional modulations of early visually evoked activity (P1 component) and decision-related positive deflection (P3 component) were attenuated under high perceptual difficulty. However, global task difficulty modulated behavioral performance without affecting these neural responses. Notably, alterations in global task difficulty did influence oscillatory events in alpha and beta band frequencies, which are thought to reflect overall task engagement and arousal states in the cortex. Taken together, our study sheds light on the distinct influences of selective attention, perceptual difficulty, and global cognitive factors on early sensory and decision-making processes.

Disclosures: P. Sawetsuttipan: None. **P. Phunchongharn:** None. **K. Ounjai:** None. **S. Pongsuwan:** None. **S. Intrachooto:** None. **N. Rungratsameetaweemana:** None. **S. Itthipuripat:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR213.06/DD28

Topic: D.06. Vision

Support: NIH R01 Grant EY028811

Title: Noise correlations and choice probabilities during learning and task switching in V4

Authors: *A. PLETENEV[1], S. LIU[1], R. M. HAEFNER[1,2], A. C. SNYDER[1,2];
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Abstract: Feedback connections are ubiquitous in sensory cortex, yet the signals they carry during perceptual decision-making remain unclear. An important clue is provided by choice-related signals and the structure of correlated neural covariability (noise correlation, *NC*). Previous studies have found *NC*s to be task-specific, which strongly suggests that they at least partly emerge during learning due to task-aligned feedback (Bondy et al., 2018). Together with the fact that they resemble information-limiting correlations (*ILC*s, Moreno-Bote et al., 2014) this leads to the counter-intuitive suggestion that *ILC*s become stronger as the behavioral performance improves. While computational models of hierarchical approximate inference predict this seemingly paradoxical effect (Haefner et al., 2016; Lange & Haefner, 2022), it conflicts with models and data from attention studies which suggest the opposite (Ni et al., 2018).

We measured choice probabilities (*CPs*) and *NCs* and examined their relationships to task sensitivity (*d'*) of neurons in monkey V4; first while learning a discrimination task between +45 and -45deg orientations, and then during trial-by-trial interleaved switching with a second orientation discrimination task, 0 vs 90deg. Since we used a chronically implanted multi-electrode array, neuron populations between sessions were not independent, and combining sessions would have created a repeated measurements problem and inflated p-values. Therefore, we employed a hierarchical bootstrap method: we bootstrapped the electrodes of the array first, followed by sessions and trials.

We estimated the strength of *ILC*s as a slope in *NC* - *d'd'* relationship. *ILC*s were statistically significant for each task, but only after the monkey learnt the task. Over learning, we observed an increase in *ILC*s, which were significantly correlated with the degree of learning. Importantly, this development was observed only in *ILC*s for the task (*d'*) being learnt, not for the alternative task.

During interleaved sessions, *ILC*s did not change between tasks on a trial-by-trial basis, suggesting that the structure of feedback was static on a short time scale. However, a neuron's *CP* did change between the two tasks in accordance with its task sensitivity: together with no change in *ILC*s, this most likely reflects a change in read-out weights.

Finally, we found the emergence of a positive *CP-d'* relationship over learning, as reported in Law & Gold, 2008 but called into question by more recent work.

In conclusion, our findings provide evidence supporting the hypothesis that feedback signals, as manifested in *CP*s and *NC*s, represent expectations during hierarchical inference.

Disclosures: A. Pletenev: None. **S. Liu:** None. **R.M. Haefner:** None. **A.C. Snyder:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

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Program #/Poster #: PSTR213.07/EE1

Topic: D.06. Vision

Support: JSPS Grant-in-Aid for Scientific Research (A) 20H00597
JSPS Grant-in-Aid for Transformative Research Areas (A) 23H04330

Title: Task dependent changes in brain-activity patterns for color stimuli in human brain

Authors: *I. KURIKI[1], T. ARIMA[1], T. HAMANO[1], S. TAKANO[1], K. UENO[2];
[1]Saitama Univ., Saitama, Japan; [2]RIKEN, Wako-shi, Japan

Abstract: [Intro] Human vision has at least two types of color perception: one is categorical and the other is mere color appearance. The former is to verbalize a color, while verbal descriptions are sometimes difficult for a pictorial perception in the latter. Although both are perceived quite naturally, not many studies have been published on the cortical representation for color appearance. A previous study reported changes in categorical representation when participant's attention was diverted away from color stimulus. The present study will report the effect of diverted attention on the cortical representation of the color appearance. [Methods] Ten healthy participants with normal color vision participated in the experiment, which was approved by institutional review board in accordance with the Declaration of Helsinki. All participants provided written informed consent. The visual stimulus was a flickering check pattern with the duration of 3 s/trial, subtended 7 deg in diameter, and colored in one of 12 hues under isoluminance. A random character was also presented every second at the center of the screen. The stimuli were presented on a calibrated LCD screen. The participants performed one of the following tasks in each run: (1) color category, (2) color appearance, or (3) two-back tasks. In the color category task, participants were instructed to report the stimulus color by pressing one of five buttons corresponding to red, green, blue, yellow, or purple. In the color appearance task, participants were asked to press buttons, correspond to red, green, blue, or yellow three times to report color more precisely. The two-back task was highly attention demanding, in which participants were asked to press a button when they identified two same letters with another letter in between. The stimulus was same across all task conditions. Functional MR images were obtained with a voxel size of 3 mm x 3 mm x 3 mm (TR = 1,000 ms, 252 volumes/run) using a GE-EPI protocol on a 3T scanner (Siemens Prisma, Germany) at RIKEN CBS. [Results] We used representation similarity matrices, sized 12 x 12 whose entries represent correlation coefficients between responses to all pairs of 12 hues, to evaluate similarities between perception and brain activity for the identification of brain areas responsive to each task. The analysis revealed distinct brain regions exhibiting higher similarity to the color tasks. The two-back task significantly affected cortical representations in a part of the inferior frontal gyrus and ventro-occipital areas, which implies that the representation of color appearance can be also affected by the attentional state.

Disclosures: **I. Kuriki:** None. **T. Arima:** None. **T. Hamano:** None. **S. Takano:** None. **K. Ueno:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR213.08/EE2

Topic: D.06. Vision

Support: 20016186

Title: Cybersickness in Augmented Reality: Gradual changes with the course of prolonged exposure

Authors: ***Z. PARK**[1], J. OH[1], M. JIN[1], S. SONG[1], S. JEON[1], H. SHIN[2], C.-Y. KIM[1]; [1]Sch. of Psychology, Korea Univ., Seoul, Korea, Republic of; [2]UCL Interaction Ctr., London, United Kingdom

Abstract: Growing interest in virtual reality (VR) and augmented reality (AR) emphasizes the need for guidelines for proper and healthy usage of such new technical instruments. Compared to the intensive evidence regarding cybersickness in VR, however, symptomatic experiences in AR are not yet thoroughly tackled (Vovk et al., 2018). Therefore, this study aims to investigate cybersickness and discomfort during the use of AR equipment while manipulating content intensity and exposure duration. Participants viewed racing simulation game videos through AR glasses. Content intensity was defined by racing speed: slow (110km/h on average) and fast (290km/h). Participants watched three 10-minute videos of one speed type on the first day and another speed type a week after. This resulted in a 30-minute AR equipment exposure per day. Cybersickness was reported subjectively with the Simulator Sickness Questionnaire (SSQ; Kennedy et al., 1993). SSQ was given before the initial exposure, after every 10-minute stimuli, after the whole 30-minute exposure, and finally, after taking off the AR glasses. While watching the video, participants performed a detection task in which they reported transitions of the video scene from color to grayscale. The SSQ results showed that as exposure duration got prolonged, the total score of SSQ showed a steady increase—scores significantly differed from the pre-test SSQ after 20 cumulative minutes of exposure. Only after taking off the glasses did the subscale scores return to the initial state. Among the three subscales of SSQ, oculomotor (O) scores were greater than nausea (N) and disorientation (D), indicating that the participants' discomfort was related most to the oculomotor domain. The behavioral results revealed that as the exposure duration increased, participants' color change detection reaction times also increased. The effect of speed was not significant on both SSQ scores and reaction times. Our study shows that prolonged usage of AR equipment can lead to an increase in cybersickness and discomfort, particularly concerning oculomotor-related issues. This shows distinguishable profiles compared to previous VR studies employing similar visual stimuli (Hughes et al., 2020), since the symptoms from VR exposure are reported to be related most with D, and least with O (Stanney

& Kennedy, 1997). These findings highlight the need for future research specifically targeting AR. We are currently investigating the related psychophysiological responses to further support these subjective reports.

Disclosures: **Z. Park:** None. **J. Oh:** None. **M. Jin:** None. **S. Song:** None. **S. Jeon:** None. **H. Shin:** None. **C. Kim:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR213.09/EE3

Topic: D.06. Vision

Support: NIH EY026924
NIH NS113073
NIH EY014800
NIH EY031477
Research to Prevent Blindness

Title: Characterizing the neuronal correlates of perisaccadic mislocalization in V4

Authors: *G. WENG[1,2], B. NOUDOOST[2], N. NATEGH[2,3];
[1]Dept. of Biomed. Engin., [2]Dept. of Ophthalmology and Visual Sci., [3]Dept. of Electrical and Computer Engin., Univ. of Utah, Salt Lake City, UT

Abstract: The brain generates a perception of the visual space through processing and interpreting the location of stimuli projecting on the retina, but the mechanism by which the brain encodes spatial information is not well understood. To understand the spatial coding of neurons, it is crucial to investigate alterations in neural activity that occur along with changes in visuospatial perception. During saccadic eye movements (saccades), altered spatial perception has been reported, including perisaccadic mislocalization: the bias in the perceived location of visual stimuli appearing near the time of a saccade. In this study, we measure perisaccadic mislocalization in rhesus macaque monkeys through combined behavioral and physiological experiments, and we aim to establish a link between perisaccadic modulations in extrastriate responses and their behavioral counterparts. We use array or single electrodes to record neuronal activity in area V4. Monkeys perform a visually guided saccade task while their eye movements are monitored with a high-resolution eye-tracking system. In each trial, the monkey makes a saccade from a fixation point to a peripheral saccade target. During fixation and saccade execution, a 50-ms visual probe stimulus is presented in one of 9 possible locations in a 3×3 grid placed around the V4 neuron's receptive field. When the probe disappears, the monkey makes another saccade to the perceived location of the stimulus. Mislocalization is measured as the deviation between the perceived location of stimuli presented around the first saccade to those presented during fixation. We found that the perceived locations of stimuli presented around the

time of saccade execution are altered. We studied how V4 neurons changed their response to the same stimulus when the animal's perception of the stimulus location was altered during saccades. In future work, we will explore computational frameworks to develop readouts of V4 responses and assess their contribution to perisaccadic alterations, which could be validated by the experimental measurements. Our approach can reveal the neural substrate underlying perisaccadic mislocalization, and the role of V4 neurons in the representation of location information.

Disclosures: G. Weng: None. **B. Noudoost:** None. **N. Nategh:** None.

Poster

PSTR213. Visual Responses During Behavior II

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Program #/Poster #: PSTR213.10/EE4

Topic: D.06. Vision

Support: NIMH Grant ZIAMH002958
NIMH Intramural Research Training Award (IRTA) Fellowship Program

Title: Behavioral detection of optogenetic stimulation in macaque V4 cortex

Authors: *R. LAFER-SOUSA, L. KELEMEN, T. SWEDAN, D. NGUYEN, R. AZADI, E. SHAHBAZI, A. AFRAZ;
Lab. of Neuropsychology, NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD

Abstract: Perturbation of neural activity in the visual system alters visual perception. Understanding the nature of the perceptual events evoked by neural perturbations is essential for bridging the causal gap between neuronal activity and vision as a behavior. This knowledge is also crucial for identifying the neural underpinnings of visual delusions in psychiatric disease and developing effective visual prosthetics for patients with severe visual impairment. While anecdotal human studies provide invaluable insights, high throughput and systematic study is impossible without the appeal to nonhuman primate research. Historically, optogenetic perturbation studies often struggle to obtain large behavioral effects in monkeys. Here we utilize Opto-Array, a novel chronically implantable array of LEDs, allowing stimulation of the same cortical sites across many sessions, and a stimulation-detection task unrestricted by prior assumptions about the tuning properties of the targeted neurons or the perceptual effect of stimulation. In contrast to prior optogenetic studies in monkeys, we have found this approach yields robust behavioral effects in inferior temporal cortex. Here we assess whether monkeys can detect optogenetic stimulation of V4 cortex. Two macaque monkeys were chronically implanted with LED arrays over a region of V4 cortex transduced with the depolarizing opsin C1V1. In one animal a second array was implanted in the corresponding region of V4 in the opposite hemisphere where no virus was injected (control site). The animals were trained to detect optogenetic stimulation while looking at different images. In each trial, following fixation an

image was displayed on the screen for 1s. In half of trials, randomly selected, a 200ms optical impulse was delivered halfway through image presentation, and the animal was rewarded for correctly identifying whether the trial did or did not contain cortical stimulation. Both animals learned to detect optical impulses delivered to their transduced V4 cortex significantly above chance within 11 and 7 sessions respectively (Chi-sq, p-values < 0.01) and improved their performance to 90% and 83% correct after 27 and 13 more days of training (Chi-sq, p-values < 0.001). The animal with the control array was not able to detect cortical illumination over the control area. The results show for the first time that monkeys can reliably detect optogenetic stimulation of V4 cortex, opening the door to systematic causal studies of V4 with optogenetic methods. Consistent with our recent studies in inferior temporal cortex, the results show that it is possible to induce large behavioral effects using optogenetics in nonhuman primates.

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Poster

PSTR213. Visual Responses During Behavior II

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Program #/Poster #: PSTR213.11/EE5

Topic: D.06. Vision

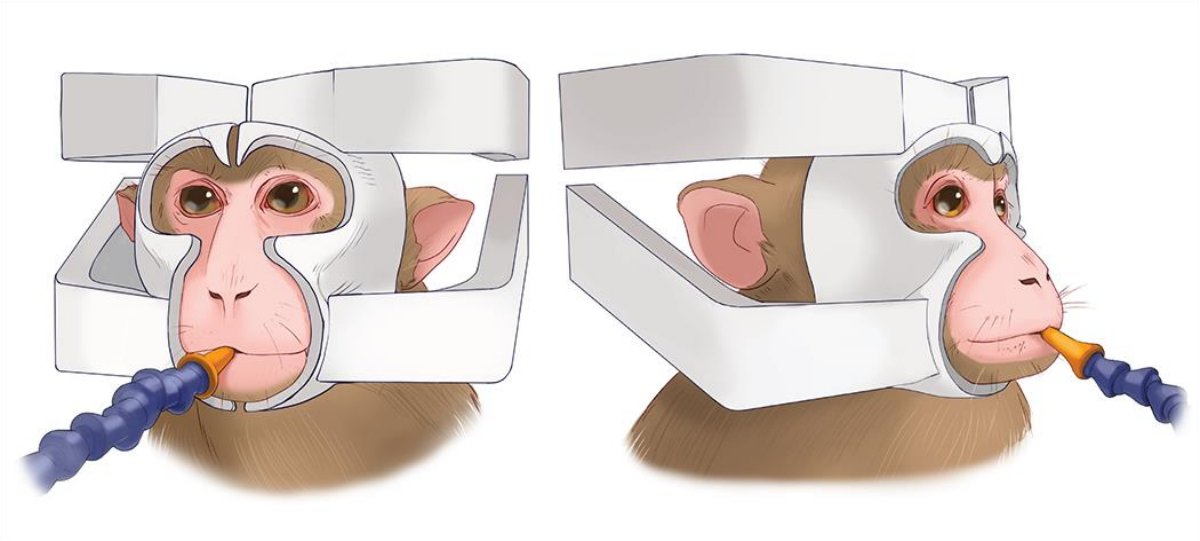
Support: NIMH Grant ZIAMH002958
NIMH Intramural Research Training Award (IRTA) Fellowship Program

Title: Custom fit non-invasive 3D-printable head immobilization system for non-human primates

Authors: *T. SWEDAN[1], E. SHAHBAZI[1], T. MA[2], R. LAFER-SOUSA[1], R. AZADI[1], A. RYAN[1], D. NGUYEN[1], A. AFRAZ[1];
[1]NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD; [2]New York Univ. Ctr. For Neural Sci., New York, NY

Abstract: Non-human primate vision research often requires head immobilization to measure eye movements and record neural signals. Researchers frequently use surgically implanted headposts for this purpose, however, these come with risks and limitations. First, headposts may fail over time and are prone to infection. Second, they take up a large footprint on the skull which imposes experimental and surgical constraints. Finally, headposts exclude designs with voluntary animal engagement, such as cage-side research. Multiple non-invasive head immobilization systems (NHIS) have been produced but lack wide adoption, perhaps because of the difficulty to construct and modify for diverse applications. We created a novel 3D-printable NHIS for macaques which is easy to customize and produce. We used a CT scan of the monkey's head and a custom software called FLoRIN to construct a 3D-printable helmet with cutouts for reward delivery, eye-tracking, and physiology hardware. The design includes an

optional backpiece that can be engaged for full restraint around the head. We investigated the NHIS's efficacy by comparing eye-tracking measurements recorded from a headpost-immobilized monkey with a helmet-stabilized monkey. This helmet-stabilized monkey was trained to voluntarily place its head in the helmet and fixate on targets presented on the screen up to 10° eccentricity for liquid reward. Without requiring the use of full restraint via the backpiece, the helmet-stabilized monkey showed high fixational precision (0.23±0.11°) and accuracy (0.47±0.21°), comparable to the precision (0.11±0.04°) and accuracy (0.41±0.24°) of the headpost-immobilized monkey. These results suggest very suitable eye-tracking accuracy for broad use of our NHIS in visual research. Furthermore, the 3D-printed nature of the system improves the ease of modification and construction over previous NHIS. These results suggest this design could facilitate a move away from surgically implanted head-immobilization systems.



Disclosures: **T. Swedan:** None. **E. Shahbazi:** None. **T. Ma:** None. **R. Lafer-Sousa:** None. **R. Azadi:** None. **A. Ryan:** None. **D. Nguyen:** None. **A. Afraz:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR213.12/EE6

Topic: D.06. Vision

Support: NIH Grant EY032900
NIH Grant OD010425

Title: Digital dual-Purkinje-image eye tracking enables precise characterization of visual receptive fields in fixating macaques

Authors: *R. K. A. RESSMEYER[1], J. YATES[3], G. HORWITZ[2];
[1]Univ. of Washington, Mercer Island, WA; [2]Univ. of Washington, Seattle, WA; [3]Optometry, UC Berkeley, Berkeley, CA

Abstract: Understanding the relationship between visual stimuli and neural activity is a fundamental goal in visual neuroscience. However, the study of visual neurophysiology in awake primates is inherently complicated by the constant occurrence of eye movements, even during periods of nominal fixation. To address this challenge, we adapted a recently developed high-resolution digital dual-Purkinje-image (dDPI) eye tracker (Wu et al., 2023), capable of accurately correcting for miniature eye movements, for use with macaque monkeys. Our eye tracker is designed to mount to a standard Crist primate chair and runs with submillisecond processing time on a 12th gen Intel CPU. To evaluate the efficacy of dDPI eye tracking for studying visual processing, we recorded single-unit extracellular electrophysiological signals from the lateral geniculate nucleus of a fixating monkey while a spatially-correlated noise stimulus was displayed. As a result of properly accounting for eye movements, the spike-triggered average pixel contrast increased by 65.8% and 90.4% in the two units analyzed thus far, while the predictive performance (R-squared) of a generalized linear model improved from 0.046 and 0.057 to 0.128 and 0.222, respectively. Additionally, the estimated center radii contracted to values equal to or smaller than those reported in the literature. Thus, our analysis revealed that the accuracy, precision, and stability of gaze estimates provided by dDPI eye tracking are sufficient to correct for fixational eye movements. In addition to tracking the Purkinje images, we simultaneously estimated the pupil center and size. Notably, correcting for eye movements using the estimated locations of the pupil center and corneal reflection—the standard method in video eye tracking—yielded worse model fits and larger receptive field sizes. This result implies that the pupil center is an inaccurate reporter of small eye movements, while the Purkinje images may be veridical. Thus, dDPI eye tracking is a powerful option for researchers interested in studying fine-scale visual processing in awake animals.

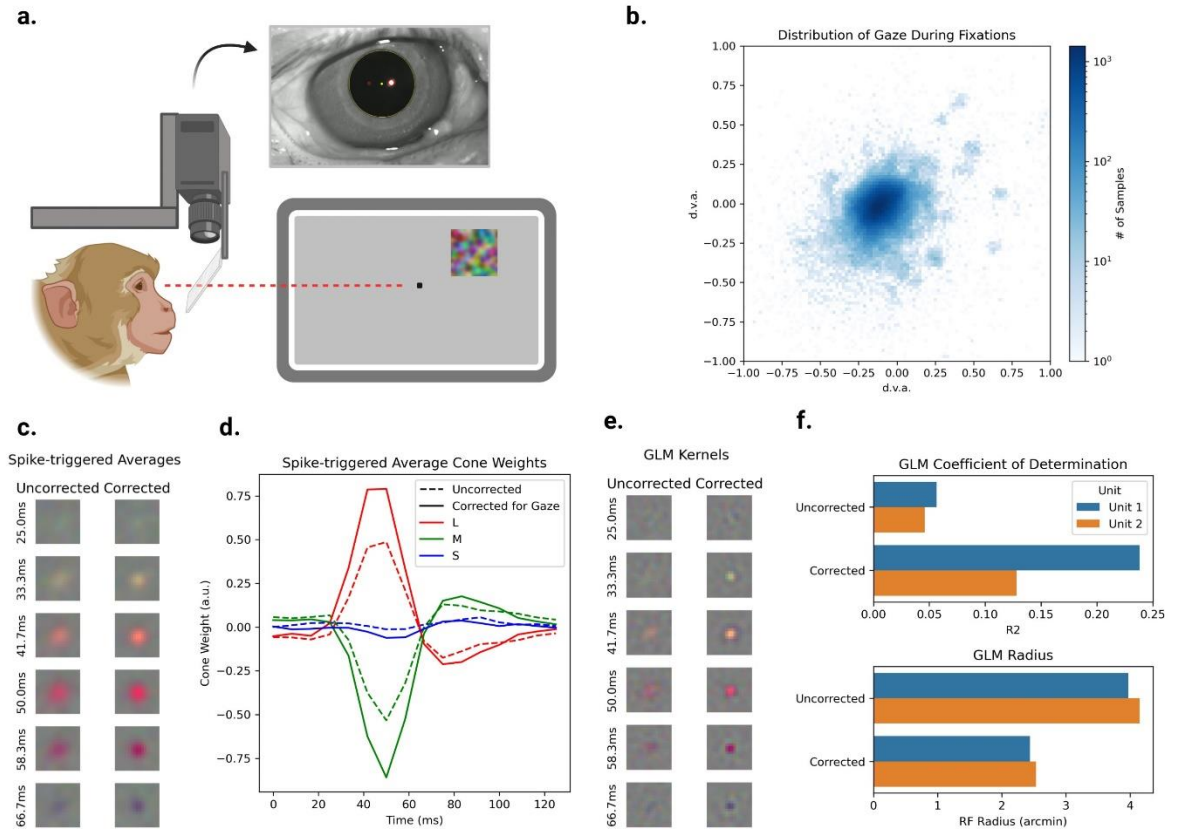


Figure 1: Overview of methods and results. (a) Visual representation of the experimental paradigm. The monkey was trained to fixate a point while a correlated noise stimulus was projected over a unit's RF. Above is a depiction of the digital recording apparatus comprising camera, mount, and hot-mirror. Inset is an example of a single frame captured from the eye tracker with identified points highlighted. (b) Distribution of gaze during periods of fixation. Any deviations of gaze from the origin will result in inaccurate analysis of neural responses if uncorrected. (c) Spike triggered averages at increasing lags for the gaze-corrected and gaze-uncorrected stimuli. (d) Temporal cone weights for the highest contrast pixel in both gaze-corrected and gaze-uncorrected conditions. Contrast increases when gaze is corrected. (e) Spatiotemporal kernels from GLMs fitted to gaze-corrected and gaze-uncorrected stimuli. (f) R^2 and RF center radii for two recorded neurons for the gaze-corrected and gaze-uncorrected stimuli. In both cases, correcting for eye movements resulted in higher R^2 and smaller radii.

Disclosures: **R.K.A. Ressmeyer:** None. **J. Yates:** None. **G. Horwitz:** None.

Poster

PSTR213. Visual Responses During Behavior II

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR213.13/EE7

Topic: D.06. Vision

Support: NIH Grant R01 EY016187
NIH Grant R01 EY025670

NIH Grant P30 EY012196
NIH Grant R01 EY026025

Title: Responses in primate ventral visual cortex track individual fixations during natural vision

Authors: *W. XIAO, S. SHARMA, G. KREIMAN, M. S. LIVINGSTONE;
Harvard Med. Sch., Boston, MA

Abstract: During natural vision, primates shift their gaze several times per second with large, ballistic eye movements known as saccades. It remains an open question to what extent visual neurons retain classical retinotopic properties, integrate information across fixations, and predictively remap during active vision in complex scenes relevant to natural behavior. We let 13 monkeys freely view thousands of naturalistic images across 4.7 million fixations, recorded over 883 hours of neuronal responses throughout the macaque ventral visual pathway, and designed flexible analyses to probe the neuronal spatial, temporal, and feature selectivity. Ventral visual neurons responded to each fixation and did not become more gaze-invariant as monkeys examined the same image for seconds. Computational models revealed that neurons continued responding to retinotopic receptive fields. The results suggest that the ventral visual cortex remains predominantly retinotopic during natural vision instead of establishing a gaze-independent representation of the world. An early version of this work is available as a preprint on biorxiv: 2023.02.08.527666.

Disclosures: **W. Xiao:** None. **S. Sharma:** None. **G. Kreiman:** None. **M.S. Livingstone:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.01/EE8

Topic: D.08. Multisensory Integration

Title: Multimodal Integration of Olfactory and Gustatory Stimuli in Locust Superior Lateral Protocerebrum Neurons

Authors: *B. KIM[1,2], M. A. STOPFER[3];
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Abstract: Multimodal Integration of Olfactory and Gustatory Stimuli in Locust Superior Lateral Protocerebrum Neurons
The experience of flavor requires both olfactory and gustatory input. Numerous studies have explored how olfactory and gustatory information are processed separately in the brain, and some behavioral studies (e.g., Elliot and Maeir, 2020) have examined the integration of these two senses. Nevertheless, the specific mechanisms by which individual neurons process and integrate

multimodal input remain unclear. We addressed this question in a simple model system, the locust Schistocerca americana. To identify neurons that receive both olfactory and gustatory information, we first mass-stained 1st and 2nd order olfactory and gustatory neurons and traced their projections deeper into the brain, seeking sites where the two chemosensory pathways converge. We stained neurons in the periphery by making small incisions into the antenna (olfactory) or palp (gustatory) and then submerged these tissues into a neurobiotin solution, and subsequently conjugated the dye with fluorophores. To stain 2nd order neurons, we inserted blunt glass microelectrodes loaded with micro ruby dye into the antennal lobe (olfactory) and the glomerular lobe (gustatory). Stained tissue was then imaged with a confocal microscope. These results and published descriptions of lateral horn neuron projections (e.g., Gupta and Stopfer 2012) revealed the superior lateral protocerebrum (SLP) as a possible convergence site. Next, with in vivo preparations, we used sharp electrodes to make intracellular recordings from SLP neurons. With a novel delivery system that controls stimuli with high temporal precision (~50 ms) we presented odors to the antenna and tastants to the palp. Our preliminary results show some SLP neurons respond to both olfactory and gustatory stimuli. Some of these neurons appeared to respond to odors and tastes with different firing patterns, suggesting they convey information about both sensory modes in their responses. Our preliminary results from simultaneous odor and taste delivery suggest integration is best described as the average of the separate inputs, as predicted by theoretical studies (Xu et al 2017). Our findings contribute to a better understanding of the dynamic integration of odor and taste and shed light on how the brain integrates complex multimodal information.

Disclosures: B. Kim: None. **M.A. Stopfer:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.02/EE9

Topic: D.08. Multisensory Integration

Title: Using Magnetoencephalography (MEG) to disentangle what happens when vision and touch converge: A multivariate source-level approach to the Rubber Hand Illusion

Authors: *M. F. A. HAUSER[1], S. COPPI[1], D. LUNDQVIST[2], H. H. EHRSSON[1];
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Abstract: A well-known example of multisensory integration is the Rubber-Hand Illusion (RHI). The presentation of a visual and a tactile touch simultaneously, respectively, on the participant's hand as well as a rubber hand that is placed in an anatomically plausible orientation, can induce the feelings that the seen and felt touch arise from the same event and that the fake hand is perceived as the own.
This phenomenon has been thoroughly investigated using functional magnetic resonance imaging (fMRI). However, this method often relies on univariate contrasts that constrain

interpretability, and secondly, they do not provide information on the dynamics underlying the unisensory processes, the multisensory integration processes, and the emergence of illusion-related processes.
To fill this knowledge gap, we conducted a large MEG study, that employed a specialized set-up with a pneumatically driven brush-robot to administer controlled touches to the real left hand and a corresponding rubber hand with temporal precision. The design, administered to a total of 46 participants, covered 9 different conditions across 2000 stimulation events, encompassing uni- and multisensory visuotactile stimulation, as well as systematic changes to the spatial congruence of rubber hand placement, and synchronicity (visuotactile synchrony or asynchrony), and a 'real hand' condition. Behaviorally, this set-up was able to induce the established illusion based on questionnaire results and changes in hand position sense towards the location of the rubber hand (proprioceptive drift).
At the neural level, we used a whole-brain approach, in which we first reconstructed brain activity at a single trial level followed by representational similarity analysis to disentangle commonalities and differences across conditions based on their belonging to a visual or tactile, a uni- or multisensory, a congruent or incongruent rubber hand placement, or whether they would result in the sensation of ownership.
The preliminary results of this approach revealed that the effect of synchronous stimulation patterns were differentiable from other conditions in frontal and parietal areas, whereas congruence was largely localized to inferior occipital areas immediately following the touch. For conditions involving a sensation of ownership, a parietofrontal network during the anticipatory phase, at around -80 ms was observed, followed by a sequence of cerebellar, pre-SMA, and bilateral premotor areas at around 160 ms after the touch. These results corroborate the involvement of brain regions often outlined in MRI studies but reveal their participation in different processes before and after the touch.

Disclosures: **M.F.A. Hauser:** None. **S. Coppi:** None. **D. Lundqvist:** None. **H.H. Ehrsson:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.03/EE10

Topic: D.08. Multisensory Integration

Support: VISTA Grant
CBB Seed Grant

Title: Examining the Relationship between GABA and GLX Neurotransmitters and Audiovisual Integration: A Transcranial Direct Current Stimulation and Spectroscopy Study

Authors: *V. UNNISA BEGUM[1], R. COHAN[2], J. K. E. STEEVES[2], D. ABULEIL[1], B. THOMPSON[1], M. BARNETT-COWAN[1];
[1]Univ. of Waterloo, Waterloo, ON, Canada; [2]York Univ., York Univ., North York, ON, Canada

Abstract: A critical aspect of human perception involves the central nervous system's capacity for multisensory integration, which fosters coherent interpretation of real-world events. Notably, audiovisual integration (AV) relies on the temporal binding window (TBW), a range of temporal offsets within which multi-modal information is perceived as simultaneous. Prior behavioral studies indicated broader TBWs in older adults relative to their younger counterparts (Bedard & Barnett-Cowan, 2016; Setti et al., 2011; Basharat et al., 2018). Such differences have been theorized to stem from age-related variations in brain excitation and neuronal chemistry (Bedard & Barnett-Cowan, 2016). Our study aimed to associate measures of AV integration (temporal order and simultaneity judgement tasks) with the brain concentrations of neurotransmitters gamma amino-butyric acid (GABA; inhibitory) and glutamate/glutamine (GLX; excitatory). We hypothesized a negative correlation between TBW width and GABA and GLX quantities. We engaged 14 healthy young adults (mean age 27.07±5.6; 4 males, 11 females) with normal vision and hearing. We conducted visual and auditory acuity tests, and participants performed simultaneity judgement (SJ) and temporal order judgement (TOJ) tasks involving auditory beeps and light flashes. Magnetic resonance spectroscopy (MRS) provided GABA and GLX estimates before and after the application of transcranial direct current stimulation (tDCS) over the visual cortex to modulate cortical excitability. Results to date reveal no significant differences in pre- and post-tDCS GABA and GLX levels. Correlation analysis, however, exposed a significant positive correlation between age and GABA and GLX concentrations under certain pre- and post-tDCS conditions. Moreover, GABA and GLX measures were inversely related to TBWs of TOJ and SJ tasks, suggesting that neurotransmitter concentrations in the visual cortex may predict AV integration. These findings set the stage for future research involving older adults to enhance our understanding of age-related AV integration decline.

Disclosures: V. Unnisa Begum: None. **R. Cohan:** None. **J.K.E. Steeves:** None. **D. Abuleil:** None. **B. Thompson:** None. **M. Barnett-Cowan:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.04/EE11

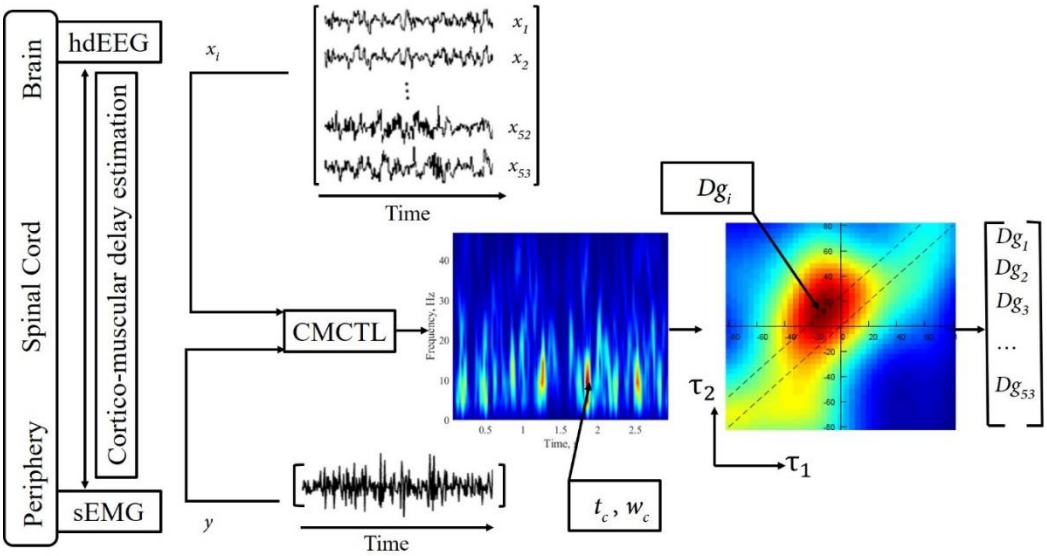
Topic: D.08. Multisensory Integration

Support: Tehran University of Medical Sciences (TUMS) with grant no. 9611345003.

Title: Computation of cortico-muscular coupling value to predict the task-related and non-task sEMG channels: A joint hdEEG-sEMG study using sparse representation of brain signals

Authors: A. KEIHANI[1], A. MOHAMMADI[2], H. MARZBANI[5], S. NAFISSI[3], A. JAFARI[4], *M. RAZA[6];
[1]Univ. of Pittsburgh, Univ. of Pittsburgh, Pittsburgh, PA; [2]Res. Ctr. for Biomed. Technologies and Robotics, [3]Dept. of Neurol., [4]Dept. of Med. Physics and Biomed. Engin., Tehran Univ. of Med. Sci., Tehran, Iran, Islamic Republic of; [5]Dept. of Biomed. Engin., Univ. of British Columbia, Vancouver, BC, Canada; [6]Neurol., Baqiyatallah Univ. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract: Cortico-muscular coherence (CMC) method is commonly used with electroencephalogram-surface electromyogram (EEG-sEMG) signals to investigate sensorimotor control during motor task. Coupling difference between non-task sEMG channels is often undetectable because noise and time delay between the two signals weaken the CMC values. To compute CMC and detect coupling for task-related and non-task sEMG signals, we used sparse representation of EEG channels. High-density joint EEG-sEMG (53 EEG channels, 4 sEMG bipolar channels) signals were acquired from 15 subjects (30.26 ± 4.96 years) during four specific hand and foot contraction tasks (2 dynamic and 2 static contraction). Bayesian optimization was employed to select best-fitted method with tuned hyperparameters on the input feeding data while using 80% data as the train set and 20% as test set. K-fold (K = 5) cross-validation method was used for evaluation of trained model. Two models were trained separately, one for CMC data and the other from sparse representation of EEG channels on each sEMG channel. Sensitivity, specificity, and accuracy criteria were obtained for test dataset to evaluate the performance of task-related and non-task sEMG channels detection. Coupling values were significantly different between grand average of task-related compared to the non-task sEMG channels ($Z = -6.33$, $p < 0.001$, task-related median = 2.011, non-task median = 0.112). Strong coupling index was found even in single trial analysis. Sparse representation approach (best fitted model: SVM, Accuracy = 88.12%, Sensitivity = 83.85%, Specificity = 92.45%) outperformed CMC method (best fitted model: KNN, Accuracy = 50.83%, Sensitivity = 52.17%, Specificity = 49.47%). To detect CMC for discerning the EMG channels involved in the contraction tasks and non-tasks, sparse representation method offers high performance.



Disclosures: **A. Keihani:** None. **A. Mohammadi:** None. **H. Marzbani:** None. **S. Nafissi:** None. **A. Jafari:** None. **M. Raza:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.05/EE12

Topic: D.08. Multisensory Integration
VA-ORD (RR&D) MERIT 1 I01 RX003523-0
NIH T32NS007421-23

Title: Multimodal Recording of Non-simultaneous LFP and BOLD Activity in a CGRP-induced Preclinical Model of Migraine

Authors: *Y. FILALI[1], M. JOHNSON[1], M. A. MATKOVICH[1], I. HULTMAN[2], N. MCCUTCHEON[1], A. O. ADEGBOYO[1], Z. J. PETERSON[3], L. HOPKINS[3], T. NICKL-JOCKSCHAT[3], S. SRIVASTAVA[2], L. P. SOWERS[4], R. HULTMAN[1];
[1]Mol. Physiol. and Biophysics, [2]Statistics & Actuarial Sci., [3]Psychiatry, [4]Pediatrics, The Univ. of Iowa, Iowa City, IA

Abstract: Introduction: Migraine is a complex, multi-system disorder, notable for its debilitating hypersensitivities to sensory stimuli. In addition to sensory dysfunction, alterations in autonomic, affective, and cognitive systems have been implicated, yet our understanding of these interacting neural substrates remains incomplete. We used a preclinical model, resting-state functional magnetic resonance imaging (rs-fMRI), and multi-site in-vivo electrophysiology to investigate these dynamics in the context of a migraine brain state. Methods: We induced a migraine-like state in adult mice (C57BL/6; >P30; N = 9) via peripheral calcitonin gene-related peptide (CGRP) administration, and later collected resting-state BOLD fMRI signals using T2* weighted echo-planar gradient-echo imaging. An additional two cohorts of adult mice (CD-1; >P30), underwent a craniotomy where tungsten wires across 16 (N = 10 male) and 8 (N = 37, 18 male, 17 female) migraine-relevant regions for electrophysiological recordings of local field potential (LFP) activity, both pre and post-CGRP administration. Data collected from the rs-fMRI sessions underwent anatomical and functional processing using a combination of neuroimaging tools (ANTs, FSL, and AFNI) in a seed voxel analysis pipeline to determine time-series cross-correlations amongst regions of interest. LFP signals were processed via MATLAB scripts to determine regional power, along with region-to-region coherence and directionality. To identify LFP network features, processed data was pushed through a supervised autoencoder-based machine learning model. Results: Both neurophysiological recording methods revealed wide-ranging brain changes in CGRP-induced migraine. Rs-fMRI showed a significant increase in BOLD activity functional connectivity between visual and somatosensory cortices during a migraine state ($P < 0.05$, Bonferroni corrected). LFP analysis within our 8-region design revealed a distinctive network that differentiated migraine from non-migraine states (AUC = 0.7), driven primarily by the basolateral amygdala and parabrachial nucleus. Within our 16-region design,

data showed evidence of trending increases in both power and coherence within the somatosensory and visual cortices. Conclusions: Our results indicate heightened connectivity between visual and somatosensory processing centers during a migraine state, which may contribute to the characteristic photophobia and hypersensitivity to touch. We also identified a distinctive network representation of migraine, highlighting the importance of coordinated brain dynamics in migraine pathology.

Disclosures: **Y. Filali:** None. **M. Johnson:** None. **M.A. Matkovich:** None. **I. Hultman:** None. **N. McCutcheon:** None. **A.O. Adegboyo:** None. **Z.J. Peterson:** None. **L. Hopkins:** None. **T. Nickl-Jockschat:** None. **S. Srivastava:** None. **L.P. Sowers:** None. **R. Hultman:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

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Program #/Poster #: PSTR214.06/EE13

Topic: D.08. Multisensory Integration

Support: Ministry of Science and Technology of China (2021ZD0202601)
National Natural Science Foundation of China (62061136001)
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National Natural Science Foundation of China (32100837)
German Research Foundation (TRR 169/C8)

Title: Neural correlates of an illusive sense of agency caused by action control of an avatar in virtual reality.

Authors: *Y. CAI[1], H. YANG[2], Z. XIONG[2], S. KÜHN[3], Y. BI[2], K. WEI[1];
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Abstract: Motor control is accompanied by the sensation that self-initiated actions lead to ensuing changes in the external environment, often referred to as sense of agency (SoA). Whether SoA exclusively relies on the processes embedded in the sensorimotor system is under debate. The comparator theory posits that the comparison between sensory prediction of action consequence and actual feedback gives rise to SoA. On the other hand, the reconstructive theory posits that SoA arise from post-movement inferential processes based on action feedback. Indeed, recent neuroimaging studies highlighted the role of motor planning regions, e.g., pre-supplementary motor area, lending direct support of the comparator model. However, the evidence to support the reconstructive theory is lacking. Here we used immersive virtual reality to modulate people's SoA and examined the accompanied neural changes by functional magnetic

resonance imaging (fMRI). Forty-four participants performed an action-effect timing task in the MRI scanner twice, before and after a VR exposure phase when they performed four gamified motor tasks by controlling an avatar. The timing task gives out a biased temporal perception, serving as a proxy of SoA. The gamified motor tasks modulate SoA by showing the controllability of the avatar. The critical difference from previous studies is that here SoA was elicited by action observation of the avatar, without self-actions and thus without action planning / prediction. Indeed, our participants showed increased SoA after the virtual reality exposure. Importantly, this SoA over the virtual body was correlated with a single cluster centered at right angular gyrus, extending to right inferior parietal lobule and the right precuneus. As a high-level associative area, angular gyrus has been recently theorized as a hub for inferential sensemaking in various tasks, including perception, language, social, and decision making. This cluster does not directly engage in motor planning, nor we did not find any significant activity in traditional motor control regions. Hence, observing putative action-related feedback can elicit sense of agency in absence of motor planning and execution, and this upregulation of SoA is subserved by neural substrate for inferential sensemaking, providing direct neural support for the reconstructive theory. Our study also suggests that sensorimotor control experience in virtual reality can affects the way how action feedback is processed subsequently.

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Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

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Program #/Poster #: PSTR214.07/EE14

Topic: D.08. Multisensory Integration

Support: IBS Grant IBS-R015-D1
KHIDI Grant HI19C1328
KBRI Grant 23-BR-01-01 and 22-BR-03-07

Title: Adult deafening induces brain-wide cross-modal plasticity in mice

Authors: *H.-J. SHIM[1,2], W. JUNG[1,3], G. KIM[4], S.-G. KIM[1,5];
[1]Ctr. for Neurosci. Imaging Res., Suwon-si, Korea, Republic of; [2]Mind and Brain Inst., Johns Hopkins Univ., Baltimore, MD; [3]Canon Med. Systems Korea, Med. Imaging AI Res. Ctr., Seoul, Korea, Republic of; [4]Korea Brain Res. Inst., Daegu, Korea, Republic of; [5]Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon-si, Korea, Republic of

Abstract: Recent evidence suggests that sensory deprivation in adulthood can lead to substantial reorganizations across cortical regions. However, the extent and time course of this adult plasticity and the underlying neural mechanisms remain poorly understood. Here, we investigated the effects of deafening in adult mice, using 15.2T blood-oxygenation-level-

dependent (BOLD) functional MRI, which allows repeated brain-wide mapping of sensory responses before and after deafening in individual animals. Deafening was induced by injecting diphtheria toxin in *Pou4f3*$^{DTR/+}$ mice (i.m., 25 ng/g at the age of ~7 weeks), engineered to express human diphtheria toxin receptors in cochlear hair cells. Deafness was confirmed by the absence of acoustic startle response 5 days after injection. BOLD responses to forepaw stimulation (4 Hz current pulse trains) or visual stimuli (5 Hz light stimuli delivered via optical fibers) were obtained in 15.2T scanner before, 1 week after, and 6 weeks after the toxin injection. A significant increase in BOLD responses to forepaw stimulation was observed in the somatosensory cortex and thalamus one week after deafening (n = 11 mice) (S1FL: 0.71±0.10% to 1.26±0.11%; VP: 0.36±0.05% to 0.56±0.07%; S2: 0.17±0.04% to 0.40±0.03%). Similarly, there was an increase in BOLD responses to visual stimuli in the visual cortex (V1: 0.69±0.08% to 0.87±0.05%). In the auditory cortex, BOLD responses to forepaw and visual stimulation became prominent one week after deafening (forepaw responses in A1: 0.12±0.04% to 0.39±0.03%; visual responses in A1: 0.03±0.01% to 0.17±0.04%). No such enhancement was observed in WT mice who also received toxin injection (n = 8 mice). Six weeks after deafening (n = 6 mice), most of the observed increases in uni-modal responses (forepaw responses in the somatosensory regions and to visual responses in the visual cortex) returned back to their pre-deafening levels, suggesting some of the deafening-induced enhancements are transient. In contrast, the increase in cross-modal responses in the auditory cortex (forepaw and visual responses in the auditory cortex) persisted even at 6 weeks post-deafening (forepaw responses in A1: 0.12±0.04% to 0.39±0.03% to 0.31±0.03%; visual responses in A1: 0.03±0.01% to 0.17±0.04% to 0.09±0.02%). Our results demonstrate that adult deafening can rapidly induce a widespread cross-modal enhancement in the auditory cortex as well as other spared sensory cortices. Our longer term imaging further reveals certain changes in BOLD responses are transient in nature, suggesting the plasticity induced by adult deafening comprises heterogeneous components.

Disclosures: **H. Shim:** None. **W. Jung:** None. **G. Kim:** None. **S. Kim:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.08/EE16

Topic: D.08. Multisensory Integration

Support: NSF DGE-1922697
Vanderbilt Brain Institute Discovery Grant

Title: Perception of auditory, visual, and audiovisual motion direction in macaque monkeys

Authors: *A. SCHOENHAUT[1], R. RAMACHANDRAN[3], M. WALLACE[2];
[2]Psychology, [1]Vanderbilt Univ., NASHVILLE, TN; [3]Hearing and Speech Sci., Vanderbilt Univ. Med. Ctr., NASHVILLE, TN

Abstract: Motion perception is a key aspect of sensory processing that enables animals to interact with the world. While extensive research exists on visual motion perception, less is known about how auditory and audiovisual (AV) motion are perceptually processed, particularly in non-human primates (NHP). This knowledge gap is especially significant given multisensory cue combination's crucial role in comprehending the environment. NHP studies in particular offer a unique opportunity to link perceptual processes to underlying neural mechanisms. We aim to establish the foundation for future neurophysiological experiments by exploring how perception of auditory, visual, and AV motion differs with changes to various stimulus parameters in macaques. Two monkeys were trained to perform a two-alternative forced choice task in which they judged the motion direction of unisensory (visual or auditory alone) and AV (both) stimuli. Visual stimuli were random dot kinematograms, while auditory stimuli were generated by amplitude-modulating a broad-band noise across two speakers and embedding it in partially-correlated noise. We systematically altered stimulus parameters including motion coherence, duration, velocity, and displacement to evaluate their respective influence on motion sensitivity. For auditory and AV motion, we found that discrimination accuracy improves with increased coherence and psychometric threshold decreases with increasing auditory dB SNR and velocity/displacement; however, the extent of these changes differed between monkeys. Additionally, psychometric slopes for visual and AV conditions were steeper than those for auditory conditions across stimulus parameters. We also assessed the behavioral benefits associated with AV cue combination. Our findings indicate increased motion sensitivity to AV stimuli when compared to their unisensory counterparts, with the magnitude of this increase varying across stimulus parameters. To our knowledge, this study represents the first concerted effort to systematically examine the impact of stimulus parameters for studies of auditory and AV motion in awake, behaving NHP. Moreover, our results provide a foundation for future investigations into the underlying neural mechanisms of multisensory motion perception.

Disclosures: **A. Schoenhaut:** None. **R. Ramachandran:** None. **M. Wallace:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

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Program #/Poster #: PSTR214.09/EE17

Topic: D.08. Multisensory Integration

Support: Gatsby Charitable Foundation GAT3361
Wellcome Trust 090843/F/09/Z and 214333/Z/18/Z

Title: Translation signals in the primary visual cortex permit the discernment of internal and external sources of motion

Authors: ***M. VÉLEZ-FORT**[1], L. COSSELL[1], C. CLOPATH[2], T. W. MARGRIE[1];
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Abstract: Locomotion requires the brain to disambiguate internally- from externally-generated motion cues during displacement in the environment. Our understanding of how the brain performs this task has been hampered by the inherent difficulty in isolating the key sensory-motor processes that are active as we navigate the real world. To address this we devised a novel setup that permits, in mice, isolation of translation (T), running (R) and external visual flow (VF) signals during linear excursions. For a given animal, we sampled speed profiles from its own running history to 'replay' ethologically-relevant epochs of passive translation with visual flow, or for visual flow alone.
Using acute Neuropixels recordings we found that visual flow increased primary visual cortex (VISp) activity when compared to the activity recorded during presentation of a static visual image. However, over the entire speed range, the combination of passive translation and visual flow significantly increased VISp responsiveness compared to the visual flow-evoked response. In the case of active translation (locomotion), VISp activity was further increased over the entire range of speed when compared to passive translation with coupled visual flow or visual flow alone. The mean population firing rate of VISp therefore reflects whether the brain is experiencing visual motion in the environment or visual motion caused by either its passive or active displacement through the environment.
To explore the effect of running and its potential interaction with translation, we compared VISp neuronal activity recorded during locomotion (R+T+VF) and static running (R+VF). At the population level, the firing rate observed for active translation with visual flow was indistinguishable (over the entire speed range) from that observed during static running with coupled visual flow. A simple biologically realistic model and data indicate that during locomotion, running suppresses translation input but that translation signals dominate VISp activity during "slip" events, when running and translation speed becomes incongruent. In VISp, slip responses occur independent of visual input and exist throughout the cortex wherever locomotion signals are observed.
We suggest that these egocentric motion signals provide an essential frame of reference for cortical areas computing the position, speed and trajectory of self and external sensory objects when moving through the world.

Disclosures: **M. Vélez-Fort:** None. **L. Cossell:** None. **C. Clopath:** None. **T.W. Margrie:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

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Title: Visual and tactile integration of object locations in the mouse barrel cortex and posterior parietal cortex

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Abstract: Multisensory integration requires transformations between coordinate systems. In the mouse brain, tactile information from the snout's vibrissae reaches the somatotopically organized primary whisker somatosensory cortex (wS1) whereas the primary visual cortex (V1) contains a retinotopic map of the visual field. The rostro-lateral (RL) area of the posterior parietal cortex is a candidate cortical region to merge these representations. However, how converging multisensory inputs of nearby objects are processed in these cortical areas remains unclear.
To address this question, here we investigate how neurons in mouse wS1, V1, and RL integrate visuotactile information about the location of a pole in reach of the whiskers. Using two-photon calcium imaging, we record neurons across the posterior cortex in L2/3 of head-fixed mice (n=11 mice, both sexes). A pole is presented either in darkness, under illuminated conditions, or a 'virtual' pole is shown on a monitor. These three conditions allow us to separate visual, tactile and multisensory signals. We track whisker-pole interactions with a high-speed camera and record the gaze direction to reconstruct sensory signals at the periphery.
We find that subsets of neurons in wS1, V1 and RL show selectivity for specific locations in the near space. This location coding in RL is driven by both visual and tactile signals and depends less on whisker kinematics compared to wS1. By fitting a shared-weight artificial neural network trained on all neurons, we are in the process of separating tactile and visual contributions to single-cell activities in the multisensory condition. Ongoing analysis addresses the influence of visual input on neural signals in wS1 to study if visual information is utilized to predict upcoming touch events. Together, this suggests that object locations in the posterior parietal cortex are represented based on visual and tactile information, potentially in a shared reference frame.

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Poster

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Title: Directionally tuned signals in mouse subicular complex during passive rotation using high-density probes

Authors: *M. DAUGUET[1,2,3], F. ALUISI[1,3], M. WEXLER[1,3], J. LAURENS[4], M. GRAUPNER[2,3], D. FRICKER[1,3];
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Abstract: Head-direction (HD) cells are neurons coding for the animal's head direction in space. They function as the brain's compass and are found in several cortical and subcortical areas. The HD system activity is generated based on vestibular signals and controlled by and anchored to visual inputs of external landmark cues. How multisensory signals are processed during passive rotation remains unclear, with the pre- and postsubiculum as a prime candidate for the integration of vestibular based anterior thalamic and visual dominated retrosplenial cortical signals.
We examined this question in a novel paradigm where we controlled both vestibular and visual cues. Head-fixed mice were rotated on a motorized stage in a pseudo-random fashion, with peak speeds of 150°/sec and accelerations up to 300°/sec2, covering all angles. Visual cues were projected on a semi-transparent dome surrounding the animal. We investigated neuronal activity of single neurons and populations of neurons in pre-, post-, parasubiculum and subiculum, using Neuropixels probes.
The recording protocol was run in the recording room as a control condition, then compared to test conditions where a single visual landmark was projected on the dome that could be shifted by 90° to examine cue control of directionally tuned signals. A darkness condition was used to examine the stability of directional signals in the absence of landmarks. Angular tuning was quantified by dividing the number of spikes by the occupancy per bin, and the Rayleigh vector (R) was calculated for each individual cell.
In a first dataset, we recorded 606 well separated units from subicular areas (n=8 mice), of which 61% showed directional tuning ($R>0.3$, $p<0.05$). Some units showed mixed selectivity, tuned to head direction and to angular head velocity. Tuning curves of subicular HD cells showed that the HD system is controlled by visual landmarks under the dome, following stripe shifts between conditions. However, no directional tuning was observed during the darkness condition. Nevertheless, using pairwise correlation analysis, we show that the same coherence of activity of HD cells from other conditions is maintained in darkness.
These results show that in animals under passive rotation, we are able to record coherently tuned signals from neurons in subicular areas that are under the control of virtual visual cues. Taken together, our work will provide new insights on multisensory processing in the presubiculum and the HD function in head-fixed paradigms.

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Poster

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Support: ERANET ANR-20-NEUR-0005 VELOSO

Title: Experimental protocol to study multisensorial integration of head direction after vestibular lesion

Authors: H. TRAN[1,2], R. EL MAHZOUM[1], F. SIMON[2], ***A. BONNOT**[1], D. FRICKER[2], I. COHEN[1];
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Abstract: The sense of orientation in space is crucial for survival and navigation in our environment. The ability to locate oneself in space comes from the detection by the vestibular organs of the inner ear of our movements (angle and speed of rotation of the head) as well as visual information that anchors orientation to landscape cues. Behavioral studies in rodents and humans, as well as neural recordings in rodents, show that vestibular damage degrades the perception of orientation in space. A number of brain structures are known to be involved, such as anterior thalamus, retrosplenial cortex, presubiculum, and lateral mammillary nuclei. Yet, how these structures interact to generate an integrated multisensorial sense of orientation remains an open question. Our study is based on a model of vestibular lesion in rat that replicates a broad spectrum of inner ear pathologies. We developed a protocol to study brain activity coupled to behavior by combining in vivo electrophysiology recordings of the brain, eye and neck muscles, together with head inertial measurement unit, video tracking and mobile functional ultrasound imaging. Orientation is challenged by rotating the animal either in the dark or with visual cues. We present the first multimodal recordings in control condition and after labyrinthectomy. We observe an acute vestibular syndrome, with typical twirling behavior in the acute phase after the lesion and progressive recovery. This project will provide crucial new data to establish a consistent model of how we locate ourselves in our spatial environment and to clarify the role of the inner ear vestibular organs in spatial orientation.

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Poster

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Title: Role of spontaneous neural activity in Hydra vulgaris

Authors: *A. HANSON[1,2], R. YUSTE[3];
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Abstract: Spontaneous neural activity is one of the most highly conserved features of all nervous systems examined to date, yet its function remains largely unknown. The development of transgenic animals expressing GCaMP in the interstitial lineage allowed simultaneous imaging of all the neurons in the freshwater polyp *Hydra vulgaris* at single cell resolution, which revealed a set of anatomically non-overlapping neural ensembles associated with specific behaviors. Interestingly, this work also revealed the presence of synchronous spontaneous activity throughout the nervous system while the animal is not engaged in any behavior (i.e., is "at rest"), suggesting spontaneous, or "resting state" neural activity is highly conserved and likely plays an important role in all nervous systems. We have now generated a transgenic animal expressing cytoplasmic GCaMP7 and nuclear tdTomato, which allows the imaging and automatic tracking of all neurons in *Hydra* at single cell resolution. In addition, we have created automatic behavioral tracking models allowing us to quantify *Hydra* behavior versus "resting state." With these new tools, we have begun exploring the role of spontaneous, or "resting state," neural activity in *Hydra*, testing the hypothesis that low frequency spontaneous neural activity may be critical for organism-wide neural and behavioral coordination.

Disclosures: A. Hanson: None. **R. Yuste:** None.

Poster

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Title: Enhanced Kalman filter model of spatial orientation formation

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Abstract: Spatial orientation (SO) refers to the perception of one's postural and motor behavioral states. It is formed through a sequential integration of time-varying sensory signals from multiple modalities, including the vestibular, visual, and somatosensory systems. Similarly, the Kalman filter, a mathematical algorithm developed by Rudolf Kalman in the early 1960s, integrates sensor measurements to provide optimal estimates of unobservable states in dynamic systems. Consequently, the Kalman filter has been utilized as a model to understand the computational mechanism underlying the estimation of behavioral states, particularly three-dimensional (3D) head motion, based on responses from the vestibular sensors, namely the semi-circular canals and otoliths. Recently, Laurens and Angelaki (eLife, 2017) proposed a unified internal model theory within the framework of the Kalman filter to resolve the paradox of active versus passive head motion sensation. In the present study, we have enhanced and adapted their Kalman filter model to provide a more realistic simulation of SO formation in response to a range of vestibular and visual sensory inputs during both active and passive scenarios. Our enhanced model retains the original state variables (3D angular head velocity, 3D linear head acceleration, and 3D gravitational acceleration in the head-fixed coordinate) while incorporating 3D visual motion stimulation and produced 3D eye movements as additional states. The model's sensory outputs include 3D retinal responses (image motion), alongside the previously incorporated semi-circular canals (3D angular head rotation) and otoliths responses (3D linear head motion and gravity). Through simulations using our model, we successfully reproduced major SO malformations, such as the somatogravic illusion frequently experienced by airplane pilots, as well as tilt-translation ambiguities and visually induced self-motion illusion (Vection). Notably, these significant SO malformations were specifically observed in scenarios where head motion was passively imposed. These findings highlight the utility of our enhanced Kalman filter model in comprehending the neural computational mechanism underlying SO formation based on vestibular and visual sensory information. We envision the application of this model in the development of artificial multimodal sensory stimulations, which can be utilized for manipulating SO in both real and virtual environments.

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Poster

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VisioNYC

Title: Effects of learning and experience on multisensory integration in primary somatosensory cortex

Authors: *D. KATO[1], R. BRUNO[2];
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Abstract: Merging information from across sensory modalities is key to forming robust, disambiguated percepts of the world, yet how the brain achieves this feat remains unclear. Recent observations of cross-modal influences in primary sensory cortical areas have suggested that multisensory integration may occur in the earliest stages of cortical processing, but the role of these responses remain poorly understood. We address these questions by testing several hypotheses about the possible functions subserved by auditory influences on the barrel field of mouse primary somatosensory cortex (S1) using *in vivo* 2-photon calcium imaging.
We first tested whether S1 encodes auditory stimulus identity in naïve mice. We found that auditory stimuli evoke spiking activity in an extremely small fraction of cells, and moreover that this sparse activity is insufficient to encode auditory stimulus identity; very few cells responded preferentially to one sound or another, and a linear classifier trained to decode auditory stimuli performed barely above chance. We then tested whether S1 encodes information about conjunctive, audio-tactile features by presenting different combinations of simultaneous whisker and acoustic stimuli. Consistent with previous results, we found that simultaneous presentation of sound has a net suppressive effect on whisker-evoked activity in S1. However, when we tested whether S1 encodes specific audio-tactile feature conjunctions, we found that classifier accuracy was within chance levels, suggesting that sound-evoked suppression of whisker responses is auditory-stimulus non-specific.
Conceivably these weak, stimulus-non-specific auditory influences on S1 could be amenable to plasticity via learning and experience. To test the idea that Hebbian-like plasticity might lead auditory stimuli to reactivate S1 ensembles representing correlated whisker stimuli, we repeatedly presented specific pairs of auditory and tactile stimuli within several milliseconds of each other over several days. We found that decoder performance for auditory audio-tactile stimuli remained unchanged, suggesting that passive experience is insufficient to induce such pattern completion. Finally, we repeated the same experiment in a rewarded setting and obtained similar results, indicating that reinforcement is also insufficient to alter the information content of auditory influences on S1. Collectively, these results suggest that auditory influences on S1 are remarkably stable and play a largely stimulus-non-specific role.

Disclosures: D. Kato: None. **R. Bruno:** None.

Poster

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Title: Examining the role of parvalbumin-expressing interneurons on audiovisual temporal processing and perception

Authors: *M. U. AL-YOUZBAKI, A. L. SCHORMANS, J. F. AHMAD, B. L. ALLMAN; Univ. of Western Ontario, London, ON, Canada

Abstract: The way we perceive our environment is largely shaped by our brain's propensity to merge information from our different senses (e.g., sight and sound); a phenomenon that is highly dependent on the relative timing of the multisensory stimuli. For example, when auditory and visual stimuli occur within ~100 ms of each other, there is a tendency to perceive the two stimuli as a single event, even though they occurred at separate moments in time. To better understand the neural basis of audiovisual temporal perception, we previously used a combination of electrophysiology, neuropharmacology and translational behavioral testing in rats to determine that a reduction of GABAergic inhibition in the audiovisual cortex was sufficient to disrupt multisensory processing across the cortical layers, and ultimately impair the rats' temporal acuity of audiovisual perception and their rapid adaptation to recent sensory experience. To extend these findings, the present study used optogenetic manipulations in a transgenic rat model to investigate the role of the most abundant GABAergic interneuron subtype, the parvalbumin-expressing (PV) interneurons, on audiovisual temporal perception. Having trained PV-Cre rats to perform an audiovisual temporal order judgment (TOJ) task (where they reported whether an auditory or visual stimulus was presented first in the pairing), we then injected a Cre-dependant adeno-associated virus into their audiovisual (V2L) cortex to express halorhodopsin; an approach that allowed us to silence PV interneurons at precise times during the TOJ task by shining in yellow (589 nm) light via chronically-implanted light fibers. Preliminary data show that PV interneurons contribute to the rats' TOJ task performance in a modality-specific way, such that their perception of the relative timing of the auditory-leading, but not visual-leading, stimuli was affected. To further investigate how PV interneurons may be directly contributing to a modality-specific disruption of audiovisual temporal processing, we have begun a series of experiments that combine electrophysiological recordings in the V2L cortex with halorhodopsin-induced silencing of PV interneurons at precise times during the presentation of auditory and visual stimuli. Overall, our ongoing studies seek to advance our understanding of the role of GABAergic inhibition, and PV interneurons in particular, on audiovisual temporal processing and perception; findings which could have mechanistic implications that extend to various clinical conditions with known alterations in either their PV interneuron function and/or multisensory processing abilities, in general.

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Poster

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Title: Real-time Optical Pattern Stimulation: A Novel Approach to Delivering Information to the Brain

Authors: *K. YAMASHIRO[1], N. MATSUMOTO[1,2], Y. IKEGAYA[1,2];
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Abstract: Various recording techniques, such as fMRI, MEG, EEG, and EcoG, are effective for observing brain activity. Analysis of signals has revealed encoded information within the brain, leading to advancements like motor cortex-responsive prosthetic hands and targeted depression treatment through electrical stimulation based on brain states. Known collectively as Brain-Computer Interface (BCI), these methods transmit brain-encoded information to a computer. However, current BCI research primarily focuses on extracting brain states from neuronal activity, with limited emphasis on information transmission from a computer to the brain. Considering that the brain's encoded information emanates from neuronal activity, effective conveyance of information to the brain can be achieved if neuronal activity can be regulated through computer intervention. In this regard, here, we directed our attention to the method of optical stimulation for neuronal manipulation. We utilized *in utero* electroporation to express ChR2 in layer 2/3 pyramidal neurons of the rat barrel cortex. A glass window was placed upon the barrel cortex, enabling wide-field optical stimulation of the targeted region. Simultaneously, an electrode was inserted to electrically stimulate the medial forebrain bundle (MFB) as a reward. A rat was positioned on a circular track with eight equidistant LED lights along its circumference. Initially, the rat learned to reach the illuminated LED lights to receive MFB stimulation. The accuracy of the behavioral task was assessed by the rat's selection of the shorter route, either clockwise or counterclockwise. Once the rats acquired the association between the visual cues provided by the LED lights and the subsequent reward, optical pattern stimulation was simultaneously applied to the barrel cortex to elicit neuronal excitation within the illuminated field. The pattern of the optical stimulation was linked to the reward's location. The brightness of the LEDs gradually diminished, compelling the rat to rely on the optically patterned stimulation to locate the reward zone. In this presentation, we elucidate the impact of patterned optical stimulation on the accuracy of the task and investigate whether rats successfully correlated the patterned stimulation of the barrel cortex with the spatial information denoted by the reward's location.

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Poster

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Title: Integration of visual and hydrodynamic flow stimuli in larval zebrafish

Authors: *A. ZHANG, F. ENGERT;
Harvard Univ., Cambridge, MA

Abstract: Larval zebrafish display a repertoire of robust innate behaviors that allow them to respond to hydrodynamic flow from as early as 5dpf. In order to efficiently navigate a changing flow environment, fish can combine streams of information from different sensory modalities, in particular vision and mechanosensation. The optomotor reflex allows fish to orient and swim with visual motion, while visually-independent rheotaxis, which persists in the dark, involves making turning decisions on the basis of lateral line mechanosensory inputs. How these streams of information are combined in a quantitative and context dependent fashion is unknown. To probe this, we have developed an arena that delivers laminar flow, where we can infer the local flow field around the fish at any given timepoint. Additionally, we project visual gratings stimuli onto the bottom of this arena, with velocities that either accelerate, equate, or dampen the perceived flow from the point of view of the fish. We compare the orienting behavior and swimming statistics of fish between these conditions and investigate the influence of stimulus reliability. We use this approach in ongoing experiments to explore the integration of these two stimulus streams in multisensory conditions.

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Poster

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Title: Separate auditory, visual and motor signals in the mouse superior colliculus

Authors: *F. TAKÁCS, C. BIMBARD, G. M. BOOTH, M. ROBACHA, T. P. H. SIT, K. Z. SOCHA, K. D. HARRIS, P. COEN, M. CARANDINI;
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Abstract: [Introduction] The superior colliculus (SC) is a layered structure that contains visual, auditory, and movement maps, suggesting a role in audiovisual integration and in motor selection. In some species, visual and auditory signals can mix in individual neurons. Does this audiovisual convergence occur also in the mouse? And do sensory signals in mouse SC mix with movement signals? Finally, does mouse SC play a causal role in audiovisual integration?
[Methods] We used Neuropixels 2.0 probes to record the responses of ~2,000 SC neurons in awake mice (N=8) to checkerboard images and pink noise sound bursts presented at varying azimuths, alone or in combination. ~1,600 of these neurons were recorded in untrained mice, and the rest in mice trained in an audiovisual localisation task (Coen et al, Neuron, 2023) using the same stimuli in the task and in a passive context (N=3). In a third cohort of mice (N=6), we expressed halorhodopsin with a viral injection and used optogenetics to unilaterally inactivate the SC while mice performed the task.
[Results] As expected, in untrained mice we observed neurons responding to visual stimuli in superficial SC, and to auditory stimuli in deeper layers. Neurons responding to both modalities were rare (~5%). In those neurons, audiovisual integration was explained by an additive model. Auditory stimuli evoked uninstructed face movements (Bimbard et al, Nature Neurosci 2023). To assess the contribution of these movement signals and of visual and auditory signals we performed linear regression (Musall et al, Nature Neurosci 2019). We found that these variables are coded in an uncorrelated manner across neurons ($r<0.05$). Preliminary results show that in mice that learned the task the overall sensory signals are similar during the task and in the passive context. Unilateral inactivation of the mouse SC promoted ipsiversive movements both during auditory and visual choices. In all trial types, including blank trials, ipsilateral choices were sped up while contralateral choices were slowed down. These behavioral changes could be explained by inactivation changing the choice bias rather than interfering with sensory processing.
[Conclusions] We conclude that auditory and visual signals rarely integrate in single SC neurons, and when they do, they integrate additively. Moreover, sensory signals rarely mix with movement signals. Mouse SC does play a causal role in performing an audiovisual localisation task, but this role is not related to sensory processing, whether auditory, visual, or audiovisual.

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Poster

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Title: Attentional demands in the visual field alter audiovisual interactions in the temporal domain: EEG correlates

Authors: *S. KOC YILMAZ[1,2], H. KAFALIGONUL[1,2,3];
[2]Natl. Magnetic Resonance Res. Ctr. (UMRAM), [3]Interdisciplinary Neurosci. Program, [1]Bilkent Univ., Ankara, Turkey

Abstract: Attention plays an important role in multisensory processing and crossmodal interactions in the temporal domain. To understand the involvement of attention in audiovisual interactions in time, we previously utilized a set of audiovisual stimuli that elicit temporal ventriloquism effects on perceived visual speed (Duyar et al., 2022). In this paradigm, two-frame apparent motion and two concurrent auditory clicks were used and the time interval between the clicks was systematically varied. The apparent motion presented with a short-interval click is typically perceived to move faster than the one with a long-interval click. We previously found a decline in the auditory time interval effects on perceived speed when attention was diverted to a stationary object in the visual field (e.g., fixation target) via a secondary task. In the present study, using the same paradigm we aimed to identify the electroencephalography (EEG) correlates of the influence of attentional demands on audiovisual interactions in motion processing. During the recordings, the participants (n = 20) performed a single task (i.e., only speed judgment on apparent motion) or a dual task (i.e., speed judgment + discrimination of fixation luminance change). Compared to the single task condition, the attentional demands and perceptual load is expected to be high in the dual task condition. Our design included unimodal (visual-only and auditory-only) in addition to bimodal (audiovisual) conditions to reveal nonlinear neural responses. In line with previous findings, the results indicated a significant reduction in the illusory behavioral effects of auditory timing on perceived visual speed in the dual-task compared to the single-task condition. The EEG waveforms indicated significant attentional modulations beyond 300 ms such that the evoked activities were larger when the attentional demand was high. These modulations were pronounced over the centroparietal sites for the visual-only conditions, whereas they became dominant over the left frontotemporal sites for the audiovisual stimulation. More importantly, attention interacted with the audiovisual processing in a later time range (450-500 ms) over the left temporoparietal electrodes in the audiovisual conditions. Our findings suggest that attention first alters neural activity elicited by visual apparent motion, and then significantly modulates audiovisual interactions at later stages of cortical processing.

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Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.21/FF1

Topic: D.08. Multisensory Integration

Support: NWO FLAG ERA DOMINO

Title: Audiovisual integration is absent in the primary visual cortex of a fragile x mouse model

Authors: *L. MONTELISCIANI, C. A. BOSMAN, U. OLCESE;
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Abstract: Autism Spectrum Disorder (ASD) has been associated with impairments in Multisensory Integration (MI). This impairment is exhibited, for instance, through a broadened temporal window for integrating visual and auditory stimuli in ASD (Foss-Feig et al., 2010). The primary visual cortex (V1) is not only essential for sensory processing but also represents the earliest cortical stage where MI has been demonstrated (Matthijs N oude Lohuis et al., 2021). However, the circuit-level mechanisms behind this altered MI in ASD are poorly understood. For these reasons, we performed Neuropixel probe recordings in V1 of wild-type (WT) mice and Fragile X syndrome mouse models (Fmr1-/- KO).We recorded in anesthetized, head-fixed mice while we presented visual, auditory and audio-visual stimuli (flashes and grating for visual stimuli and white noise and pure tones for auditory ones). To study how temporal integration differentially is represented in the two mouse lines, we presented visual and auditory stimuli at five different values of stimulus onset asynchrony (SOA), from -250ms to + 150ms. Preliminary analyses were performed on 180 single neurons recorded from 3 WT and 3 Fmr1-/- KO mice. We first investigated how responses to flashes varied based on the SOA at which auditory white noise was presented. Relative to audio-visual responses, V1 neurons in WT mice showed a significant response depression when the auditory stimulus was presented before the flash (WT, SOA Negative vs. SOA 0, $p = 0.0499$; SOA Negative vs. SOA Positive, $p = 0.0188$ – linear mixed effect model with post-hoc Tukey correction). On the other hand, in Fmr1-/- KO mice, visual responses were not modulated by auditory stimuli. Therefore, audio-visual integration is apparently present in WT mice but not in Fmr1-/- KO mice. Moreover, we observed that neurons in Fmr1-KO mice presented stronger responses to visual stimuli compared to WT mice (Fmr1-/- KO vs. WT, $p = 0.0406$), suggesting a unisensory imbalance which numerous studies connect to deficits in multisensory integration. These preliminary results will be expanded by studying responses to more complex visual stimuli (e.g., gratings) and by investigating the microcircuit-level mechanisms of MI. This study will help us better understand the cortical microcircuit of MI and to characterize the differences present in neurodevelopmental disorders such as Fmr1-KO.

Disclosures: L. Montelisciani: None. **C.A. Bosman:** None. **U. Olcese:** None.

Poster

PSTR214. Cross-Modal Processing and Multisensory Integration

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR214.22/FF2

Topic: D.08. Multisensory Integration

Support: NIDCD Grant R01DC020363
NIDCD Grant R01DC017532

Title: Saccade-related eardrum oscillations are altered by surgical denervation of the middle ear muscles

Authors: *S. LOVICH (SCHLEBUSCH)[1], D. KAYLIE[1], C. KING[1], C. A. SHERA[2], J. GROH[1];
[1]Duke Univ., Durham, NC; [2]USC, Los Angeles, CA

Abstract: The visual and auditory systems work together to ensure surrounding stimuli are perceived accurately. Information about eye movements is necessary to this process because eye movements shift the relative positions of the visual and auditory sensory organs. We have recently reported an oscillation of the eardrums time-locked with the onset of a saccade and in the absence of auditory stimuli, suggesting that such oculomotor information is available as early as the auditory periphery (Eye movement-related eardrum oscillations or EMREOs; Gruters, Murphy et al. PNAS 2018; Lovich et al. in press 2023). However, the underlying mechanical causes of EMREOs are still unknown. Here, we sought to determine the role of the middle ear muscles in producing these saccade-associated eardrum oscillations.
We recorded EMREOs in two rhesus monkeys during spontaneous saccades before and after surgical transection of the middle ear muscles. The monkeys were head-restrained in a dark room, and eye movements were tracked with a video eye tracker (1000 Hz sampling rate) while eardrum oscillations were recorded using microphones placed in the ear canals of both ears. We report that surgical transections of either the stapedius muscle or the tensor tympani muscle cause changes in the EMREO. Transection of the stapedius causes the EMREO to be significantly diminished in amplitude, but it is not eliminated. Transection of the tensor tympani muscle, in contrast, causes the EMREO to be significantly *increased* compared to the pre-surgery baseline data. This data corroborates parallel findings collected in our lab in humans with stapedius and/ or tensor tympani dysfunctions. Together, these findings suggest that there may be multiple contributors to the EMREO in a complex system within the ear, and that the middle ear muscles may work as a push and pull mechanism with opposite functions to stabilize the system.

Disclosures: S. Lovich (Schlebusch): None. **D. Kaylie:** None. **C. King:** None. **C.A. Shera:** None. **J. Groh:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.01/FF3

Topic: E.01. Eye Movements

Title: Evidence of an exogenous input to Superior Colliculus (SC) as a source of saccadic inhibition

Authors: *P. M. DAYE[1], A. SANGARÉ[2], B. M. GAYMARD[2], P. POUGET[2];
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Abstract: Saccadic inhibition refers to the strong temporary decrease in saccadic initiation observed when a visual distractor appears shortly after the onset of a saccadic target. One classical interpretation is that the saccadic inhibition phenomenon results from a competition between the target and the distractor in visuomotor maps within SC (Reingold & Stampe, 2002, 2004). In addition to being involved in the initiation processes, these maps in SC also contain information regarding saccade metrics. Interestingly, previous studies either reported epicene or no accompanying amplitude changes (Reingold & Stampe, 2002, 2004; Edelman and Xu et al. 2009; Guillaume 2012). One alternative hypothesis required more attention. Exogenous input to SC may suppress antagonistic oculomotor signals by sending strong inhibitory output to the superior colliculus (SC). We further examine this second hypothesis by performing visual, auditory, and manual response tasks. In the oculomotor task, the subject is required to trigger a saccade to a target appearing randomly leftward or rightward. A short duration after the target appearance a structured masked covers the target. In the visuomotor task, the subject uses a computer mouse to move to the target instead of a saccadic task. Finally, in the auditory task, the subject is blindfolded. A stereo sound acts as a fixation followed by a silence gap. Then a sound is played randomly either in the left or the right ear. A short duration after that single-ear sound, a stereo sound is played to both ears, acting like the visual mask. Our results show that a decrease in motor initiation following a distractor presentation is observed in all tasks. Furthermore, the depth of the response time distribution appears at a similar delay and with comparable amplitude. This points to a common source of inhibitory signal acting on SC (for the saccadic response) but that this inhibitory signal is unlikely to be generated at the level of SC.

Disclosures: **P.M. Daye:** A. Employment/Salary (full or part-time):; NeuroClues by P3Lab. **A. Sangaré:** None. **B.M. Gaymard:** None. **P. Pouget:** F. Consulting Fees (e.g., advisory boards); NeuroClues by P3Lab.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.02/FF4

Topic: E.01. Eye Movements

Title: Identifying the neural origin of pupil responses to isoluminant visual events

Authors: V. MARSHEV, H. G. FREY, ***J. W. BRASCAMP**;
Psychology, Michigan State Univ., East Lansing, MI

Abstract: The pupil constricts following visual events even if those events do not change average luminance. Whether these 'isoluminant constrictions' have a retinal or cortical origin is debated. We examined this by measuring pupil responses to the onset of grating stimuli (space-averaged luminance same as background) that were presented following adaptation to high-contrast gratings. In two conditions the adapting gratings were oriented either parallel or orthogonal to the gratings of interest. Participants reliably reported weaker grating perception following parallel adapters than following orthogonal ones, confirming the presence of orientation-specific contrast adaptation. As orientation-specific contrast adaptation arises post-retinally, a difference in constriction response between those conditions would signal a post-retinal origin of the response. Indeed, data collected so far (8 participants) show robust constrictions following orthogonal adapters, yet substantially reduced constrictions following parallel ones. This indicates that these constrictions arise at least partly post-retinally, in agreement with the view that they have a cortical origin.

Disclosures: V. Marshev: None. **H.G. Frey:** None. **J.W. Brascamp:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.03/FF5

Topic: E.01. Eye Movements

Support: NIH Grant EY027373

Title: Sensory reliability and task engagement interact nonlinearly to drive predictive sensory-motor behavior

Authors: *S. W. EGGER[1], S. BEHLING[2], B. BOWELL[1], S. LISBERGER[2];
[1]Neurobio., Duke Univ. Sch. of Med., DURHAM, NC; [2]Neurobio., Duke Univ., Durham, NC

Abstract: Our actions are not driven solely by our immediate sensory experience, but also by our past experience and predictions of what will happen in the future. Current theories of sensory-motor control invoke a feedback pathway where motor commands predict future sensory inputs. Sensory inputs and predictions are then integrated according to their relative reliability. We study the mechanisms of this integration in smooth pursuit eye movements. In natural smooth pursuit, visual motion drives the initiation of eye movements. However, once the eye’s speed matches the target's speed, motion on the retina is reduced and motor feedback maintains pursuit velocity. To probe this feedback pathway, we had a monkey pursue a moving patch of dots while controlling motion visibility. Intermixed with trials where the patch was persistently visible, we presented trials where the patch was briefly extinguished, either by disappearing (e.g. a blink) or passing behind a virtual occluder. To control sensory reliability, we adjusted the fraction of dots in the patch that moved coherently in the direction of patch motion whenever it was visible. Together, these manipulations allowed us to measure strength of the feedback

pathway as a function of sensory reliability through eye movements in the absence of visual motion. Across reliability conditions, the speed of pursuit decayed upon extinguishing the dot patch. However, the rate of decay depended on both motion reliability and how the patch was extinguished. During blink trials, the rate of decay was nonmonotonic with motion reliability. In contrast, the rate of decay decreased monotonically with increasing sensory reliability during occlusion trials. Finally, the decay of pursuit speed during occlusion depended on experience with the task. When first exposed to the occluder, eye speed decayed at a rate similar to that in blink trials. After several days of experience in the task, pursuit during occlusion persisted better, suggesting the monkey learned to better engage the pursuit system during occlusion. Together, these results demonstrate that current theories of sensory-motor integration are insufficient to explain smooth pursuit. While the strength of the motor feedback pathway depends on sensory reliability, it also depends nonlinearly on engagement with the sensory-motor task.

Disclosures: S.W. Egger: None. **S. Behling:** None. **B. BoWell:** None. **S. Lisberger:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.04/FF6

Topic: E.01. Eye Movements

Support: ERC 755745

Title: Population coding of categorically distinct behaviors in the frontal eye field

Authors: *M. CAIN, M. JOSHUA;
Edmond & Lily Safra Ctr. for Brain Sci., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: How does a neural population encode multiple behaviors? One possibility is that the population can be classified based on the categories of behavior; however, cortical neurons do not easily fit into such categories. Thus, coding of behavior might arise from the organization of the population. Accordingly, we aim to study how a population of neurons represents categorically distinct behaviors. We recorded the activity of 1200 neurons from the frontal eye field (FEF) of two monkeys while they were engaged in eye movement tasks. These tasks included: a smooth pursuit task, where monkeys tracked a moving target; a pursuit suppression task, where monkeys maintained fixation on a target at the center of the screen while a second target followed a trajectory similar to the pursuit paradigm; and a saccade task. All tasks were performed in eight directions. We used a measure of effect size to quantify the activity modulation. Single neurons tended to respond in all three tasks, indicating that the FEF is not organized into clusters that respond to the different tasks. To characterize the population response, we used dimensionality reduction methods. We identified the low-dimensional spaces that contained most (>80%) of the population activity during each behavior and measured the overlap between these spaces across behaviors. We found that pursuit and saccades mostly

occupied orthogonal subspaces, as projection across subspaces significantly reduced the activity. By contrast pursuit and the suppression of pursuit showed high overlap. Next, we studied if and how the overlap between pursuit and its suppression is reflected in the coding properties of the single neuron across tasks. Neurons tended to have the same preferred direction in both tasks and a population vector constructed based on these directions predicted similar magnitudes of output from the frontal eye field during pursuit and its suppression. We confirmed that the residual eye movement during suppression could not explain the similarity in activity during pursuit movement and suppression. Thus, our results suggest that the decision whether to suppress movement occurs mostly downstream to the FEF. Finally, as expected from the lack of overlap of the population subspace, we found only weak relation between the coding of direction during saccade and pursuit. These residuals were in accordance with coding of the visual properties of the stimulus rather than coding of movement direction. Taken together, our analysis shows that in a specific area, distinct behaviors can exhibit either separate or overlapping population codes, emphasizing the necessity of conducting a system-level dissection of behavior.

Disclosures: M. Cain: None. **M. Joshua:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.05/FF7

Topic: D.07. Visual Sensory-Motor Processing

Title: Neuronal response of monkey peri-arcuate area during smooth pursuit eye movement with textured background

Authors: *Y. KODAKA, A. MURATA, M. INASE;
Physiol., Fac. of Medicine, Kindai university, Osaka-sayama, Japan

Abstract: We recorded the neuronal activities at peri-arcuate area (including frontal eye field and area 6) on adult rhesus monkeys (*Macaca mulatta*) during performing smooth pursuit eye movements with/without random dot textured background. We investigated the preference direction of the neural activities to the smooth pursuit eye movements toward eight direction. Some neurons activated at the start of the pursuit eye movement (phasic response). Some neurons activated during smooth pursuit eye movement (tonic response). Neurons showed the preference direction at the initiate and/or during the smooth pursuit eye movement. The preferred direction of the smooth pursuit activity did not effected by the background. The preferred direction of the neurons in frontal area was one direction (mono pole) and the selectivity was broad (not sharp) at the most neurons.The short latency small eye movement induced by the brief motion of the background during smooth pursuit eye movement (Kodaka et. al. 2003). Some neurons responded to these background motion. Perturbation of the background (60ms) induced the phasic neuronal response just before the eye movement response.

These data suggest the neurons at the frontal area code not only target spot information but large field motion information and it link to short latency eye movement.

Disclosures: **Y. Kodaka:** None. **A. Murata:** None. **M. Inase:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.06/FF8

Topic: E.01. Eye Movements

Support: DFG Grant SPP 2205

Title: Probing correlates of saccadic suppression in the primate superior colliculus and primary visual cortex using simulated saccades

Authors: *M. BAUMANN, Z. M. HAFED;
Univ. of Tübingen, Tuebingen, Germany

Abstract: Visual sensitivity is strongly impaired around the time of saccades, in a phenomenon known as saccadic suppression of visual sensitivity. Recently, we linked the origins of perceptual saccadic suppression to the rapid visual flow that appears on the retina during saccadic eye movements (Idrees and Baumann et al. 2020, Idrees et al. 2022). Specifically, we found that the visual appearance of the background (e.g. low or high spatial frequency content) that is translated on the retina by saccades can affect both the strength and duration of perceptual saccadic suppression, and that this effect already starts in the retina. Here, we investigated how visual flows created by rapid image shifts (simulated saccades) affect the superior colliculus (SC) and primary visual cortex (V1), both downstream of the retina. We recorded SC and V1 neural activity from one rhesus macaque monkey. The monkey fixated while we presented a saccade-like image displacement of different textured backgrounds (similar to Idrees and Baumann et al., 2020 with either a dominant low or high spatial frequency content). At different times after the texture displacement, we presented a brief probe flash (luminance pedestal) within the visual receptive fields (RF's) of the recorded neurons. We analyzed multi-unit activity and uncovered differences in how the two areas responded to both the texture displacements and the flashes. Both brain areas showed suppression of the flash response depending on the timing of presentation relative to the texture displacement, consistent with our earlier retinal and perceptual results. However, the areas exhibited important differences. The SC was highly selective in responding to the texture displacement itself, only responding to low spatial frequency image translations. Moreover, regardless of whether there was a strong response to the texture displacement or not, the response to the flash was suppressed immediately after the displacement. In V1, responses to the texture displacements and flashes were decidedly more varied. In most recorded channels, there were no differences in responses between the different displaced textures. Additionally, if there was no response to the texture displacement itself, there

was also no suppression of the subsequent flash response. Thus, in both the SC and V1, we observed correlates of "saccadic suppression" of flash-induced visual responses after global image displacements (simulating the visual flow of saccades). Moreover, we saw more variability in V1 responses. These results help elucidate the visual component of saccadic suppression, and they highlight functional specializations along the V1-SC processing axis.

Disclosures: M. Baumann: None. **Z.M. Hafed:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.07/FF9

Topic: E.01. Eye Movements

Support: CORE Grant P30EY08098 to the Department of Ophthalmology, the Eye and Ear Foundation of Pittsburgh, and unrestricted funds from Research to Prevent Blindness, New York, NY.

Title: Visual spatial attention alters V4 neural activity regardless of microsaccades

Authors: *S. WILLETT[1], J. MAYO[2];
[2]Dept. of Ophthalmology, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Recent work has implicated small fixational eye movements, called microsaccades, in the gating of attention-related modulation of neuronal activity. In cortical area V4, it was reported that visual spatial attention modulated neural activity only after a microsaccade was made toward the attended location. Related work reported a similar direction dependency in attentional modulation of neural activity in the superior colliculus (SC). However, in the SC attention-related enhancement of neuronal activity occurred prior to and in the absence of microsaccades. What is the origin of the discrepancies between the gating of attentional modulation by microsaccades in area V4 and the SC? It may be that the discrepancy exists because the two studies focused on cortical versus subcortical brain areas. Here, we investigate this possibility by probing attention-related modulation of V4 in relation to microsaccades while monkeys performed a task similar to the task used to probe SC attention-related modulation.
We investigated attention-related changes in neuronal population activity in V4 around the time of microsaccades while monkeys performed a visual-spatial attention task. Monkeys detected an orientation-change in one of two simultaneously presented oriented Gabors. Monkeys fixated and reported an orientation change by saccading to the changed stimulus. Attention was cued (80% validity) to a stimulus location using a visual cue on instruction trials at the start of each block of trials. We recorded over 3500 V4 single- and multi-units from Utah microelectrode arrays implanted in area V4.
We compared the average population response aligned to microsaccade onset when monkeys attended a target within neural receptive fields versus when they attended a target outside of

neural receptive fields. Contrary to previous reports in V4, we found that attention modulated V4 activity prior to and in the absence of microsaccades. Although our results largely agree with previous reports in SC, we found no evidence for a gating of attentional modulation by microsaccade direction. We extended the analysis by performing de-mixed principal component analysis on each recording session to extract attention-related latent factors aligned to microsaccade onset. Overall, our results suggest that attentional modulation in V4 can occur independent of microsaccades. More broadly, it is likely that certain task parameters may be key in determining microsaccade-attention interactions.

Disclosures: S. Willett: None. **J. Mayo:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.08/FF10

Topic: E.01. Eye Movements

Title: Monocular viewing during fixation reveals independent smooth drift control

Authors: *S. N. WATAMANIUK[1,2], S. HEINEN[2], A. CHANDNA[2], D. SINGH[2];
[1]Psychology, Wright State Univ., Dayton, OH; [2]Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: It is generally thought that the two eyes are yoked and rotate together, driven by single commands for both conjugate and vergence movements (Hering's Law). We recently published evidence challenging Hering's Law for vergence during smooth pursuit of a midline target during monocular viewing (Chandna et al., 2021). Here we investigate fixational eye movements during monocular viewing and show that smooth drift in each eye during fixation can be remarkably different and appears not governed by a unitary command. We recorded binocular eye movements in eight human observers fixating the center of a small "X" during either binocular viewing, or with one eye occluded with an infrared-pass filter. Each trial lasted 20 seconds and viewing condition was varied from trial-to-trial. We found that the occluded eye showed greater position variability than the viewing eye as quantified with the bivariate contour ellipse area (BCEA). Curiously, the BCEA of the viewing eye during monocular viewing was also larger than the BCEA of either eye during binocular viewing. These differences were due, in part, to increased drift speed and excursion since across subjects, drift speed was highly correlated with BCEA magnitude. In contrast, microsaccades were always conjugate, and their rate and frequency were identical in both eyes in both binocular and monocular viewing conditions. The results suggests that visual information from the covered and viewing eyes interact to guide the eyes when visual input is degraded. The results also suggest that drift during fixation is controlled independently while microsaccades remain conjugate, consistent with our new binocular control model comprising independent and conjugate channels.

Disclosures: **S.N. Watamaniuk:** None. **S. Heinen:** None. **A. Chandna:** None. **D. Singh:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.09/FF11

Topic: E.01. Eye Movements

Support: NIH R01 NINDS NS125843
Duke Institute for Brain Sciences (DIBS) Incubator Award

Title: Characterization of projection patterns to superior colliculus using rAAV2-retro in non-human primates

Authors: ***M. O. BOHLEN**[1], M. HASSE[2], M. RUDZITE[4], M. A. BASSO[5], L. N. KATZ[6], R. J. KRAUZLIS[6], G. D. FIELD[7], J. MOVSHON[3], M. J. HAWKEN[8], K. RITOLA[9], M. SOMMER[4]; [1]Duke Univ. Neurobio. Grad. Program, Durham, NC; [3]Ctr. for Neural Sci., [2]New York Univ., New York, NY; [4]Duke Univ., Durham, NC; [5]Univ. of Washington Primate Ctr., Seattle, WA; [6]Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD; [7]UCLA, Los Angeles, CA; [8]Ctr. for Neural Sci., New York Univ. Ctr. For Neural Sci., New York, NY; [9]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Background: Optogenetic technologies promise unprecedented insights into neuronal circuit function. However, their application in non-human primates (NHPs) has remained minimal. While actuator derived behavioral perturbation remains elusive, photosensitization of neurons is achievable. One promising approach is to use viruses that travel retrogradely to photosensitize neurons in distant areas that project to the injection site, an approach called "photo- or opto-tagging with projection targeting". A limiting factor in this approach is the characterization of transduction profiles. To address this, we provide a comprehensive anatomical characterization of rAAV2-retro in the NHP model. Here we describe results of mapping the biodistribution and transduction profiles of rAAV2-retro following injections into the superior colliculus.
Results: Our preliminary data across 4 macaques reveal an intricate neuroanatomical expression atlas for rAAV2-retro. This includes several structures known to project to the superior colliculus, indicating successful vector transduction. Notably, we found that the patterns of labeling vary in some structures based on the promoter utilized. Comparing CAG, closely related CBA, and the pan-neuronal promoter hSyn, we have found that all three promoters successfully drive expression both at the injection site and in corticotectal projecting populations, but some subcortical projection patterns are contingent upon promoter choice.
Conclusion: Given that the same capsid was used for all injections, these observations suggest that promotor-capsid interactions are important for determining transduction profiles. Thus, these results suggest an additional layer of regulatory complexity. This work establishes the

anatomical groundwork required for physiological experiments targeting tectal-projecting neuronal populations. These developments pave the way for more refined studies in systems neuroscience, setting the stage for hypothesis-driven studies within this field. By using projection targeting, we can now physiologically determine the information conveyed through individual circuits to the superior colliculus, providing new insights into its role in visual and visuomotor behaviors.

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Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.10/FF12

Topic: E.01. Eye Movements

Support: JSPS KAKENHI Grant Number 22H03492

Title: Effect of vergence and vertical eye movements on velocity perception

Authors: *Y. YOSHIMURA[1], T. KIZUKA[2], S. ONO[3];
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Abstract: In daily life or sports situations, we often visually track an approaching target with convergence eye movements and judge the velocity of the target in order to avoid colliding it or to intercept it. In those situations, since the target does not always move at the same height, the vertical eye movements are not always the same, even though convergence eye movements (horizontal component) are the same. Thus, not only convergence eye movements but also vertical eye movements could be crucial in determining the velocity of an approaching target. Previous studies have reported that convergence eye movements influence the velocity perception of an approaching target. However, it is unclear whether vertical eye movements that coincide with convergence movements influence the target velocity perception. Therefore, the main purpose of our study was to reveal the effect of convergence and concurrent vertical eye movements. Participants performed the velocity perception task for an approaching target. In the velocity perception task, participants judged whether the target was “faster” or “slower” in comparison to all other targets they had seen until then. The target moved at a distance of from 240 to 40 (far condition) or 240 to 20 (near condition) at one of the three different velocities (220, 250 or 280 cm/s). The velocity perception tasks were also implemented under two conditions: one in which the target moved at a vertical distance of 5 cm from a participant’s eyes to the horizontal screen, and the other in which the target moved at a vertical distance of 10 cm from a participant’s eyes to the horizontal screen. Participants performed a total of 480 trails (2

depths × 3 velocities × 2 vertical distances × 40 trails). The results showed that participants perceived the target velocity as slower when fast convergence and vertical eye movements, such as saccadic eye movements, occurred than when eye movements did not occur in the near condition only. Therefore, fast convergence and vertical eye movements could influence the velocity perception of an approaching target.

Disclosures: Y. Yoshimura: None. **T. Kizuka:** None. **S. Ono:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

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Program #/Poster #: PSTR215.11/FF13

Topic: E.01. Eye Movements

Support: NIH Grant EY030669

Title: Inhibitory tagging facilitates efficient saccade target selection in the superior colliculus during visual search

Authors: *C. CONROY[1], R. NANJAPPA[2], R. M. MCPEEK[1];
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Abstract: It has been suggested that, during difficult visual search tasks involving time pressure and multiple saccades, an inhibitory-tagging mechanism helps to facilitate efficient search by inhibiting saccades to objects in the scene once they have been searched and rejected. Recent findings from our lab are consistent with an influence of such a mechanism on the activity of superior colliculus (SC) neurons. Because the SC is involved in target selection, such findings suggest, in turn, that an inhibitory-tagging mechanism that seeks to promote efficient search may do so by modulating the target selection process in the SC. The purpose of this study was to examine this hypothesis. Rhesus monkeys performed a difficult visual search task while we recorded from individual SC neurons. The search task was designed to promote relatively long multi-saccade sequences on each trial, and therefore to provoke the placement of inhibitory tags. We found that, during the brief fixation period between saccades in these multi-saccade sequences, target selection by SC neurons was both more rapid and more robust when an uninspected saccade-target object was selected from among inspected (i.e., searched and rejected) as opposed to uninspected nontarget objects in the display. In other words, we found evidence that, consistent with the above hypothesis, inhibitory tagging both speeds and strengthens saccade target selection at the level of individual SC neurons. Thus, we conclude that an inhibitory-tagging mechanism that seeks to promote efficient visual search may do so by facilitating saccade target selection in the SC.

Disclosures: C. Conroy: None. **R. Nanjappa:** None. **R.M. McPeek:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.12/FF14

Topic: E.01. Eye Movements

Support: CIHR Grant MOP-FDN-148418

Title: How Subjects Learn to Optimize Blinking in an Interleaved Pro/Anti-Saccade Task

Authors: *I. C. PITIGOI, B. C. COE, O. G. CALANCIE, D. C. BRIEN, R. YEP, H. C. RIEK, R. H. KIRKPATRICK, D. P. MUNOZ;
Queen's Univ., Kingston, ON, Canada

Abstract: Spontaneous eye blinking is strategically timed to limit the obstruction of important or interesting visual stimuli and thus maintain efficiency in the visual system. It is unsurprising then that the behaviour has complex relationships to visual attention, cognitive control, and information processing. Yet, it takes no conscious effort to achieve optimal blink organization within a behavioural task and this adaptation happens relatively quickly. Here, we analyzed blink metrics and eye movements from a large cohort of normative participants (n=608, aged 5-93 years, 390 F) on an interleaved pro- and anti-saccade task to demonstrate how optimal blink timing is learned. The task contains an equal number of pro- and anti-saccade trials in a randomized order, totalling 240 trials. Previously, we revealed highly consistent patterns of blink suppression in preparation for visual stimulus appearance on this task. Blinks were most likely during the middle of the intertrial interval and fixation epoch, where they were least likely to interfere with stimulus perception. In the present study, we demonstrate that subjects achieve this stable temporal structure within the first 30 trials, and that it is well maintained for the rest of the task. Despite ocular fatigue increasing blink rates across the task, surplus blinks were contained within the intertrial and fixation intervals where they would be least disruptive to task performance. Analysis of sex and age also revealed group differences on blink organization and learning behavior; females between 20 - 55 years blinked more often than males, but the timing of blinks maintained the temporal structure described above. Overall, our findings suggest that spontaneous blinking is remarkably adaptive to predictable task structures so that timing is quickly optimized relative to attentional demands.

Disclosures: I.C. Pitigoi: None. **B.C. Coe:** None. **O.G. Calancie:** None. **D.C. Brien:** None. **R. Yep:** None. **H.C. Riek:** None. **R.H. Kirkpatrick:** None. **D.P. Munoz:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.13/FF15

Topic: E.01. Eye Movements

Support: The Adaptive Mind, funded by the Excellence Program of the Hessian Ministry of Higher Education, Research, Science and the Arts

Title: Sensorimotor Learning while Navigating Virtual Environments

Authors: *B. KRETZMEYER[1], M. MCMANUS[1], C. ROTHKOPF[2], K. FIEHLER[1]; [1]Justus Liebig Univ. Giessen, Giessen, Germany; [2]Tech. Univ. of Darmstadt, Darmstadt, Germany

Abstract: Navigating in naturalistic environments requires route planning and adaptive movement adjustments to sudden changes. To reduce adjustment costs, humans use predictions to guide sensorimotor decisions. For example, eye gaze deployment is used to reduce uncertainty about the potential movement paths of other people in the environment and to accordingly adjust one's own movement. This way, not only crashes, but also greater and more frequent movement adjustments in the near future can be avoided. Using virtual reality, we investigated how well humans can learn environmental regularities to optimize their gaze deployment and movement decisions. Participants were instructed to take the shortest path to one of two exit doors while navigating through a virtual museum and to avoid a suddenly appearing virtual museum guest who blocked either path. We manipulated the expected reward of a path by increasing obstacle frequency on one of the paths (90% vs. 10% probability of path blocking) within all trials of an experimental block. In the control block, each path was blocked randomly (50% probability of path blocking). Participants performed 6 experimental and 3 control blocks consisting of 20 trials each. We expected participants to learn and exploit the statistical regularity of obstacle appearance by adjusting their eye and body movements predictively towards the less frequently blocked path in later trials of an experimental block. While approximately half of the participants indeed adjusted their movement and gaze in the direction of the expected learning effect prior to obstacle appearance, i.e., they directed their gaze and movement towards the more likely free path, the others did not. The lack of an effect in these participants indicates interindividual differences in either learning capability or route planning strategies and requires further investigation. Our results demonstrate the human ability to adapt their information gathering and navigation behavior to statistical regularities in the environment. To gain a better understanding of the underlying cognitive processes in both groups of participants (learners and non-learners), we now aim to infer moment by moment navigation decisions by applying inverse reinforcement learning to our eye and body movement trajectory data.

Disclosures: B. Kretzmeyer: None. **M. McManus:** None. **C. Rothkopf:** None. **K. Fiehler:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.14/FF16

Topic: E.01. Eye Movements

Support: NSERC RGPIN-2019-04440, D.S.M.

Title: How self-confidence about terrain and energetic cost interact to influence gaze behavior

Authors: *V. DA EIRA SILVA[1], D. S. MARIGOLD[2];
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Abstract: When walking, people use vision to gain information about possible path choices. To make the best possible decision, people direct gaze to different features of the available paths that will help them to solve uncertainties about the level of safety, length, required level of skill, and expected energetic cost of walking that path. Usually, people prefer the path that is least effortful. Terrain complexity and increased stepping energetic cost can cause people to adapt their gaze behavior. Recently, we showed that self-confidence about walking across a path influences gaze behavior when choosing which path to take. However, in that experiment, we only manipulated the types of terrain people had to walk on, while keeping path length constant. Here we determined how self-confidence with terrain affects gaze behavior and path choice when deciding between paths with different energetic costs. Participants (n=6) had to choose between one of two paths to walk across. The paths in each of 8 environments varied in length (1.2 or 2 m, low- and high-cost paths, respectively) and consisted of realistic images of different types of terrain (e.g., dirt, rocks, mud). We quantified gaze behaviour during the approach phase. After performing the walking trials, we asked participants to rate terrains based on how confident they were about walking across them without losing balance as though they had to step on them in real life. When facing situations where the paths only differed in level of self-confidence (different terrain types, same length) participants directed a greater number of fixations ($p=6.57e-8$) and gaze time ($p=1.29e-11$) on the high confidence (6.65±2.88; 0.46±0.84s, respectively) versus the low confidence option (3.19±2.53; 0.17±0.61s, respectively). When facing situations where the paths only differed in level of energetic cost (same terrain types, different length) participants directed a greater number of fixations ($p=0.0006$) and gaze time ($p=0.0009$) to the low cost (6.12±2.7; 0.41±0.92s, respectively) versus the high-cost option (3.75±2.94; 0.23±1.06s, respectively). Next, we determined participant's gaze behavior when facing different confidence level and cost for each path (different terrain types and length). The disparity in self-confidence between the high- and low-cost path predicted gaze behavior on the high-cost path (number of fixations: $R^2=0.50$, $p=4.6e-6$; gaze time: $R^2=0.55$, $p=6.7e-7$), suggesting that self-confidence influenced information-seeking gaze behavior. Together, our preliminary results show that the brain considers both energetic cost and self-confidence when deciding where to direct gaze.

Disclosures: V. da Eira Silva: None. **D.S. Marigold:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.15/FF17

Topic: E.01. Eye Movements

Support: NEDO, JPNP20016
JST SPRING, JPMJSP2106

Title: Neural correlates of sense of agency in temporally delayed gaze-contingent display: an fMRI Study

Authors: *J. KIM, T. YOSHIDA;
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Abstract: Sense of agency (SOA)—the feeling of authoring actions and outcomes— is posited to be disturbed by discrepancy of action and sensory feedback (mismatch). While prior SOA studies focus on hand or limb movements as action, eye movements have barely been studied. This study is the first to adopt eye movements with temporal mismatch (delay). Since the subjective report of SOA is known to be attenuated by delay (Wen, August 2019), we examined delay-correlated neural activation using parametric modulation analysis with functional magnetic resonance imaging (fMRI) and eye tracking. In a block-designed fMRI experiment, 23 human participants conducted a visual search for Chinese letters with a gaze-contingent window, showing high-resolution in the fovea, blurring the periphery. The window was delayed at 0, 100, 300, and 1000 ms relative to eye movements. We implemented a no-authorship condition so that participants could not feel any authorship. In this condition, 300 and 1000 ms delay were pseudo-randomly set within a block so that participants could not predict the delay. Also, spatial incongruity was set between the window and participant's gaze position. Participants reported their perceived degree of control over the window (full, partial, or no control) per trial block. The proportion of "full" responses gradually decreased with the delay and lost its dominance to "partial" from 300 ms, then reached near zero in 1000 ms. "No" responses were barely reported with delay and only dominant in the no-authorship condition. These results are supported by the two GLMMs in delay conditions. The delay had significant main effect in the "full" vs. "partial" comparison ($p = .000$), and no main effect ($p = .055$) in the "no" vs. "partial" comparison. Overall, while participants clearly perceived the delay and reported fewer "full" responses with the delay, "partial" authorship was maintained even with a 1000 ms delay. To examine delay-correlated activations, delay was used as a parametric regressor at the individual level, followed by a group-level t-test. The right angular gyrus (Ag) was significant (FEW, $p = .018$), indicating stronger activations with temporal mismatch. Identified brain regions, the right Ag, align with Farrer et al. (May 2008) where stronger activation was reported when the visual feedback was delayed after hand movements. Also, there is consistency with subjective reports (Farrer et al., March 2008). Our research demonstrates parallels to SOA's neural activation with hand movements, suggesting that the eye can also be experienced as a causal initiator.

Disclosures: J. Kim: None. **T. Yoshida:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.16/FF18

Topic: E.01. Eye Movements

Title: The eye meets hand: plastic effector-specific and domain-general motor representations in human precentral gyrus

Authors: *C. FENG[1], E. ZHANG[3], K. XU[2], J. ZHU[1];
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Abstract: Human precentral gyrus is crucial for skeletomotor control, but it remains unknown whether it is also involved in oculomotor representations and how human oculomotor interacted with skeletomotor at the neuronal level. Here, we examined neuronal activity in human hand knob area when a participant performed either an eye or hand tracking task. We found neural tuning to the direction and spatial location of eye and hand tracking movements defined in extrinsic Cartesian coordinate system. The interaction between the direction and location showed the representations of eye and hand movements in an intrinsic mirror coordinate system. Population activity exhibited similar but not identical representations for the eye and hand movements. Projecting population activity into the first three dimensions of the state space, we identified two largely orthogonal neural components: a movement-coding subspace shared by eye and hand, and an effector-coding dimension that specified the eye and hand independently of their movements. Training in hand tracking task resulted in multiple forms of plastic changes in hand knob area, which occurred in parallel with the learning transfer to eye tracking movements. Manual training recruited more neurons whose activity correlated with the eye tracking performance. The training also refined representations of eye and hand movements in the shared subspace. Interestingly, manual training rotated a hand subspace to be orthogonal to an eye subspace. Such an effector-specific change in representation geometry allowed the refined representations to be applied to the effector-specific motor control without interfering with each other. These findings suggest the coexistence of effector-specific and domain-general movement representations in human hand knob area, which may facilitate skill generalization across effectors by optimizing both the movement representations and readout of task-relevant information.

Disclosures: C. Feng: None. **E. Zhang:** None. **K. Xu:** None. **J. Zhu:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.17/FF19

Topic: E.01. Eye Movements

Support: JSPS KAKENHI Grant Number 22H03492

Title: Evaluation of cortical visuomotor function using smooth pursuit eye movements in athletes

Authors: *S. ONO[1], T. MIYAMOTO[2,3], K. MIURA[4], T. KIZUKA[1];
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Abstract: The purpose of this study was to determine the effects of regular exercise in athletes on visuomotor function as estimated from smooth pursuit eye movements. Fifty-four participants were included in the study, and their smooth pursuit eye movements were measured. Participants were instructed to track a moving target. Smooth pursuit is known to be generated by either first- or second-order motion stimuli. Target stimuli using theta motion, in which the Gaussian window and the random-dot texture move in the opposite direction at the same speed, are known to induce lower smooth pursuit gain compared with first-order motion. The first-order motion is defined by the low-level visual motion such as luminance, whereas the second-order motion including theta motion is defined by the higher-level visual motion such as contrast, and flicker of motion. To examine the effects of regular exercise on cortical visuomotor function, the participants were divided into athlete and non-athlete groups, and their eye movements were compared. Athlete and non-athlete groups were classified based on exercise history and VO2max. As a result, the athlete group showed higher performance in pursuit gain and velocity than the non-athlete group. These results suggest that regular exercise in athletes may influence cortical activity related to visuomotor function. Furthermore, in the different experiments, we attempted to clarify the effect of theta motion on EEG-based event-related potential (ERP) and smooth pursuit eye movements. ERP amplitude was determined in the first 100 ms after the target motion onset to evaluate the eye or retinal motion-related activity. Our results showed that the theta motion yielded smaller ERP amplitude at parietal electrodes (P3 and P4) as well as smaller eye velocity/acceleration. It is known that the P3 and P4 EEG electrodes are situated over human MT and surrounding areas such as human homologs of area MST. Therefore, these results suggest that individual differences in pursuit gain to theta motion are related to the function of the visuomotor system, at least in the parietal areas.

Disclosures: S. Ono: None. **T. Miyamoto:** None. **K. Miura:** None. **T. Kizuka:** None.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: E.01. Eye Movements

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Support from P30 CORE award EY0809 through NIH NEI (MB & JPM via Pitt Ophthalmology))
Supporting funds from The Research to Prevent Blindness Inc, NY (MB & JPM via Pitt Ophthalmology)
Supporting funds from the Eye & Ear Foundation of Pittsburgh (MB & JPM via Pitt Ophthalmology)

Title: Horizontal and Vertical Sinusoidal Smooth Pursuit After Childhood Hemispherectomy

Authors: *M. Z. CHRONEOS[1], J. MAYO[2], M. BEHRMANN[2];
[1]Psychology, Carnegie Mellon Univ., Pittsburgh, PA; [2]Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Successful smooth pursuit eye movements, which involve a bilateral network of cortex and subcortex (Sharpe, 2008, Lencer et al., 2008), are crucial for tracking moving visual stimuli. Previous studies have shown that adults with unilateral frontal or posterior lesions or hemispherectomy execute saccades rather than smooth pursuit when pursuing a horizontally target moving towards their ipsilesional hemifield (Morrow et al., 1995, Thurston et al., 1988, Troost et al., 1972). Given the potential for plasticity in childhood, an open question is whether similar deficits are evident in children with large cortical lesions. Individuals with drug-resistant epilepsy and subsequent childhood hemispherectomy offer a unique opportunity to delineate the deficits and potential reorganization of the neural correlates of eye movements. We recorded eye movements during smooth pursuit in 14 such individuals (n = 14, 10 left [LH] and 4 right [RH] hemispherectomies, age at surgery: <1 year-8 years, age at test: 12-32 years) and 7 age-matched controls. Participants tracked targets moving in both horizontal and vertical sinusoidal trajectories (frequency = 0.3 Hz, amplitude = 10 degrees). Both LH and RH patients demonstrated pursuit that was significantly interrupted by ipsilesional saccades during horizontal trials, but no consistent asymmetries during vertical pursuit. In contrast, controls showed smooth eye traces in all directions. In measuring pursuit speed, both patient groups again showed reduced median ipsilesional eye speed across trials but intact contralesional speeds horizontally, and intact speeds in both vertical directions. Controls, in comparison, matched the speed of the target in all pursuit directions. Single subject data in controls correspondingly revealed that peak pursuit speeds relative to the target within expected time windows remained near the speed of the target, with similar speeds between the two horizontal and two vertical directions. By contrast, 12/14 (9 LH; 3 RH) patients showed markedly reduced ipsilesional peak pursuit speeds relative to contralesional peak pursuit speeds, which were more similar to controls. No consistent vertical asymmetries were noted in any group, though LH patients tended to have slightly reduced peak pursuit speed in either direction. Together, these findings elucidate a persistent but specific deficit of horizontal smooth pursuit following extensive childhood cortical resection. One possible explanation for this selective pattern is the presence of hemianopia and resultant sensorimotor dysfunction, but other accounts remain to be investigated.

Disclosures: **M.Z. Chroneos:** None. **J. Mayo:** None. **M. Behrmann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of Precision Neuroscopics.

Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.19/FF21

Topic: E.01. Eye Movements

Support: SEAMO AFP Innovation Fund Award SEA-17-004
Canadian Institutes of Health 1627 Research Grant MOP-FDN-148418

Title: Impulsivity and Eye-Tracking Measures in Borderline Personality Disorder with and without Comorbid ADHD: A Comparative Study

Authors: *O. CALANCIE[1], A. C. PARR[4], D. C. BRIEN[2], J. HUANG[5], I. C. PITIGOI[2], B. C. COE[5], L. BOOIJ[6], S. KHALID-KHAN[3], D. P. MUNOZ[7];
[2]Ctr. for Neurosci. Studies, [3]Dept. of Psychiatry, [1]Queen's Univ., Kingston, ON, Canada; [4]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [5]Ctr. for Neurosci. Studies, Queens Univ., Kingston, ON, Canada; [6]Psychiatry, McGill Univ., Montreal, QC, Canada; [7]Ctr. for Neurosci. Studies, Queen's Univ. Ctr. For Neurosci. Studies, Kingston, ON, Canada

Abstract: Borderline Personality Disorder (BPD) affects 1-2% of the population and symptoms peak during adolescence, interfering with normal psychosocial development. Impulsivity is a prominent trait in BPD and a strong predictor of diagnosis long-term. In the clinic, impulsivity is characterized by excessive spending, engaging in risky sexual behaviors, substance abuse, and self-harming tendencies, however, it remains unknown if persons with BPD demonstrate increased impulsivity in the absence of social/emotional stimuli. Literature presents mixed findings, possibly due to the high comorbidity of ADHD (~50% incidence rate) and inadequate control in behavioral analysis. Here, we test whether impulsive behavior via response inhibition and temporal prediction differs among adolescents with BPD and BPD with comorbid ADHD ('ADHD/BPD') against matched controls in a computer-based eye-tracking task using the Eyelink 1000 Plus. We also examine whether pupillary and oculomotor measures (saccade; blink) vary in BPD as a function of an ADHD comorbidity. 24 BPD (Mean: 16 ± 1.4 yrs; *Female*), 25 ADHD/BPD (16 ± 1.5 yrs; *F*), and 66 control (15 ± 1.6 yrs; *F*) participants were recruited to complete the *metronome task* (a visual target alternated at a steady interstimulus interval [ISI] between two fixed locations), *random task* (ISI changed at each target step) and *interleaved pro-/anti-saccade task* (a central fixation cue informed participants to make a pro-saccade to or an anti-saccade away from a target). Saccade, blink and pupil data were marked offline using custom Matlab scripts. Main effects of group x impulsivity were observed, with ADHD/BPD participants making significantly more direction errors on anti-saccade trials (p=0.015) and anticipatory saccades (i.e., guesses of target appearance) in the random task

(p=0.004), whereas performance of BPD and control groups did not differ. Larger baseline pupil sizes were noted in BPD and ADHD/BPD groups in all three tasks (p<.001). Further, ADHD/BPD participants had higher blink rates than controls in the metronome task (p=0.002) and increased blink probability during fixation epoch of the interleaved pro-/anti-saccade task (p<.001). Eye-tracking performance supports increased impulsivity under conditions devoid of social/emotional stimuli in BPD with comorbid ADHD, but not in BPD alone. Larger pupil sizes in BPD groups suggests elevated sympathetic tone, perhaps contributing to the increased threat sensitivity and aggression observed in this disorder. Lastly, blink parameters differentiated ADHD/BPD from BPD participants, perhaps serving as a biomarker of ADHD pathology.

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Poster

PSTR215. Eye Movements: Central Mechanisms, Perception, and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR215.20/FF22

Topic: D.07. Visual Sensory-Motor Processing

Support: IBS-R015-D1
MSIT Grant 2023R1A2C2005290

Title: Interplay between temporal and directional expectations on sensorimotor processing

Authors: *S. WOO[1,2,3], S. BAE[1,2], J. KIM[1,2], G. KIM[1,2], Y. HWA[1,2], J. LEE[1,2];
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Abstract: The ability of humans to make optimal decisions relies on their expectations concerning time and direction in the world, and previous research has shown the profound influence of each expectation on behavior. Higher temporal expectations yielded faster reaction times, while greater directional expectations led to biased behavior toward the expected direction and reduced variability. However, it remains unknown how their interaction exists and affects our behavior. This study aims to explore the existence of the interaction between these two expectations and propose a Bayesian observer model as the underlying mechanism. Thirty human participants are involved in a smooth pursuit eye movement task, while electroencephalography (EEG) and eye movement data were recorded simultaneously. We confirmed a close relationship between temporal and directional expectations through correlation analysis, which revealed a significant negative correlation between the reaction time of eye movements and directional bias. To explore a mechanism about the interaction, we developed two alternative hypotheses and employed the Bayesian observer model for both: (1) Temporal and directional expectations increase simultaneously, or (2) considering the limitations of our

cognitive capacity, as more resources are allocated to temporal expectations, fewer resources remain for processing the direction of moving target, resulting in reduced reliability of sensory information and an increased reliance on directional expectations. Behavioral variability serves as the pivotal factor in differentiating between the two hypotheses. Through the modeling, we determined which hypothesis provides a more accurate explanation for the bias and variability observed in the direction of movement. When using the Akaike's weight as a measure to evaluate the goodness of fit, we observed that most participants supported the first model. To examine the neural mechanism for this, EEG frequency analysis was conducted. As the stimulus appeared later, there was a decrease in Beta power in the posterior region and an increase in Theta power in the anterior region. We anticipate that the distinct alterations in frequency power between the two regions might contribute to the mechanisms underlying the interaction between temporal and directional expectations.

Disclosures: S. Woo: None. **S. Bae:** None. **J. Kim:** None. **G. Kim:** None. **Y. Hwa:** None. **J. Lee:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.01/FF23

Topic: E.04. Voluntary Movements

Support: Facebook Technologies Grant 00144033

Title: Can we learn a motor control task by decomposition?

Authors: *E. GRIESSBACH[1], A. M. HAITH[2], C. BRADY[2];
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Abstract: The acquisition of novel motor skills from scratch is fundamental to a myriad of human tasks, from operating complex machinery to performing intricate surgeries. Despite its importance, the mechanisms through which humans efficiently learn high-dimensional control tasks, a challenge not yet solved in engineering, remain elusive. One proposed strategy is learning by composition, wherein complex tasks are broken down into components, learned separately, and then recombined. Although observable in sequential tasks like piano playing, its application in continuous motor control tasks, like car driving, is less established. Hence, we focused on composition as a potential strategy of generalization within the context of a two-dimensional (2D) bimanual control task (Haith et al., 2022). This task required participants to control cursor movements on a screen, where each hand corresponded to one dimension, thus providing a natural separation for learning the two dimensions independently. We have previously shown that participants learn this skill by acquiring a new control policy "de novo" rather than adapting an existing controller. We divided participants into two groups: a composition group and a control group. The composition group had targets during the learning

phase appearing solely in cardinal directions (1D task), whereas the control group saw targets appearing in all directions similar to the test conditions (2D tasks). Both groups performed a pretest and posttest requiring cursor movements in all directions (2D task). An ANCOVA was used to analyze performance, with trial completion time as the dependent variable and initial block performance as the covariate. Contrary to our hypothesis, training in cardinal directions did not facilitate subsequent performance in non-cardinal directions. The control group performed better in the posttest than the generalization group. Moreover, the initial 2D performance showed no significant difference between groups, indicating a failure to successfully compose the learned ability to move along the x- and-y-axes in all movement directions. Our results suggest that generalization may be more limited in our task, and this may account for the typically slow time course of learning complex, high-dimensional skills.

Disclosures: E. Griessbach: None. **A.M. Haith:** None. **C. Brady:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.02/FF24

Topic: E.04. Voluntary Movements

Title: Implicit adaptation is modulated by the sense of agency

Authors: *X. ZHANG, K. WEI;
Peking Univ., Beijing, China

Abstract: Motor adaptation refers to the process that our motor system adapts to changes in the environment or the body to maintain stable performance. The popular visuomotor rotation (VMR) paradigm perturbed reaching movements via a rotated visual representation of the hand. The resulting adaptation consists of both explicit and implicit processes. To isolate the implicit process, the other popular paradigm, the error-clamp (EC) paradigm, presents the same visual rotation but requires the subject to ignore it and to reach the target with their invisible hand directly. In the EC paradigm, the visual cursor is not under the agent's control. Currently, most researchers maintain that the implicit adaptation elicited in the EC paradigm was equivalent to that in the VMR paradigm, given their similar rates and magnitude (Morehead et al., 2017; Kim et al., 2018; Tsay et al., 2022). However, implicit adaptation is promoted by monetary reward and motivation (Galea et al., 2015; Nikooyan et al., 2015). The sense of agency (the feeling that the movements and their sensory outcomes are the results of our own actions) oftentimes acts as an intrinsic reward and thus promotes action control (Naour et al., 2023; Penton et al., 2018), which could lead to higher implicit adaptation. We notice that the crucial difference between the VMR task and the EC task is whether the cursor is under the agent's control. Thus, we hypothesize that implicit adaptation should be reduced in the EC task when compared to the VMR task, given the absence of agency and thus intrinsic reward. In separate sessions, our participants adapted to random EC or VMR perturbations, and their implicit adaptation was

quantified by single-trial corrections. Despite that both tasks had identical visual feedback and perturbation schedules, we found that implicit learning in the VMR task was significantly higher than that in the EC task. The reaction time also increased in the VMR task, possibly caused by more cognitive resources or mental efforts involved. Our results thus support that the sense of agency, endowed by task instructions here, modulates the implicit process during motor adaptation.

Disclosures: X. Zhang: None. **K. Wei:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.03/FF25

Topic: E.04. Voluntary Movements

Support: NSERC Alexander Graham Bell Canada Graduate Scholarship - Doctoral
NSERC Discovery Grant

Title: Adopting an external focus of attention impedes visuomotor adaptation

Authors: *D. O. WIJEYARATNAM, E. K. CRESSMAN;
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Abstract: Previous work has consistently demonstrated the benefit of an external focus of attention compared to an internal focus of attention in motor skill acquisition (see Wulf, 2013 for a review). In the current experiment we asked if learning benefits observed in the skill acquisition literature extend to visuomotor adaptation. In particular, we asked if an external focus of attention benefits visuomotor adaptation. Participants were divided into 3 groups: (1) External focus (EXT; n = 27), (2) Internal focus (INT; n = 27) and (3) Control (CTL; n = 29). The EXT group was instructed to focus on the path taken by a cursor on the screen, while the INT group was instructed to focus on the path taken by their hand. The CTL group was not provided with an attentional focus instruction. For all participants, the task goal was to perform a shooting movement such that the cursor landed on the target. Reach training trials were performed within a virtual environment when (1) a cursor accurately represented participants' hand motion (48 aligned cursor trials), or (2) a cursor was rotated 40 degrees clockwise relative to participants' hand motion (160 rotated cursor trials). Attentional focus instructions were provided at the start and after every 40 rotated reach training trials (i.e., 4 times in total; trial 0, trial 40, trial 80 and trial 120). Following rotated reach training, participants reached to the targets in the absence of cursor feedback when instructed to (1) engage in strategic reaching (i.e., assessment of explicit adaptation) and (2) reach directly to the target (i.e., assessment of implicit adaptation). Analyses of angular errors at peak velocity revealed that participants in the INT and CTL groups were able to adapt their reaches to a similar extent over the course of rotated reach training trials. Surprisingly, participants in the EXT group adapted significantly less compared to the INT and

CTL groups across mid-to-late rotated reach training trials. Analysis of no cursor reaches revealed that the EXT group was more limited in their ability to engage in a reaching strategy (i.e., had less explicit adaptation) in comparison to the INT and CTL groups. However, there was no difference in implicit adaptation across the three groups. Together, these results suggest that an external focus of attention impedes the extent of visuomotor adaptation achieved, potentially by interfering with one's ability to engage in strategic reaching. These results are in contrast to typical results found in motor skill acquisition literature, supporting the suggestion that visuomotor adaptation is a particular form of motor learning distinct from motor skill acquisition (see Krakauer et al., 2019).

Disclosures: D.O. Wijeyaratnam: None. **E.K. Cressman:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.04/GG1

Topic: E.04. Voluntary Movements

Support: United States - Israel Binational Science Foundation (BSF) Grant 2021323

Title: Motivation upregulates the adaptive response in motor learning

Authors: S. KHATIB[1], V. S. CHIB[2], ***F. MAWASE**[1];
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Abstract: Motivational state plays a critical role in our ability to learn new motor skills, yet little is known about the mechanisms by which motivation influences motor learning. We designed a motor adaptation paradigm that systematically decoupled the effects of motivation from performance-contingent outcomes, by making these two components statistically independent. We implemented a force-field perturbation setup that allowed us to dissociate the adaptive response of each of the two components by fully randomizing external perturbations, and thus motor errors, and making the external motivational cues orthogonal to performance. Healthy young participants (n=13) were instructed to make rapid reaching movements with the dominant hand toward a single target that appeared 10 cm from the home position, holding onto the handle of a two-joint robotic manipulandum. In a multi-phase force-field motor adaptation task, participants first performed a baseline phase for familiarization in which no external force-field perturbation was applied. Participants were then exposed to pseudo-random velocity-dependent force-field perturbations. Critically, within these perturbation trials, some trials were associated with monetary cues that were uniformly sampled, but statistically independent of the perturbation schedule. These cues were used to set either a low ($3) or high ($30) motivational state in each trial. By quantifying the trial-by-trial adaptive response with and without motivation, our results indicated that motivation significantly interacted with the adaptive

response (Repeated-measure ANOVA, F=28.32, *P<0.0001*) -- participants compensated more for an error when the motivational state was high. Our findings reveal that motivation affects motor learning by upregulating the rate of the adaptive response, essentially increasing individuals' speed of learning. These results provide experimental support for the importance of motivation in facilitating error-based motor learning in humans.

Disclosures: S. Khatib: None. **V.S. Chib:** None. **F. Mawase:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

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Program #/Poster #: PSTR216.05/GG2

Topic: E.04. Voluntary Movements

Support: Indian Institute of Technology Hyderabad
DST-CSRI grant to Neeraj Kumar

Title: Role of working memory in immediate versus delayed interlimb generalization of a newly learned motor skill

Authors: G. YADAV[1], S. MATKAR[2], R. PAL[2], ***N. KUMAR**[2];
[1]Univ. Catholique de Louvain, Brussels, Belgium; [2]Indian Inst. of Technol. Hyderabad, Hyderabad, India

Abstract: Newly learned motor skills can generalize/transfer to the untrained arm. Such interlimb generalization of skilled movements has been shown to be symmetric in nature (interlimb transfer of learning from right to the left arm and vice versa), and is thought to be mediated by cognitive processes that can influence the newly acquired skill memory. Recently, it has been demonstrated that working memory (visuo-spatial) can interact with skill memory, particularly when the skill is acquired in a variable task environment. However it is unclear how working memory interacts with a newly formed skill memory and influences immediate versus delayed interlimb generalization. To test this idea, we recruited right-handed young healthy subjects who learned a novel motor skill task. Subjects were required to make spatio-temporal accurate reaching movements to one of five randomly presented targets with their right arm using a stylus on a digitizing tablet. Vision of the arm was blocked by a horizontal semi-silvered mirror, which is placed just above the hand. Visual feedback was provided by a computer-generated display that projects target positions and a cursor representing the hand position on the mirror. Performance feedback was provided after each trial - visual feedback indicating spatial and temporal error, and a numeric motor error score. Subjects were divided into 4 groups- i) Group Imm_WT: Learned skill task (160 trials), performed working memory task (corsi-block tapping task, 90 trials) with right arm followed by interlimb generalization test of motor skill with the left arm (160 trials), ii) Group Imm_CT: Learned skill task, performed control version of corsi-block task with right arm followed by interlimb generalization test of skill with the left

arm, iii) Group Late_WT: Learned skill task and working memory task with right arm on day1, followed by interlimb transfer 24hours later and iv) Group Late_CT: Learned skill task on day 1 followed by the control task with right arm. Interlimb transfer was assessed 24hours later. Interlimb transfer was observed when the untrained left arm is tested immediately in Group Imm_WT and Imm_CT. On the other hand, performing a visuo-spatial working memory task (Group Late_WT), but not the control task (Group Late_CT), immediately after learning a new motor skill impairs interlimb generalization of the skill when the generalization test is at 24 hr following skill acquisition. These results indicate the possibility of distinct neural mechanisms involved in immediate versus delayed interlimb generalization of a newly learned skill.

Disclosures: G. Yadav: None. **S. Matkar:** None. **R. Pal:** None. **N. Kumar:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

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Program #/Poster #: PSTR216.06/GG3

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 NS052804-01A2

Title: Assessing the Role of Cognition during early Learning of a New Motor Skill

Authors: *M. V. COLAVITO[1], C. S. YANG[2], J. LIU[1], E. GRIESSBACH[1], J. W. KRAKAUER[1], A. M. HAITH[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Neurosci., Lockheed Martin, Arlington, VA

Abstract: When learning a new motor skill, cognitive resources are thought to be crucial for skill performance, particularly during the early stages of learning. If so, diverting cognitive resources to a secondary task should disrupt performance. For example, when first learning to drive a car, trying to hold a conversation while driving can be difficult or even dangerous. The cognitive demand of motor skills is thought to lessen with increased practice though, allowing for other tasks to be performed in parallel. However, there is little direct evidence for this in the context of continuous motor skills. We conducted a study to investigate the role of cognition in the early stages of learning a novel continuous motor skill. Participants learned to control an on-screen cursor using a non-intuitive bimanual mapping. Our prior studies demonstrated that this mapping requires about five days of practice for performance improvements to plateau and is learned “de novo” (rather than through adaptation). We employed a dual-task approach to assess the involvement of cognition in performance of this task during early learning. We focused on spatial working memory (SWM), which has been proposed as a critical cognitive resource for learning visuomotor skills, using two distinct tasks. The first task was a mental rotation of the target reach position, and the second task involved reaching to the remembered positions of a sequence of 5 targets. If participants' ability to control the cursor declined compared to the baseline while performing the additional tasks, it would indicate that the cognitive resources

engaged by these tasks were necessary for performance. Surprisingly, when probed after one day of practice (after 600 trials) we found no difference in the impact of the additional tasks on bimanual control compared to the baseline. Importantly, we did observe interference when participants attempted to perform the two SWM tasks concurrently, ruling out the possibility that the tasks were not sufficiently demanding. These results led us to suspect that we had assessed participants too late in the learning process, so we repeated the experiment. This time, participants (n = 16) were given only 100 trials of initial skill training. Once again, we found minimal disparity in the effect of the additional tasks on the de novo control of the cursor compared to the baseline. Notably, we did find a correlation between baseline performance on the sequence task and early de novo performance (first 100 trials). However, this advantage was not sustained later in learning. These findings suggest, surprisingly, that SWM demands during motor skill performance are minimal, even in the early stages of learning.

Disclosures: M.V. Colavito: None. **C.S. Yang:** None. **J. Liu:** None. **E. Griessbach:** None. **J.W. Krakauer:** None. **A.M. Haith:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.07/GG4

Topic: E.04. Voluntary Movements

Support: University of Minnesota Foundation
National Center for Advancing Translational Sciences Award No. UL1TR002494

Title: How does cognitive load affect upper limb motor control in adults?

Authors: *S. A. L. JAYASINGHE[1], P. THAPA[1], S. TIMANUS[2];
[1]Rehabil. Med., [2]Computer Sci. and Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Previous studies have shown that laterality can depend on task and/or environmental demands. Task complexity, such as object manipulation vs. simply picking up an object, can result in interlimb differences in movement time and coordination that show higher proficiency for right hand performance (Bryden et al., 2007). According to the dynamic dominance hypothesis, in right-handers, the dominant left hemisphere is specialized for the control of intersegmental dynamics while the nondominant right hemisphere is specialized for postural control (Sainburg, 2005). Previous studies have shown that cognitive load may affect hand selection and reaction time without necessarily influencing the linearity (i.e., quality) of the trajectory (Liang et al., 2018). However, it is not known whether cognitive load differentially affects lateralized motor control processes in each limb. We hypothesized that interlimb asymmetry in motor performance and coordination would increase with higher levels of cognitive load. We will recruit 20 right-handed adults for this study. We designed an upper limb

reaching task on the Kinereach system to be completed with each of the left and right hands, in a randomized order. Participants sat in front of a table with a mirrored screen that occluded view of the arms placed on the glass table. An inverted TV projected the task onto the screen. Sensors placed on the hand and upper arm recorded position and orientation, and EMG sensors placed on the posterior deltoid, pectoralis, biceps long head, and triceps lateral head recorded surface muscle activity at the shoulder and elbow. The task was an upper limb reaching task combined with a cognitive challenge. Each trial involved two screens - the first screen provided a set of pictorial instructions that needed to be committed to working memory within 2 s; the second screen had an array of objects to choose from. Participants had to reach for the correct object based on the instructions on the first screen. We increased the cognitive load on the participant by varying the number of items to remember and/or changing the complexity of the object array. Preliminary analysis of the kinematic data shows that the right hand has longer reaction times compared to the left hand, possibly because of longer time needed to accurately prepare for a movement. Movement linearity and accuracy change with cognitive load. Analysis of both the kinematic and EMG data is ongoing. We expect the results to be informative in expanding our understanding of the neural control of movement.

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Poster

PSTR216. Influences on Motor Learning and Execution

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Program #/Poster #: PSTR216.08/GG5

Topic: E.04. Voluntary Movements

Support: NIH T32-NS082128-06

Title: Executive function overload increases dysmetria but not intra-individual variability

Authors: *J. C. HUBBARD[1], J. J. KIM[2], S. DELMAS[2], Y. CHOI[2], S. SALAMEKH[2], B. YACOUBI KEYHANI[2], E. A. CHRISTOU[2];
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Abstract: Executive function is defined as the cognitive processes involved in regulating analytical skills (e.g., selective attention, workload capacity, working memory) and metacognitive skills (e.g., problem-solving, decision-making, strategic planning). The Flanker Task is a validated cognitive test used to evaluate an individual's executive functioning capabilities. The brain centers activated by the Flanker task (the dorsolateral prefrontal cortex and the anterior cingulate cortex) overlap with the brain centers used to control movement; however, it remains unknown if overloading the frontal lobe (through simultaneous performance of the Flanker Task) can impact endpoint accuracy and variability in the motor output. Here, we compare the endpoint control of healthy young adults when performing open-loop goal-directed movements with and without simultaneous completion of the Flanker test. Nine healthy

participants (21.9 ± 3.8 years) performed 4 sets of 100 ankle dorsiflexion trials with their non-dominant foot. Each set of 100 trials alternated between two conditions: Flanker_ON and Flanker_OFF (control). The order was counterbalanced amongst participants. A visual stimulus prompted subjects to dorsiflex their ankle to a spatial target of 9 degrees and a temporal target of 160 ms. Participants were provided with visual feedback after each trial. To avoid differences in error-based learning and adaptations, we used the last block of 25 trials to quantify spatial and temporal errors of the movement, as well as the intra-individual spatial and temporal variability (coefficient of variation). During the Flanker_ON condition, participants exhibited significantly greater spatial errors (OFF: 0.32°, ON: 1.07°, $p<0.05$) and overall error (OFF: 3.54%, ON: 11.9%, $p<0.05$) than with the Flanker_OFF condition. There were no significant differences between the two conditions for temporal errors, and spatial and temporal variability. These findings suggest that overloading the frontal lobe in healthy young adults increases endpoint errors (dysmetria), but not variability in the motor output. These results elucidate how analytical and metacognitive skills impact and interact with the motor system. These findings can help us understand how frontal lobe disturbances can change the motor output in individuals with cognitive deficits.

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Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.09/GG6

Topic: E.04. Voluntary Movements

Support: Meta Reality Labs

Title: The effect of practice variability on learning and generalization during de novo skill learning

Authors: K. KITA[1], S. SEKI[3], M. V. COLAVITO[2], *A. HAITH[2];
[1]Dept. of Neurol., [2]Johns Hopkins Univ., Baltimore, MD; [3]Kyoto Univ., Kyoto, Japan

Abstract: Learning a new skill can often involve learning new relationships between our actions and their outcomes that are arbitrary and unfamiliar. For instance, when driving a car, we must turn a wheel with our hands to steer or press a pedal with our foot to stop. This kind of learning - termed “de novo” learning - requires extensive practice to become proficient. The conditions of practice can affect how quickly we learn and how readily we can generalize our learning to new contexts. In particular, task variability during practice is thought to be critical for promoting broad generalization. We performed an experiment to examine how variability of practice impacted de novo skill learning. We used a task in which participants controlled a cursor through a non-intuitive mapping between the hands and cursor position: the forward-backward

movement of the left hand controls the cursor's left-right movement, while the left-right movement of the right hand controls its forward-backward movement (the “De Novo” mapping). We asked whether participants could learn this new skill in a narrow context, with minimal task variability and successfully generalize it to broader, unexperienced contexts. Over the course of four days, a group of nine healthy participants learned to control the cursor using the De Novo mapping by performing only center-out reaches from a consistent starting location in each trial (the narrow-practice group). Another group of 25 healthy participants learned to control the cursor using the De Novo mapping through practice involving a series of point-to-point movements starting from different positions around the workspace (the varied-practice group). Participants in both groups gradually improved their performance through practice. On the first day of practice, however, participants in the narrow-practice group exhibited worse performance and higher initial errors in cursor direction compared to participants in the varied-practice group. After four days of practice, the cursor direction error of the narrow-practice group converged to the same level as the varied-practice group. On the fifth day, we tested whether participants in the narrow-practice group were able to generalize their learning to the full task that the varied-practice group experienced. We found that participants could generalize very easily and performed comparably to participants in the varied-practice group. In summary, while narrow practice led to initially slower skill acquisition but, after extensive practice, the variability of practice had minimal effect on the learned skill, and participants were easily able to generalize narrow training to a broader range of contexts.

Disclosures: K. Kita: None. **S. Seki:** None. **M.V. Colavito:** None. **A. Haith:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

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Program #/Poster #: PSTR216.10/GG7

Topic: E.04. Voluntary Movements

Title: Trade-offs between Effort and Complexity in Sensorimotor Control

Authors: *X. DENG[1], F. LU[2], A. HAITH[1];
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Abstract: Theories of motor control propose that our actions are selected to optimize costs, which might include task performance, effort, and time. Typically, behavior reflects a trade-off between these competing costs. For instance, we could move more quickly, but at a greater cost of effort. Here, we propose a further cost that is relevant in sensorimotor control: the cost of control *complexity*. In the context of cognitive decision-making tasks, it has been argued that the complexity of a policy provides a critical constraint on our ability to select actions. The best possible course of action may be infeasible to follow given limited resources available for making a decision. Instead, it is argued that people may behave in a way that is optimized given these resource constraints. Here, we examine the possible role implications of similar complexity

costs in human sensorimotor control. We reasoned that a key strategy by which the complexity of a controller might be might be simplified is through cocontraction. Under strong cocontraction, the endpoint of an effector becomes largely independent of the precise initial state of the limb, significantly simplifying the control problem at the cost of expending greater effort. We show through simulations that this insight bears true under the information-theoretic formulation of complexity costs. To test in more detail whether people actually trade off control complexity for effort, we designed a virtual inverted pendulum balancing task. In this task, participants are asked to balance the inverted pendulum through applying forces to with their two fingers, which control two muscles arranged as an agonist/antagonist pair, so that participants could either keep the pendulum upright through continual "cocontration" of the two muscles (a low-complexity/high-effort solution), or by continuously responding to momentary fluctuations in the pendulum's state (a low-effort/high-complexity solution). We vary difficulty of this task by varying the strength of gravity, in order to assess how participants would vary their control strategy and, in particular, how they trade complexity for effort. We predict that: when the difficulty is low, the participants will engage in low effort and low complexity strategy; when the difficulty is moderate, the participants will first increase the complexity of their strategy, while still applying low efforts; when the difficulty is high, the participants will switch to high effort and low complexity strategy instead.

Disclosures: **X. Deng:** None. **F. Lu:** None. **A. Haith:** None.

Poster

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Program #/Poster #: PSTR216.11/GG8

Topic:

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Title: Influence of variability on search for new coordination patterns in de novo motor learning

Authors: *A. MEHTA[1], D. TRAVIESO[2], J. SMITH[1], R. BONGERS[1];
[1]Univ. of Groningen, Univ. Med. Ctr. Groningen, Groningen, Netherlands; [2]Fac. of Psychology, Univ. Autonoma de Madrid, Madrid, Spain

Abstract: Learning a novel motor task involves searching within the joint space for formation of new coordination patterns to accomplish the task goal. The process of search is the systematic change in joint angle coordination over time which requires variability in the joint coordination patterns. Motor learning studies have widely reported benefits of practice variability on improvement in task performance and have evaluated search processes primarily at the task level but not at the underlying joint level. Therefore, this exploratory study aims to identify differences

in learning patterns in the task space as well as joint space between variable and blocked practice that can be discerned through differences in search strategies. We hypothesize that participants performing variable practice will display more search behavior as compared to blocked practice. The increased search behavior in the variable group should be reflected in more variability of joint configurations within the solution space than the blocked group. To evaluate this, we perform a learning task with blocked and variable practice groups, followed by a transfer task. So far we measured 11 participants (Age: 23.8±3.2 yrs, 8M & 3F) who learned a virtual lateral interception task where the paddle used to intercept the downward moving ball was controlled by elbow and shoulder movements. A novel redundant mapping was introduced between the movement signals measured by the sensors (goniometer in elbow and IMU in shoulder) and the paddle position in VR. Learning the mapping to accurately move the paddle requires the formation of new coordination patterns. Results of the study show that participants learn the mapping and thereby the necessary movement coordination in both practice conditions. Success in pretest was 2.7±1.5 while in posttest was 11.7±3.3 out of 16 trials in each session. The SD of joint angles at interception was higher in variable practice as compared to blocked practice for each phase. The uncontrolled manifold (UCM) analysis will be performed to evaluate the structure of variability in the joint space. It is expected that UCM like structure will be present in the last phases of learning even when the error at task level was small. This indicates that an optimum solution within the joint space is not achieved and there is continued search behavior within the solution space. The search behavior will be characterized by parameters derived from the UCM analysis. The variable group would display higher search behavior and more variance in the joint configurations to intercept the ball. The preliminary analysis indicates differences in the two practice groups aligned to our hypothesis.

Disclosures: **A. Mehta:** None. **D. Travieso:** None. **J. Smith:** None. **R. Bongers:** None.

Poster

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Program #/Poster #: PSTR216.12/GG9

Topic: E.04. Voluntary Movements

Title: Assessing the dynamics of conflict resolution and conflict adaptation through a forced-response task

Authors: *S. LEE[1], A. HAITH[1], J. XU[2];
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Abstract: During action selection, we are often faced with multiple conflicting responses to a single environment cue. How these conflicts are resolved, and how the process of conflict resolution can be altered through experience, have been widely studied as a model of cognitive control. When participants frequently encounter conflicting stimuli, they become able to resolve the conflict more effectively, leading to lower reaction times to incongruent stimuli. Existing

approaches to studying conflict resolution and conflict adaptation are limited by relying primarily on reaction times. This unitary measure makes it impossible to fully infer the potentially multi-faceted dynamics underlying action selection and conflict resolution. We studied conflict resolution and conflict adaptation in a variation of the Simon task, where participants learn a pairing between a small number of symbolic cues and corresponding spatial goals, making center-out reaching movements in response to the cue. By presenting the stimuli inside one of the four possible targets, we created a conflict between the spatial location of the stimulus and the instructed goal location.To overcome the limitations of measuring performance based on reaction time alone, we adopted a forced-response approach in which allowed preparation time could be varied on a trial-by-trial basis, from 0 ms to 1000 ms. This allowed us to reveal the underlying dynamics of conflict resolution based on how participants' responses evolved as a function of allowed preparation time. When participants were forced to respond to an incongruent cue with very limited preparation time (300-600ms), they reliably responded according to the spatial location of the cue, rather than the instructed goal location.To assess how this process was altered during conflict adaptation, we tested participants in two conditions in which trials were either mostly congruent (75%) or mostly incongruent (25%). To ensure that any conflict adaptation effects were not due to lower-level, stimulus-specific learning, but due to higher-level changes in cognitive control, we also included a distinct set of "diagnostic" symbols for which the frequency of congruent trials was consistent across conditions (50%). We found that experiencing incongruent trials more frequently led to more rapid preparation of the appropriate response. However, it also led to a delay in the preparation of the spatial response. These effects were comparable for the diagnostic stimuli. Therefore, varying the proportion of congruent trials modulated the preparation of responses to both the task-relevant and task-irrelevant components of the stimulus, in a complementary manner.

Disclosures: S. Lee: None. **A. Haith:** None. **J. Xu:** None.

Poster

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Program #/Poster #: PSTR216.13/GG10

Topic: E.04. Voluntary Movements

Support: ARC Co-Action

Title: Partial interference between adaptive and flexible control policies indicates shared neural mechanisms

Authors: *A. DOYEN, P. LEFÈVRE, F. CREVECOEUR;
UCLouvain, Louvain-la-Neuve, Belgium

Abstract: During reaching movements, humans can quickly adjust their goal according to task rule changes. Humans are also able to adapt to force fields (FF). These mechanisms are at play at

similar quick timescales within an ongoing movement. But do these mechanisms involve similar neural structures? To answer this question, 3 experiments were conducted with 40 right-handed participants aged between 18 and 30yrs and randomly assigned to each experiment: 15 in each of the first two experiments and 10 in the third (8 women in each group). In these experiments, reaching movements were performed using the Kinarm end-point robotic device. The first experiment reproduced our previous work and examined the ability of participants to flexibly react to a switch in target shape (square or rectangle) during the movement. In the second experiment, participants were exposed to a velocity-dependent force field in addition to the target switch. In these two experiments, we used step mechanical loads as probe trails. In the last experiment, participants adapted to the same FF without any change in target shape to evoke standard adaptation of reaching control. The two first experiments were compared to highlight motor adaptation's influence on flexible control. The means of end-point coordinates following rightward mechanical perturbation showed that motor adaptation did not significantly affect participants' ability to respond to changes in target shape. When the final target switched from a square to a rectangle, participants were able to exploit the redundancy of the target in the same way independently of the presence of the FF (one-tailed t-test, $t = 1.31$, $p=0.1$). The second and third experiments were used to see the influence of target shape on motor adaptation. We showed based on the evolution of maximal deviation in the direction of the FF that the presence of randomly changing targets affected the extent of adaptation. A bootstrap method with 1000 iterations was used to derive confidence intervals on the fit parameters of the standard learning curve. The curves obtained indicated that the extent of learning was reduced (final asymptotes of 1.7 (standard FF) and 2.4cm with no overlap of the 95% CI) even if the participants could learn at comparable rates (overlap of the 95% CI). This last result indicates that when responding to changes in environmental dynamics, the presence of visually and mechanically perturbed movements reduced the ability of participants to compensate for the new dynamics. We interpret these findings as a sign that the neural structures involved in motor adaptation and in flexible control at least partially overlap, resulting in interference between these two systems.

Disclosures: **A. Doyen:** None. **P. Lefèvre:** None. **F. Crevecoeur:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.14/GG11

Topic: E.04. Voluntary Movements

Support: JSPS Grant 20J13734
JSPS Grant 23K10739
JSPS Grant 17H00874
JSPS Grant 21H04860

Title: A redundant bimanual stick-manipulating task: Online error corrections are influenced by task-irrelevant perturbations

Authors: *T. KOBAYASHI, D. NOZAKI;
Grad Sch. of Edu, Univ. of Tokyo, Tokyo, Japan

Abstract: Redundancy between multiple controlled variables and low-dimensional task goals is inherent in motor tasks (Bernstein, 1996). This redundant structure separates controlled variables into task-relevant and task-irrelevant dimensions. However, it remains unclear whether and how the motor system rapidly responds to unexpected errors in each dimension. Although previous studies on arm-reaching movements have shown that humans can achieve online correction of errors with a latency of 100-200 ms (Scott, 2016), the nature of the redundant system remains to be fully explored. In this study, we developed a realistic stick manipulation task. Participants bimanually held a virtual stick (distance: 15 cm) and moved the right tip of the horizontal stick (length: 40 cm) from a starting point to a target (15 cm). During the training phase (280 trials), 21 participants moved the tip to each of 7 targets (0°: horizontal direction, ± 30°, ± 60°, ± 90°). They tilted the stick in a clockwise (CW) or counterclockwise (CCW) direction when aiming at a CW or CCW target direction, respectively. During the subsequent test phase (160 trials, Exp. 1; 360 trials, Exp. 2), visual perturbations were introduced. In Exp. 1 (N = 11), the stick angle was abruptly rotated by 6° around the tip when the tip was 1 cm away from the starting point (stick rotation). The participants need not respond to this perturbation because it did not affect the tip position. However, they quickly corrected stick angle errors (latency: 187 ms). Interestingly, this unnecessary correction of the CW or CCW stick angle error was temporarily accompanied by a forward or backward tip error. In Exp. 2 (N = 10), we tested whether the task-irrelevant perturbation influenced task-relevant error correction. The entire stick was shifted perpendicularly to the target direction by 3 cm (normal shift). The participants corrected the tip position while tilting the stick (latency: 156 ms). Moreover, we randomly added stick rotation at the same time as the shift. That is, the shifted stick was rotated inward (iRot shift) or outward (oRot shift). Based on the results of Exp. 1, we expected that stick rotation would either accelerate (iRot) or decelerate (oRot) the tip error correction. A repeated-measures one-way ANOVA followed by a Bonferroni post-hoc test did reveal significant differences in corrective speed (iRot vs. normal, p = 0.041; iRot vs. oRot, p < 0.001; normal vs. oRot, p = 0.047). In summary, we show that task-irrelevant perturbation can influence task-relevant motor control. We speculate that the motor system cannot ignore the task-irrelevant error due to the mutual relationship between task-relevant and task-irrelevant dimensions.

Disclosures: T. Kobayashi: None. **D. Nozaki:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

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Program #/Poster #: PSTR216.15/GG12

Topic: E.04. Voluntary Movements

Support: NIH Grant NS112367

Title: Effect of different learning types on properties of complex skilled movement

Authors: *R. BABU, H. BLOCK;
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Abstract: Learning a motor skill may involve multiple underlying processes, including use-dependent learning, reinforcement learning, and error-based learning. Each depends on distinct neural substrates and time scales of action, but they work together to account for skill learning. The relative contribution of each learning type varies depending on the task and may differently affect skill-associated parameters like acquisition, consolidation, transfer, hand-eye coordination, and proprioception. Here we ask how these learning types differentially affect the parameters associated with skill learning. We tested 4 groups of healthy young adults learning a maze navigation skill on a KINARM End-Point manipulandum with their right hand. The groups differed based on the type of feedback received during training. Subjects in the use-dependent learning (UDL) group practiced the movement without any feedback. Two groups trained using reinforcement feedback, receiving a point to indicate success on the trial if their accuracy was above a threshold. Subjects in the RL group had a consistent threshold while those in RLA had an adaptive threshold. Finally, subjects in the error-based learning (EBL) group received a score for their accuracy and online feedback about their position throughout the movement. Assessments of skill, transfer, and proprioception were done before and after training. Skill was assessed with the speed-accuracy function (SAF) by having subjects perform the same maze at 6 different speeds. Transfer was assessed via SAF on the left hand. Proprioception was assessed using a 2 alternate forced-choice shape discrimination task and computing the point of subjective equality (PSE) and just noticeable distance (JND). Movement kinematics and gaze information were collected throughout. Preliminary results (~10 per group) suggest that subjects in the EBL group undergo skill learning while the UDL group gets worse as observed by changes in accuracy and variability of the movement. All groups show a clear speed-accuracy relationship but the differences pre to post are small. EBL shows a reduction in variability post learning for all speeds. There is a pattern of improvement in the left hand SAF in all groups except UDL. Groups EBL and RLA also show a reduction in variability for transfer, both of which could indicate that EBL and RLA show some transfer of skill. PSE reduces for RLA and increases for RL while JND reduces for RLA and EBL and increases for RL and UDL, suggesting that proprioceptive measures improve for EBL and RLA and worsen for UDL and RL. Early results support the idea that skill parameters are differentially affected by the learning types.

Disclosures: R. Babu: None. **H. Block:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.16/GG13

Topic: E.04. Voluntary Movements

Title: Frequency-dependent long-term de novo learning of continuous control

Authors: *C. AVRAHAM, F. MAWASE;
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Abstract: Learning a motor skill from scratch requires the formation of a completely new controller, a process termed as de novo learning. In most real-world motor tasks, we continuously control our actions in response to ongoing external and internal cues. For example, controlling a ball passed to a soccer player depends on being able to rapidly respond to the speed and direction of the ball, as well as to obstacles from a defender. On one hand, learning new skills in the initial stages often requires us to establish an arbitrary mapping between our actions and their outcomes. This initial learning is thought to depend on the use of time-consuming cognitive strategies. On the other hand, continuous control following long-term practice allows producing rapid responses without extensive deliberation. However, the mechanisms underlying the development of de novo continuous motor skills over an extended period of practice remain unclear. In this study, we investigated the de novo learning process of healthy, right-handed participants (n=8) over a 5-day period. The participants learned to counter a mirror reversal of visual feedback in a continuous tracking task, where a target moved along a pseudo-random sinusoidal trajectory at frequencies ranging from 0.1 to 2.15 Hz. First, we evaluated the overall learning by calculating the mean squared error (MSE) between the hand and the target, observing a significant improvement in performance with practice. To assess whether participants selectively moved at the same frequencies as the target, we converted their trajectories to a frequency-domain representation via the discrete Fourier transform, which decomposes the hand trajectory into sinusoidal components of different amplitudes, phases, and frequencies. We found distinct frequency-dependent learning processes. At slow frequencies, participants rapidly mastered the mirror transformation. Interestingly, learning at high frequencies was initially absent but gradually emerged with practice. A potential explanation for the observed data is that participants used corrective feedback responses primarily at low frequencies. This feedback control acted early in learning and improved with practice. At high frequencies, the rapid movement of the target may have hindered the effectiveness of feedback control, relying mainly on inappropriate feedforward responses. Nevertheless, over time, the feedforward controller gradually developed through practice. Overall, our findings highlight the frequency-dependent nature of de novo continuous motor learning and contribute to our understanding of the mechanisms through which the brain acquires real-world motor skills.

Disclosures: C. Avraham: None. **F. Mawase:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

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Topic: E.04. Voluntary Movements

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Beijing Natural Science Foundation (5194024)
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Scientific Planning Projects from Beijing Education Committee (KM202110029002)

Title: Reward and punishment have an opposite effect on reinforcement-based motor generalization

Authors: *C. YIN;
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Abstract: Reward and punishment have long been recognized as potent modulators of human behavior. Although reinforcement learning is a significant motor learning process, the exact mechanisms underlying how the brain learns movements through reward and punishment are not yet fully understood. Beyond the memory of specific examples, investigating the ability to generalize to new situations offers a better understanding of motor learning. This study hypothesizes that reward and punishment engage qualitatively different motivational systems with different neurochemical and neuroanatomical substrates, which would have differential effects on reinforcement-based motor generalization. To test this hypothesis, two groups of participants learn a motor task in one direction and then relearn the same task in a new direction, receiving only performance-based reward or punishment score feedback. Our findings support our hypothesis, showing that reward and punishment had an opposite effect on motor generalization: reward promoted while punishment impaired generalization. These behavioral differences may be due to different tendencies of movement variability in each group. The punishment group explored more than the reward group during the initial learning phase, possibly due to loss aversion. In contrast, the reward group explored more actively than the initial learning phase during the generalization test phase, seeking to explore the strategy that led to reward. These results suggest that reward and punishment may engage different neural mechanisms during reinforcement-based motor learning and generalization, with important implications for practical applications such as sports training and motor rehabilitation.

Disclosures: C. Yin: None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.18/GG14

Topic: E.04. Voluntary Movements

Support: CIHR Grant MOP126518

Title: Sensorimotor adaptation and de-adaptation recruit shared explicit learning processes

Authors: *D. STANDAGE[1], A. DE BROUWER[2], J. FOOKEN[3], D. GALE[1], J. Y. NASHED[1], J. FLANAGAN[1], J. P. GALLIVAN[1];
[1]Queens Univ., Kingston, ON, Canada; [2]lululemon research, Vancouver, ON, Canada; [3]Queen's Univ., Queen's Univ., Kinigston, ON, Canada

Abstract: Sensorimotor mappings are learned associations between sensation and action. Sensorimotor adaptation (SA) is the recalibration of these mappings when a stimulus-response relationship is perturbed, a process that is fundamental to flexible behaviour. It is widely believed that SA reflects a slow, implicit component that reduces sensory prediction errors, and a fast, explicit component that corresponds to a strategy. There is intense research interest in these processes during adaptation, but relatively little attention has been given to them during de-adaptation, when a stimulus-response relationship reverts to its initial state. We investigated de-adaptation with data from two published visuomotor rotation (VMR) experiments, in which participants moved a cursor to a target that appeared at equidistant locations from a starting point. On each of two days, a 'baseline' block was followed by a learning block (adaptation), followed by an unlearning block (de-adaptation). The hand-cursor mapping was rotated by 45 degrees during learning, when a strategy is to aim in the opposite direction to the rotation. During learning on Experiment 1, we divided participants into 'aimers' and 'non-aimers' by the angle of their eye movements from the starting point (gaze angle). Gaze angle not only differentiated the two subgroups during learning on both days (de Brouwer et al, J Neurophysiol, 2018) when aimers had a lower error than non-aimers, but it also differentiated them during unlearning, when aimers again had a lower error and their gaze angles reversed direction. These results predicted that neural processes recruited preferentially by fast learners during learning will be recruited preferentially during unlearning.
Experiment 2 tested this prediction with functional magnetic resonance imaging (fMRI). We clustered participants behavioural data during learning to derive subgroups of fast and slow learners, and we derived functional networks linked to learning on each day (Standage et al, Cereb Cortex, 2022), referred to as 'the strategy network' and 'the recall network' since fast relearning is believed to involve the recall of a successful strategy for learning. On Day 1 (Day 2) recruitment of the strategy network (recall network) not only differentiated the subgroups during learning, when fast learners had a significantly lower error than slow learners, but it also differentiated them during unlearning, when fast learners again had a lower error. Thus, the predictions by our eye movement dataset were confirmed by our fMRI dataset. These results provide strong evidence that cognitive strategies for adaptation are generalised to de-adaptation.

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Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.19/GG15

Topic: E.04. Voluntary Movements

Support: NSERC to SD
NSERC to DYPH

Title: The Time-Course of Implicit Adaptation in the Context of Rotation Size and the Time-Course of Explicit Adaptation

Authors: *S. D'AMARIO, B. M. 'T HART, D. Y. P. HENRIQUES;
York Univ., Toronto, ON, Canada

Abstract: Motor adaptation relies on explicit and implicit learning processes, and involves changing our movements in response to errors. Research has found implicit learning in visuomotor adaptation is limited to 15° regardless of rotation size, but the time course of implicit learning remains poorly characterized. Building on a previous study from our lab that demonstrated a rapid emergence of implicit aftereffects, our study aimed to investigate the potential impact of perturbation size on the rate of implicit learning. We used continuously visible cursor feedback and varied the rotation sizes to 15°, 30°, 45°, and 60°. As a measure of explicit awareness, participants underwent 8 aiming trials in late training. We used an exponential function to estimate rates of change and asymptotes, comparing conditions through bootstrapped confidence intervals. Our findings revealed that the asymptotes of implicit reach aftereffects increased with larger rotation sizes (15°: 12.9°, 30°: 21.3°, 45°: 27.9°, and 60°: 28.7°), while the relative rates of change decreased (15°: 16.9%, 30°: 13.2%, 45°: 8.4%, 60°: 9%). However, in absolute terms, the first trial elicited around 2.5 degrees of change in all conditions, suggesting the rate of change in response to the perturbation was independent of its size. Aiming trials in late training showed a greater explicit contribution for larger perturbation sizes. This led us to investigate the effects of continuous aiming throughout the experiment, along with the no-cursor reaches, to directly compare the timecourses of implicit and explicit adaptation. The ‘continuous aiming’ group, utilizing a 45° rotation, exhibited a higher rate of implicit adaptation (14.9% vs. 8.4%) although a lower extent of implicit adaptation (21° vs 27.9°) compared to the 45° counterpart described above. Thus, explicit strategy at the end of training was 80% greater in the continuous aiming group compared to the group with aiming measured at the end (23.4° vs 14.7°). Also, the rate by which cognitive strategy emerges (23.2%) was only slightly faster than implicit aftereffects for the same group. This suggests that consistently reporting an aimed location can facilitate a higher implicit and explicit learning rate. In summary, implicit aftereffects emerge quickly, with rotation size influencing the relative speed but not the absolute speed of implicit learning, and it also impacts the overall extent of implicit learning, contrary to previous findings.

Disclosures: S. D'Amario: None. **B.M. 't Hart:** None. **D.Y.P. Henriques:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

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Program #/Poster #: PSTR216.20/GG16

Topic: E.04. Voluntary Movements

Support: NIH Grant 1R15AG059095-01

Title: Using a self-report aiming method to estimate explicit and implicit contributions to visuomotor rotation in older adults

Authors: *K. TREWARTHA[1], A. WATRAL[1], B. WOOLMAN[1], R. RANGANATHAN[2]; [1]Cognitive and Learning Sci., Michigan Technological Univ., Houghton, MI; [2]Kinesiology, Michigan State Univ., East Lansing, MI

Abstract: Research has suggested that sensorimotor adaptation involves explicit and implicit mechanisms that work together to support motor learning. One common approach to dissociate the contributions of these two processes is to utilize a self-report aiming method in a visuomotor rotation task to estimate the explicit and implicit contributions across movement acquisition. This method has provided important insights into the cognitive mechanisms involved in sensorimotor adaptation. Research has suggested that aging is associated with diminished learning and retention during sensorimotor tasks, due in part to age-related cognitive declines. There is evidence that aging may differentially affect the explicit and implicit contributors to motor learning. However, the self-report aiming method has not been previously employed to estimate the relative contributions of those processes to age differences in learning. Here, we recruited 23 younger adults (18-34 years old, *M* = 19.52 years, 11 females) and 26 healthy older adults (56-85 years old, *M* = 67.48 years, 16 females) to perform a visuomotor adaptation paradigm with the self-report method for estimating the explicit and implicit components. During adaptation, younger adults performed better than older adults overall, but this effect was not significant, and the two groups learned at similar rates. However, estimates of the explicit component, derived from the participants' self-reported aiming direction prior to each movement showed better learning in the younger adults compared to older adults. Likewise, the implicit estimates showed better learning in younger than older adults. These findings are consistent with previous literature showing that age differences in sensorimotor adaptation may be due to changes in both explicit and implicit cognitive mechanisms involved in motor learning. However, it is important to note that seven of the older adults (representing ~27%) tested were unable to complete the self-report aiming task because they never perceived a difference between their hand position and the cursor. These results highlight the need to understand the role of proprioception in the self-report aiming method and suggest that caution should be exercised when using this method in special populations.

Disclosures: K. Trewartha: None. **A. Watral:** None. **B. Woolman:** None. **R. Ranganathan:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

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Program #/Poster #: PSTR216.21/GG17

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant

Title: Effects of immersive visual environment-change cues on motor learning during a virtual-reality target hitting task

Authors: *S. MODCHALINGAM, A. KING, D. Y. HENRIQUES;
Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: When performing motor tasks, we improve future movements by detecting and correcting for motor errors. Errors are determined by comparing predicted outcomes of internal models of the motor interaction with the observed outcomes. When errors are detected, we correct for them by either updating existing internal models of the movement interaction, or by creating, switching to, and switching from new internal models. Assignment of the error's source, termed error attribution, can impact whether we update existing or create new internal models. Since the cause of an error is often ambiguous, sensory cues about the effector, the object being interacted with, and the environment in which the interaction occurs are used to estimate the likely source.

In this study participants attempted to hit targets by rolling a ball along a surface. We tested whether informative visual cues about environment changes, represented by the horizontal slant of the surface, could successfully facilitate model creation and switching - characterized by fast, one-trial decay of learning when environment changes are detected - when adapting to two types of errors. We induced errors by either modifying the mapping between the arm movement and the initial movement of the ball (a visuomotor rotation), or by applying a constant acceleration to the ball's travel path only after the initial release of the ball. The surface-slant visual cues were informative of the direction of the visuomotor rotation, and the direction and magnitude of the acceleration perturbation.

The error induction method alone, and not the visual cues, determined whether errors led to model creation and switching, or model updating. Visuomotor rotations led to model updating and acceleration perturbations led to model creation and switching. Additionally, in follow-up experiments, we found internal models that are updated consider both the hand used in the movement, and the physical properties of the environment on which the movement occurs. That is, the internal model being updated is not purely a model for the control of limb movement, but an interaction model.

Disclosures: S. Modchalingam: None. **A. King:** None. **D.Y. Henriques:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

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Topic: E.04. Voluntary Movements

Support: FRS-FNRS FRIA PhD Grant
Concerted Research Action of UCLouvain (ARC, “coAction”)

Title: Adaptation to visuomotor delays and its transfer to feedback control during reaching

Authors: *A. K. E. HOFFMANN[1,2], S. ARUSI[3], C. AVRAHAM[3], I. NISKY[3], F. CREVECOEUR[1,2];
[1]Inst. for Information and Communication Technologies, Electronics and Applied Mathematics, Univ. Catholique De Louvain, Louvain-la-Neuve, Belgium; [2]Inst. of Neurosci., Univ. Catholique De Louvain, Brussels, Belgium; [3]Dept. of Biomed. Engin., Ben-Gurion Univ., Beer-Sheva, Israel

Abstract: Studying motor adaptation to artificial feedback delays can shed light on how the nervous system compensates for intrinsic, sensory delays. Exposure to artificial visual delays has been shown to result in increases in movement amplitudes. A previous study concluded that this effect could be explained under the hypothesis that humans adapt to the difference between proprioceptive and visual feedback as if they were spatially related by a gain factor, rather than by a shift in time (Avraham et al. 2017, 10.1523/ENEURO.0179-17.2017). However, this conclusion was based on unperturbed movements in the absence of visual feedback. Here, we investigated whether adaptation to visuomotor delays also influences feedback control of movements. We performed two experiments using a virtual Pong-Game with a robotic-arm. Participants moved the robot-handle with their right hand to control the movement of a virtual paddle to hit a ball. Over time, we added an increasing artificial visual delay between hand and paddle movements (0ms, 50ms, 100ms, 150ms). At the beginning of the experiment, participants performed 40 trials without delay and afterwards, the delay was increased every 40 trials. Pong trials were randomly interleaved with reaching movements to a target. To assess if adaptation to the delay affected feedback control mechanisms, we probed feedback corrections to 5cm left- and rightward visual cursor jumps (exp. 1, N=16, 11 female) or to ±5N mechanical perturbations (exp. 2, N=16, 10 female) during 50% of all reaches. We removed the visual delay during perturbed movements, while half of the unperturbed movements were performed with increasing visual delays. As expected, the presence of visuomotor delays led to an increase in reach amplitudes that scaled with the size of the added delay. Conversely, the unexpected removal of the delay resulted in a decrease of the amplitudes. Interestingly, during the perturbed reaching movements, we observed an increase in the speed of corrective responses to visual perturbations during delay adaptation, but this effect was not present during force perturbations. Our findings suggest that the exposure to visual delays influenced corrections mediated by the visual feedback circuit. We will compare these differences in kinematic profiles with predictions from computational models using either a spatial gain- or a time-based adaptation to provide further insight into the potential neural representation of sensory feedback delays.

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Poster

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Program #/Poster #: PSTR216.23/GG19

Topic: E.04. Voluntary Movements

Title: Using tools as cues for dual adaptation to opposing visuomotor rotations in virtual reality

Authors: *A. KING[1], L. MIKULA[2], S. MODCHALINGAM[2], B. M. 'T HART[3], D. HENRIQUES[4];
[1]York Univ., Oakville, ON, Canada; [3]Ctr. for Vision Res., [2]York Univ., Toronto, ON, Canada; [4]York Univ., Vaughan, ON, Canada

Abstract: Humans are experts at designing and utilizing unique tools to accomplish various tasks, like wielding an axe to chop wood. Humans are also capable of using different tools to accomplish opposing motor tasks simultaneously (using a fork and knife to cut food). While a lot is known about motor adaptation with changed visual feedback of the hand, we rarely consider how adaptation may differ in novel situations requiring pairs of tools with similar or different movement patterns. Here we test whether having two tools that require different movements to accomplish a similar goal would serve as sufficient cues that allow dual tool-use adaptation, akin to lead-in movements in dual motor adaptations. We ran an immersive virtual reality experiment where participants used one of two sets of tools; a motor incongruent pair, comprised of a paddle (forward motion) and a slingshot (backward motion), or a motor congruent pair, where participants used a paddle and a curling tool (both requiring forward motions). Participants swapped between tools every 8 trials. After a familiarization phase, we added visually opposite perturbations to the ball after it was launched from each tool (30^{o} clockwise or counterclockwise rotation). In addition to the motor congruent and incongruent groups, we also collected participants for a single adaptation version which involved the presentation of the incongruent tools successionally, where only one tool and its corresponding perturbation were learnt at a time. Finally, we added a "control" pairing of tools composed of a red and blue colored paddle that were functionally identical to one another. We found that the motor incongruent, motor congruent, and single adaptation groups had reduced angular error in the exposure phase, suggesting that participants were able to form distinct motor memories for both pairs of tools. However, the reduction of angular error in the motor incongruent group was 27% larger than that of the congruent group, which reinforces the notion that internal cues are important markers for the formation and retrieval of motor memories. Additionally, there was no significant reduction of angular error in the control condition, supporting the literature that visual cues are not strong enough to facilitate the encoding and retrieval of motor memories. These findings suggest separate motor memories are more reliant on the movement profile of a perturbation than any associative visual cues.

Disclosures: A. King: None. **L. Mikula:** None. **S. Modchalingam:** None. **B.M. 't Hart:** None. **D. Henriques:** None.

Poster

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Program #/Poster #: PSTR216.24/GG20

Topic: E.04. Voluntary Movements

Support: NIH Grant 1R15AG059095-01

Title: Age differences in long-term retention in a force-field adaptation task

Authors: *B. WOOLMAN[1], A. T. WATRAL[1], K. TREWARTHA[1], R. RANGANATHAN[2]; [1]Cognitive And Learning Sci., Michigan Technological Univ., Houghton, MI; [2]Kinesiology, Michigan State Univ., East Lansing, MI

Abstract: Sensorimotor adaptation tasks have been frequently used to investigate how people acquire a novel motor skill. This type of motor learning is thought to unfold through two learning process that operate on different timescales: a fast process that leads to rapid improvements in performance, and a slow process that leads to more gradual refinement of the movement. Several studies have shown that these fast and slow learning phases may be driven by different memory processes, with the fast process relying on explicit, spatial working memory, and the slow process relying more on procedural memory that may solidify the retention of the skill. A single learning session can lead to lasting movement adaptation that is retained over days, weeks, and even months. Research has suggested that aging is associated with diminished short-term, within-session retention in a force-field adaptation (FFA) task. This is likely due to age-related changes in the underlying memory processes that support learning. However, little is known about how aging affects long-term retention in this context. Here, we tested the hypothesis that older adults exhibit diminished retention 24-hours after initial learning compared to younger adults due to well-known changes in long-term memory in later adulthood. We recruited 26 younger adults (18-34 years old, M = 20.15 years) and 29 healthy older adults (55-83 years old, M = 70.4 years) to perform two sessions of a FFA task, 24-hours apart. Older participants also completed a neuropsychological test battery that provided an overall assessment of cognitive ability and a screening tool for identifying patients with signs of dementia. During the first session, younger and older adults learned to adapt generally at a similar rate, but short-term retention was diminished in the older group. On Day 2, younger participants learned faster than they did on Day 1 showing that they exhibited significant retention over the 24-hour period. Older adults showed no difference in learning on Day 1 and Day 2 suggesting that their long-term retention was diminished. Savings scores calculated to quantify long-term retention were correlated with cognitive performance in the older adult group but not specifically with independent measures of memory function. These findings reveal age differences in long-term retention in a sensorimotor adaptation task that are correlated with levels of overall cognitive function.

Disclosures: B. Woolman: None. **A.T. Watral:** None. **K. Trewartha:** None. **R. Ranganathan:** None.

Poster

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Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.25/HH1

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 AG041878

Title: Learning rate upregulation enables temporally volatile savings for same and opposite-polarity sensorimotor learning

Authors: *A. S. NYANTAKYI, A. M. HADJIOSIF, M. A. SMITH;
Harvard Univ., Cambridge, MA

Abstract: Savings refers to faster improvement when one relearns a previously learned motor skill. A longstanding assumption has been that savings originates from a latent stable long-term memory that reemerges during relearning. However, recent findings indicate that temporally-persistent (TP) implicit memory, defined by stability over a short 60-second period, displays slower rather than faster relearning, and thus acts to reduce rather than promote savings. In contrast, it is temporally-volatile (TV) implicit memory, which decays away in 60 seconds or less and thus cannot contribute to stable long-term memory, that displays faster relearning and thus drives savings in implicit learning. The mechanism, however, for this faster relearning of temporally-volatile implicit memory is not yet clear. Thus here we test whether the faster relearning of this TV memory arises from (1) a specific ability to efficiently rebuild recently lost TV memory, which would resemble in a way the reemergence of a stable long-term memory in the traditional view of savings, versus (2) a general ability to learn TV memories more rapidly, independent of whether this learning rebuilds recently lost memory or instead forms entirely new memories based on, for example, oppositely-directed changes in motor output. To test these possibilities, we compared relearning and oppositely-directed learning following a period of initial learning for a visuomotor transformation (a 30° visuomotor rotation (VMR)) followed by washout of this learning for either 40 or 800 trials. Remarkably, we found that opposite learning (n=24, n=11) yielded TV savings similar in magnitude to same-polarity relearning, which is consistent with a general ability to learn TV memories more rapidly. In the opposite learning period, TV savings was present at 25.1±10.5 % and 23.5±11.8% in the 40 & 800-trial washout experiments, respectively ($p<0.04$, $p<0.05$); this was similar to the savings previously found for same-polarity relearning (27.8±4.9% and 28.8±5.1%). This demonstration of substantial TV savings during opposite learning suggests that TV savings arises from a general increase in learning rate and is thus not restricted to the re-learning of an identical task.

Disclosures: A.S. Nyantakyi: None. **A.M. Hadjiosif:** None. **M.A. Smith:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.26/Web Only

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Effects of tool use and perturbation during motor adaptation on hand localization

Authors: *M. KHAN[1], S. MODCHALINGAM[2], A. KING[3], B. 'T HART[2], D. HENRIQUES[2]; [1]York Univ., London, ON, Canada; [2]York Univ., Toronto, ON, Canada; [3]York Univ., Oakville, ON, Canada

Abstract: Effects of tool use and perturbation during motor adaptation on hand localization Maryum Khan[1], Shanaathanan Modchalingam[1], Andrew King[1], Bernard Marius 't Hart[1], Denise Y.P. Henriques[1] [1]Centre for Vision Research, York University, Toronto, Canada Our brain has a remarkable capacity for learning movements and adapting them to accomplish a motor goal. In many adaptation studies, participants move in a 2D plane while their hand is represented by a cursor. When visual feedback of hand position is misaligned, people can quickly compensate for this perturbation, show persistent reach aftereffects, and even misestimate the location of the unseen hand in the direction of previous visual training. However, it is unknown how well this generalizes to real-world settings or to the tools we use every day. Here we will use immersive virtual reality to test if end-effector shifts are also observed in more naturalistic virtual reality environments and if they extend to tools as end effectors. In Study 1, we replicated our previous work where we found shifts in end-effector localization after adapting reach movements to a 30° visuomotor rotation of the hand, showing a similar magnitude of both shifts in unseen hand location and reach aftereffects following training to the perturbation in the VR environment. In the next condition, Study 2, we extend this paradigm to investigate how well people can adapt when aiming with a common tool, like a pen, and whether the tool location is also recalibrated. Participants will reach to the same targets using both a physical and virtual pen, whose movements will also be deviated by 30°, and we will measure the extent that the unseen location of hand-held tool, as well as the hand (in separate trials) recalibrates with adaptation. Our results will provide insight into the adaptative processes involved when learning to wield tools in more complicated, realistic environments.

Disclosures: M. Khan: None. **S. Modchalingam:** None. **A. King:** None. **B. 't Hart:** None. **D. Henriques:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.27/HH2

Topic: E.04. Voluntary Movements

Support: NSERC
IRTG: Brain in Action

Title: Investigating event-related potentials during movement preparation and outcome error processing to compare between motor adaptation and de novo learning

Authors: *R. Q. GASTROCK[1], E. ODY[2], D. Y. P. HENRIQUES[1], B. M. 'T HART[1];
[1]York Univ., Toronto, ON, Canada; [2]Philipps - Univ., Marburg, Germany

Abstract: When people encounter movement errors, they process these errors to correct ensuing movements. This error processing contributes to motor learning, either when we are adapting well-known movements or acquiring new motor skills (de novo learning). While the behavioral mechanisms underlying these two motor learning types have been investigated before, we understand less about the neural correlates of each type. Here, we investigated event-related potentials (ERPs) during movement preparation and outcome as participants performed reaching movements. We distinguished the two motor learning types by having participants (N = 32) train with two perturbations in counterbalanced order: a 30° visuomotor rotation to investigate adaptation and a mirror reversed cursor feedback for de novo learning. Before training with each perturbation type, participants completed a control condition, where they experienced a random rotation with magnitudes of ±15°, ±25°, or ±35°. Participants learned to compensate for both the fixed rotation and the mirror reversal, but not the random perturbations. For movement outcome, we time-locked to feedback onset at the end of the movement. We found a negative-going ERP before feedback onset in fronto-central and parietal electrodes, with perturbed reaches showing more negativity compared to aligned baseline reaches. However, the ERP amplitude did not scale with perturbation type nor error magnitude, suggesting that it was only processing the presence of an error. Furthermore, we found a larger P3 component after feedback onset in the perturbed conditions compared to aligned baseline reaches, suggesting more attention allocation for perturbed reaches. For movement preparation, we time-locked to the go signal onset before the movement. We found a Readiness Potential (RP) that depended on whether participants moved to the right or left side of the workspace. We then quantified changes in movement preparation across learning, using a Lateralized Readiness Potential [LRP = (right C3 - right C4) - (left C3 - left C4)] for different blocks of trials during training in each of the perturbation types. We found less pronounced LRPs for the random perturbation than in aligned reaches, suggesting weaker preparatory activity for such unpredictable trials. However, we found no LRP differences between the fixed rotation and mirror reversed reaches. Thus, although the ERPs we investigated represent movement preparation and outcome error processing, these markers are unable to distinguish between motor adaptation and de novo learning. We will further investigate other markers in relation to these two motor learning types.

Disclosures: R.Q. Gastrock: None. **E. Ody:** None. **D.Y.P. Henriques:** None. **B.M. 't Hart:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR216.28/HH3

Topic: E.04. Voluntary Movements

Title: Task Error vs. Sensory Prediction Error: Autonomic Nervous System Responses in Reaching Movement

Authors: *W.-P. WU[1], T. HAYASHI[2], D. NOZAKI[2];
[1]Grad. Sch. of Educ., [2]The Univ. of Tokyo, Tokyo, Japan

Abstract: It is well known that the Autonomic Nervous System (ANS) responds sensitively to negative feedback or unexpected outcomes when performing cognitive tasks (Kastner et al., 2017; Iris et al., 2018; Noordewier et al., 2021). Similarly, individuals can experience a sense of unexpectedness from errors that occur during movement. However, it has not been thoroughly investigated whether such an ANS response can also be observed in motor task errors. In Experiment 1 (n=18), we investigated the Heart Rate (HR) response to the visual rotation (0, ±15, and ±6 deg) imposed on a cursor representing the hand position while performing reaching movement (movement distance 20cm; target diameter 1cm). The HR was evaluated by Inter-beat Interval (IBI) (time interval between two adjacent heartbeats). During the reaching movement, the IBI typically increased prior to the movement onset and then returned to the baseline level within approximately 3 seconds. Yet, for larger perturbations, the IBI remained more elevated for 1 and 2 IBIs after the movement onset. Indeed, the post-hoc test after the ANOVA indicated that there was a significant difference ($p=0.026$) in IBI2 (2nd IBI after the movement onset) between 0 and 15 perturbation conditions. The trend in the IBI fluctuation might indicate that ANS also responds to errors in movement, as reported in previous studies using cognitive tasks. Notably, the error induced by visual rotation consists of two distinct components: the Sensory Prediction Error (SPE) and the Task Error (TE). The SPE represents the discrepancy between the predicted and observed sensory information, whereas the TE represents the failure to achieve a task (Kim et al., 2019). To dissociate the effects of these errors, we further performed Experiment 2 (n=20), in which two different target types (narrow (width 1cm) and wide (width 30 cm) targets) were adopted. The visual rotation (±15 deg) induced the SPE for both target types but did not induce TE for the wide target because the perturbed cursor safely reached the target. We examined how IBI change between adjacent IBIs was influenced by the target type conditions. From IBI1 to IBI2, we found that the IBI was marginally significantly elevated for the narrow target condition ($p=0.054$) with 15 deg perturbation, but such an elevation was not observed for the wide target condition ($p=0.361$). Thus, in the absence of TE, the IBI elevation on perturbed trials would reduce. These results suggest that the ANS responds to the TE rather than the SPE, implying that ANS activity is more affected by the outcome of the movement rather than sensory information about the movement.

Disclosures: **W. Wu:** None. **T. Hayashi:** None. **D. Nozaki:** None.

Poster

PSTR216. Influences on Motor Learning and Execution

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Program #/Poster #: PSTR216.29/HH4

Topic: E.04. Voluntary Movements

Support: Jérôme Lejeune Foundation (2020-2021)
Instituto Carlos III (ISCIII) Sello Excelencia ISCIII-Health
IHMC22/00026

Title: The impact of allelic variation on hyperactivity in mouse models for Down syndrome

Authors: *R. A. J. CRANS, J. L. MUSOLES-LLEÓ, M. SABARIEGO-NAVARRO, K. BAGHIZADEH, M. DIERSSEN;
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Abstract: Down syndrome (DS, or trisomy 21) is the most common genetic disorder leading to intellectual disability. Most individuals with DS carry a complete or partial extra copy of the human chromosome 21 that bears around 234 protein-coding genes, which results in the elevated expression levels of those transcripts and proteins. This leads to the manifestation of major clinical features, although with varying severities, such as cognitive impairments, hyperactivity/impulsivity, a small-sized brain, and early-onset Alzheimer's disease. Several genotype-phenotype studies have postulated that this phenotypic variability between DS individuals is associated with genomic variations in trisomic as well as in non-trisomic (euploid) genes. The goal of this study is to identify specific allelic variations that influence behavior in two mouse models for DS with mixed allelic backgrounds.
To this aim, behavioral test batteries were performed in two mouse models for DS (*i.e.*, Ts65Dn and TcMAC21 mice) and their wild-type (WT) littermates. The behavioral test consisted of measuring the circadian activity for 24 hours, the novel object recognition (NOR) test and the elevated plus maze (EPM). Each individual behavioral outcome was stratified into low, medium, or high-performance groups. Subsequently, the allelic background for each individual mouse was genotyped by an SNP array, which was able to dissect over 3,000 substrain specific alleles in the genome within the mouse models.
Remarkably, WT animals showed to have a broad spectrum from a low to high nocturnal activity, while DS mice were solely categorized into groups of medium and high activity. The genetic background of WT littermates from two distinct DS mouse models had a significant influence on their nocturnal activity. More specific, a strong correlation was observed between the proportion of C57BL/6 alleles and nocturnal activity levels in WT littermates of Ts65Dn mice (R^2 = 0.1793 and *p*-value = 0.0311) and showed a tendency in WT littermates of the TcMAC21 mouse model (R^2 = 0.1101 and *p*-value = 0.1530). These results may explain the discrepancies observed between previous studies, which were not always able to detect a hyperactive phenotype for Ts65Dn mice. This could be due to the group of WTs used that were or weren't hyperactive due to their allelic background (*i.e.*, proportion of C57BL/6 alleles). Overall, these data suggest that an allelic background play an important role for the behavioral test results of hyperactivity in DS and may be used to predict phenotypical outcomes.

Disclosures: R.A.J. Crans: None. **J.L. Musoles-Lleó:** None. **M. Sabariego-Navarro:** None. **K. Baghizadeh:** None. **M. Dierssen:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.01/HH5

Topic: E.04. Voluntary Movements

Support: R21NS125571-01

Title: Visualizing the sensory information processing of oral cavity

Authors: *A. MATUNIS[1], E. STACY[1], Z. HUBBARD[1], R. IWAMOTO[1], K. ABE[1], S. TAMURA[1], T. K. SATO[2], T. R. SATO[1], Y. KAMBE[2];
[1]Neurosci., Med. Univ. of South Carolina, Charleston, SC; [2]Kagoshima Univ., Kagoshima, Japan

Abstract: The oral cavity is essential for eating, which requires intricate interaction of sensory processing and motor control. However, current knowledge on coordinated neural information processing of the oral cavity is limited both at the level of the brainstem and the cortex. In the current project, we performed anatomical tracing and established technical frameworks using mice as a model system.
First, we injected AAV1-CAG-Fluorescent protein (FP) and AAV-retro-FP in the tongue, the gum, and a tooth. We found that the injection of AAV1-CAG-FP into the gum labeled neurons in the trigeminal ganglion and their axons to the spinal trigeminal nucleus. In contrast, unexpectedly, the injection of AAV1-CAG-FP into the tongue resulted in retrograde labeling of the neurons in the hypoglossal nucleus that controls tongue movements. However, we did not observe such labeling following the injection of AAV-retro-FP in the tongue.
Second, to investigate how sensory information from the gum is processed, we developed a technique to monitor the activity of neurons in the brainstem and in the cortex. For the brainstem, we performed a craniotomy on the occipital bone and removed the overlying tissues. For the cerebral cortex, we performed a standard craniotomy that extended laterally, and then inserted microprisms to visualize the lateral and ventral portions of the cortex (near perirhinal areas). In both sets of experiments, we labeled the neurons via retro-orbital injections of AAV-PHPeB-GCaMP7s 3-5 weeks in advance. We are currently identifying the precise locations of the areas that encode the gums.
In addition, we will label the neurons of the hypoglossal nucleus with ChR2 and GCaMP to manipulate and monitor the neural circuits that control the tongue movements.

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Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.02/HH6

Topic: E.04. Voluntary Movements

Support: R01 DC017439

Title: Retention of Newly Learned Speech-Acoustical Mapping Following Formant Perturbation in Humans

Authors: *N. RAO[1], Y. HUA[1], D. J. OSTRY[1,2];
[1]Dept. of Psychology, McGill Univ., Montreal, QC, Canada; [2]Child Study Center, Yale Sch. of Med., Yale Univ., New Haven, CT

Abstract: Adaptation to altered auditory feedback is widely used to characterize the mechanistic underpinnings of speech motor learning. Recent findings underscore the effector-specific mechanisms that distinguish adaptation in speech motor learning from that in hand-reaching tasks. However, the durability of retention of a newly learned speech-acoustical mapping with time alone or with time and sleep remains unclear. As observed in upper limb movement, we hypothesized enhanced retention with the passage of time and sleep (24 hours group) versus only the passage of time (8 hours group) using a formant perturbation task as an experimental model. We recruited healthy young adults (all fluent English speakers, 18-40 years of age) to read aloud words displayed on a computer monitor, one at a time. The words were consonant-vowel-consonant sequences, ‘bep’, ‘dep’, or ‘gep’, which were presented in a pseudorandomized sequence. During visit-1, participants performed baseline trials with no formant perturbation, followed by a set of trials in which the first formant frequency (F1) was gradually increased over trials to 30% and, on each trial, played back to participants in real-time through headphones. Participants returned either 8 or 24 hours later (visit-2) and completed a set of relearning trials with similar formant perturbation as in visit-1. As expected, trends among participants in both the 8- and 24-hours groups showed similar levels of speech motor adaptation to the perturbed F1 during visit-1. Retention of learning was observed in both experimental conditions, with the 8 hours group showing similar retention to that observed in the 24 hours group, contrary to the expectation of sleep-enhanced consolidation of newly acquired memories. We also assessed differences in retention when the perturbation was introduced either abruptly or gradually over trials. For this purpose, we recruited additional participants who underwent a similar number of trials during visit-1 as described above, but with an abrupt F1 perturbation following the baseline. Tests of retention were conducted either 8 or 24 hours after learning. Results to date indicate equivalent learning and retention in both abrupt and gradual conditions. Taken together, our findings suggest the robustness of newly acquired speech-acoustical mappings with time and no additional gains with sleep or with the introduction of perturbation in a gradual or abrupt manner. These findings could guide modeling of the effector-specific mechanisms underlying consolidation and learning of newly acquired speech-acoustical mapping.

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Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.03/Web Only

Topic: E.04. Voluntary Movements

Support: MOST 110-2314-B-A49A-518 -MY3

Title: A Novel Test for Assessing Oral Sensorimotor Adaptation to Masticatory Perturbation

Authors: *C.-S. LIN[1], T.-C. CHEN[2];
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Abstract: *Background:* To maintain adequate masticatory function is pivotal to oral health and nutrient intake for older adults. Mastication can be interfered with by intraoral perturbation, such as hard food or a newly installed denture. Oral adaptation to masticatory perturbation, as complex sensorimotor processing, has not been fully investigated. *Aims and hypotheses:* We developed the masticatory perturbation task (MPT) to assess the perturbation effect during mastication and quantify the degree of oral adaptation to masticatory perturbation in younger and older adults. *Methods:* 38 older adults (OA, mean age=69.2 years) and 38 younger adults (YA, mean age=28.1 years) performed the MPT, which consists of three trials of assessment of masticatory performance (MP) without perturbation (i.e., the baseline condition) and three trials with perturbation (i.e., the perturbation condition). Perturbation was implemented by concurrently chewing test food on the preferred side and a drinking straw on the non-preferred side. The perturbation effect was estimated as the change of mean MP between the baseline vs. perturbation condition. The adaptation effect was quantified by the index Adapt(%), as the change of MP between the last vs. the first trial. A smaller value of Adapt(%) reflects better adaptation to perturbation (i.e., the MP of the last trial returning to the level of the first trial). Finally, subjective experience of masticatory adaptation was assessed using the Masticatory Adaptative Experience Questionnaire, which consists of six questions regarding the frequency of using adaptative approaches (e.g., spending more time chewing food) during mastication. *Results:* (A) The mean MP was lower in the perturbation condition, compared to the baseline condition (Wilcoxon test, two-tailed $p<0.001$), suggesting the perturbation was valid to interfere with mastication. (B) In the YA group, the MP of the last trial returned to the level of the first trial (Wilcoxon test, two-tailed $p=0.99$). In contrast, in the OA group, the MP of the last trial remained lower than that of the first trial (Wilcoxon test, two-tailed $p=0.001$). Furthermore, the OA group showed a lower degree of oral adaptation (i.e., higher Adapt(%)) compared to the YA group. (C) In the OA group, Adapt(%) was positively correlated with the MAEQ score ($rho=0.35$, $p=0.032$), suggesting that better oral adaptation to masticatory performance was associated with more experience of adopting adaptive approaches during mastication. *Conclusions:* Our findings demonstrated the MPT as a valid test for assessing oral adaptation to masticatory perturbation and revealed the association between age and masticatory adaptation.

Disclosures: C. Lin: None. **T. Chen:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

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Program #/Poster #: PSTR217.04/HH7

Topic: E.04. Voluntary Movements

Support: IRP of NINDS
IRP of NIAAA

Title: Exploring the Correlation between Stuttering and Sleep Disorders in a Mouse Model

Authors: *A. Z. TURK[1,3], M. MILLWATER[1], M. WEINHOLD[1], A. J. KESNER[2], S. SHEIKHBAHAEI[1];
[1]Natl. Inst. of Neurolog. Disorders and Stroke, [2]NIH, Bethesda, MD; [3]Georgetown Univ. Sch. of Med., Washington, DC

Abstract: Developmental stuttering affects the verbal fluency of about 10% of preschoolers worldwide. While most cases resolve on their own, for some, dysfluency continues into adulthood and poses a detrimental problem to communication. Although genetic mutations in a family of genes involved in cellular trafficking, including the *GNPTAB* gene, have been found in some individuals who stutter, the neurological basis of developmental stuttering is not entirely understood.
Population studies that have examined sleep difficulties in people with developmental stuttering suggest that sleep disturbances seem to be common in developmental stuttering. In addition, anecdotal data indicate poor sleep in individuals who stutter, and the stuttering phenotype worsens after a poor night's sleep. However, data on the relationship between sleep disturbances and stuttering phenotype is limited.
To study this relationship, we induced sleep deprivation (SD) in a well-characterized stuttering mouse model, *Gnptab*-mutant model, and evaluated the vocal behaviors of mice. We analyzed sleep characteristics using an electroencephalogram (EEG). Histology techniques and computer-aided morphometric analysis of astroglia we used to study the effect of SD on glial morphology.
Our preliminary data suggest that sleep duration is generally decreased in *Gnptab*-mutant mice compared to control littermates by about 60% (n=3 per group). SD affects vocalization and breathing in both *Gnptab*-mutant and control mice. The frequency of compound vocalization increased by ~ 30%, and breathing frequency increased by 50% (n=3 per group) in *Gnptab*-mutant mice compared to control littermates. Lastly, our previous research indicates decreased astrocyte complexity in *Gnptab*-mutant mice in various brain regions compared to control astrocytes. Following sleep deprivation, we found that this trend holds in certain regions, such as periaqueductal gray; however, in other areas, such as central amygdala, we found no differences in astrocyte complexity.
Our results suggest that the *Gnptab*-mutant model can be used to study the effect of SD on vocalization and breathing behaviors. In the future, we plan to evaluate sleep behavior in individuals who stutter to confirm the findings in animal models. Together, these studies in human and non-human animal models may expand our understanding of the development of stuttering.

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Poster

PSTR217. Oral and Vocal Motor Control

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Program #/Poster #: PSTR217.05/HH8

Topic: E.04. Voluntary Movements

Support: HL090554
HL126523
HL144801
HL151389

Title: Swallow-related sympathetic nerve activity in mice exposed to chronic intermittent hypoxia

Authors: *M. KARLEN-AMARANTE[1], A. HUFF[2], L. M. OLIVEIRA[3], J. M. RAMIREZ[3]; [1]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., SEATTLE, WA; [2]Seattle Children's Res. Inst., [3]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: The homeostatic maintenance of arterial blood pressure involves tonic sympathetic drive to control the contraction of blood vessels. Clinical evidence demonstrates that patients with obstructive sleep apnea (OSA) are hypertensive and exhibit excessive sympathetic activity to the cardiovascular system. Studies in rodents showed that chronic intermittent hypoxia (CIH), a model of OSA, is associated with hypertension and dysphagia (difficulty swallowing). Here we show that the preBötzinger complex (preBötC), a medullary region critical for the generation of inspiration, and the Postinspiratory Complex (PiCo) a region critical for postinspiration and swallow-breathing coordination, also contribute to the generation of sympathetic activity. We characterized the cervical sympathetic nerve response to water swallow stimulation as well as optogenetic stimulation of the brainstem in an *in vivo* mouse preparation under control and CIH conditions for 21 days (5% O_2 - 80 bouts/day). Recordings of diaphragm (DIA), submental and laryngeal muscles complex, hypoglossal (HN), vagus (cVN), and cervical sympathetic nerves (cSN) were performed in spontaneously breathing, anaesthetized (urethane 1.5mg/kg) adult mice in baseline conditions, during swallow stimulation by injecting 0.1cc of water into the mouth and swallow by optogenetic stimulation of preBötC and PiCo neurons. During water swallows and optogenetic stimulation of PiCo and preBötC, cSN area under the curve response was increased in both control and CIH groups with a stronger response in the CIH mice. This suggests that swallow triggers an excitatory vasomotor sympathetic pathway which involves the activation of neurons in preBötC and PiCo.

Disclosures: M. Karlen-Amarante: None. **A. Huff:** None. **L.M. Oliveira:** None. **J.M. Ramirez:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

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Topic: E.04. Voluntary Movements

Support: NIH Grant U01 DC018671-01A1
NIDCD Grant K99 DC020235
NCATS Grant 5TL1TR001871-05

Title: Cortical dynamics underlying speech sequence planning

Authors: *J. R. LIU[1,3,2], **L. ZHAO**[1,2], P. W. HULLETT[1,2,4], E. F. CHANG[1,3,2];
[1]Neurolog. Surgery, [2]Weill Inst. for Neurosci., Univ. of California San Francisco, San Francisco, CA; [3]UC Berkeley - UCSF Grad. Program in Bioengineering, Berkeley, CA; [4]Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Fluent speech production requires the planning and articulation of accurately sequenced speech sounds. This process is commonly thought to result from the progression of activity across cortical areas, each of which has a specific function in the planning and execution of articulation. The classic model is that Broca's area sends planning commands to the precentral gyrus which in turn sends motor commands to the vocal tract. However, recent evidence suggests that Broca's area is not critical for articulation, and the neurological basis of how speech is sequenced is unknown. To address this, we used high-density direct cortical recordings (electrocorticography) from distributed speech cortical areas to investigate the dynamics of cortical activity while participants were cued to read, wait a short delay, and speak simple or complex syllable sequences. While we found activity related to execution and feedback, we found unexpectedly prominent sustained activity across multiple cortical areas that lasted throughout all periods of the task. Sustained activity was found in the middle precentral gyrus (mPrCG), posterior superior temporal gyrus, supplementary motor area, supramarginal gyrus, and the inferior and middle frontal gyri. Sustained neural activity reflected distinct internal states that transition between the encoding, delay, pre-speech, and execution periods. Trial-averaged sustained population activity also showed distinct trajectories associated with each of these task phases. Encoding and execution period trajectories occupied roughly 2D planes, which were not parallel to each other, suggesting that activity prior to production does not directly trace the eventual neural trajectory associated with execution. Increased sequence complexity was associated with greater sustained activity, most prominently in the mPrCG. Pre-speech activity in the mPrCG also correlated with behavior, predicting single trial reaction times. These results suggest that speech production planning involves sustained cortical dynamics supporting complex speech sequence execution. Importantly, we identify the mPrCG as a novel node of speech-motor planning.

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Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.07/HH10

Topic: E.04. Voluntary Movements

Support: NIH F32DC019538
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NIH R01DC017091
NIH R01DC013979
NIH R01NS100440
NIH P50DC019900

Title: Neurophysiological evidence of prediction errors driving sensorimotor adaptation

Authors: *K. S. KIM[1], S. NAGARAJAN[2], J. HOUDE[2];
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Abstract: The human sensorimotor system has a remarkable ability to quickly and efficiently learn movements from sensory information. One prominent example is sensorimotor adaptation, learning that occurs when the sensorimotor system experiences sensory errors and adjusts future movements to compensate for those errors. Despite its fundamental nature in maintaining and fine-tuning motor control, mechanisms underlying sensorimotor adaptation remain unclear. Previous studies have suggested that an implicit component of adaptation (i.e., the learner is unaware of the learning process) may result from sensory prediction errors-the discrepancies between predicted sensory consequences of motor commands and actual sensory feedback. However, although assuming the existence of prediction errors explains behavioral data, direct neurophysiological evidence of prediction errors driving adaptation has not been documented to date. Here, we examined prediction errors via magnetoencephalography (MEG) imaging during speech sensorimotor adaptation to altered auditory feedback (e.g., Houde & Jordan, 1998), an entirely implicit adaptation task (e.g., Lametti et al., 2020; Kim & Max, 2021). Specifically, we measured Speaking-Induced Suppression (SIS)—suppression of auditory responses (M100) to self-produced speech compared to the responses to passively heard speech—which is thought to represent prediction errors (Houde et al., 2002). The results indicated that SIS was reduced (i.e., larger prediction errors) during the early learning phase compared to the non-adaptive phase, and the amount of reduction in SIS correlated with the amount of adaptation, suggesting that larger prediction errors were associated with more learning. In contrast, such a reduction in SIS was not found in a control experiment during which participants were not adapting (i.e., speaking with non-altered auditory feedback). In addition, in a subgroup of participants who reached a plateau in late learning, SIS was increased (i.e., smaller prediction errors) during the late learning phase compared to the early learning phase, demonstrating that prediction errors were minimal when there was no further adaptation. Together, these findings provide neurophysiological evidence for the hypothesis that prediction errors drive the implicit adaptive process.

Disclosures: K.S. Kim: None. **S. Nagarajan:** None. **J. Houde:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.08/HH11

Topic: E.04. Voluntary Movements

Title: Sensitivity to auditory feedback and individual variability

Authors: *M. OZKER SERTEL, L. GIGLIO, J. WESTER, P. HAGOORT;
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Abstract: Monitoring auditory feedback is important for fluent speech production as it enables correction of vocalization errors. Influence of auditory feedback is best illustrated by manipulating it during speech production. A common temporal manipulation technique is delaying auditory feedback (DAF), which disrupts speech fluency, and a common spectral manipulation technique is perturbing the pitch of auditory feedback, which elicits vocal changes. Interestingly, not everybody is equally sensitive to auditory feedback manipulations, however the reason for this individual variability is unknown. We aimed to understand whether there is a correlation between sensitivity to temporal versus spectral manipulations of auditory feedback. And whether less sensitive individuals rely less on auditory feedback when an alternative sensory feedback (e.g. visual) is available. We collected data from 40 native Dutch speakers during both a DAF and a pitch perturbation task. In the DAF task, participants repeated auditorily presented sentences. Auditory feedback was presented either simultaneously or with 200ms delay. In half of the trials visual feedback was presented additionally via a webcam. Voice recordings were analyzed using linear mixed effect (LME) models to test the effects of delay, visual feedback, gender and trial structure on articulation duration, voice intensity and voice pitch. In the pitch perturbation task, participants phonated the vowel /a/ for 4 seconds and pitch of the auditory feedback was shifted by ±100 or ±200 cents. LME models were used to test the effects of shift magnitude, shift direction and gender on compensatory response magnitude, response latency and percentage of opposing responses. We found that DAF significantly prolonged articulation duration and increased both voice pitch and intensity. In contrary to our expectations, visual feedback did not ameliorate but reinforced the disruptive effects of DAF. For the pitch perturbation task, we only found that larger pitch shift elicited less compensatory responses. We used articulation duration and compensatory response magnitude to measure sensitivity to different auditory feedback manipulations. There was a large individual variability in sensitivity to feedback manipulations for both tasks, however there was no correlation between the sensitivity profiles between tasks, suggesting that these features are processed differently. We also showed that, possibly because visual feedback is not naturally available to us during speech production, it is less likely integrated with auditory feedback to aid speech monitoring.

Disclosures: M. Ozker Sertel: None. **L. Giglio:** None. **J. Wester:** None. **P. Hagoort:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.09/HH12

Topic: E.04. Voluntary Movements

Title: Data driven decomposition reveals neural sub-processes that link speech perception and production

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Abstract: While typically studied as separate processes, speech perception and production are intrinsically linked in the brain. Evidence for this comes from conduction aphasia (CA) patients with intact speech comprehension and production but deficits with repetition. While traditional models propose a single pathway as the anatomical locus of this linkage, there is evidence that CA cannot be attributed to any one neuroanatomical substrate (Buchsbaum, 2011). Furthermore, there is no one-to-one mapping of sound properties in speech perception to motor properties in speech production (Liberman, 1967). This suggests multiple parallel pathways and neural sub-processes orchestrate the link between speech perception and production (Hickok, 2022). We therefore sought to investigate these neural sub-processes using a method with high spatio-temporal precision: intracranial recordings. We collected data from 23 subjects undergoing intracranial monitoring for surgical treatment of epilepsy (mean age = 28, 13 female). Subjects performed a repetition task where they listened to a single word or sentence that they repeated after a delay, mimed after a delay, or passively listened to (270 trials). Statistical significance of the high gamma response (HG, 70-150 Hz) for each time point on each electrode served as an index of local neural computation (cluster-corrected at $p < 0.05$). We first replicated previous results (Cogan et al. 2014, 2017) by showing distinct auditory (AUD - 117 electrodes), production (PROD - 552), and sensory-motor responses (electrodes with both auditory and production responses: SM - 307). We next investigated the morphology of neural sub-processes by performing a novel unsupervised decomposition of the spatio-temporal profile of HG responses within each electrode category. Results from SM electrodes revealed four distinct components that overlap in time with perception and production processes: a primarily visual response to the instruction/go cue (localized to the occipital and inferior temporal cortex), an early auditory response that also peaked during late production (posterior superior temporal cortex), a later auditory response that also peaked at pre/early production (pre/motor and parietal cortex), and a working memory component that showed sustained higher HG delay activation in the repetition vs. passive listen condition (prefrontal, parietal, and superior temporal cortex). Our results show that speech repetition can be broken down into neural sub-components, which

supports the overarching hypothesis that the link between speech perception and production is supported by multiple parallel pathways and neural sub-processes.

Disclosures: **A. Earle-Richardson:** None. **D. Southwell:** None. **S.R. Sinha:** None. **M. Vestal:** None. **G. Grant:** None. **M. Zafar:** None. **G.B. Cogan:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.10/HH13

Topic: E.04. Voluntary Movements

Support: ERC StG 335880
ERC CoG 864491
Evangelisches Studienwerk Villigst

Title: Neural representations of the content and production of human vocalization

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Abstract: Speech is the spoken form of language and allows to encode information in sound (overt) and thought (covert). The phenomenon of inner (covert) speech implies a functional independence between speech content and motor production. However, it remains unclear how content and production are represented neuronally and how the brain achieves a flexible mapping between the two. To address this, we recorded magnetoencephalography (MEG) in human subjects (n=24) performing a rule-based vocalization task. Content (vowel /u/ or /ʊ/) and production (overt or covert) were instructed sequentially and in random order. We applied multivariate pattern analysis to analyze the format, overlap and temporal dynamics of neural content and production representations. We found robust and dissociable neural information about the vocalization content and production several seconds before vocalization onset. The strength of the neural information correlated with effort, i.e. the degree of motor involvement. The production representation changed once the content was known, whereas the content representation remained stable throughout the trial. Together, our results provide insights into the neural dynamics underlying human vocalization and open a new window for noninvasive speech research in health and disease.

Disclosures: **V.A. Voigtlaender:** None. **F. Sandhaeger:** None. **D.J. Hawellek:** None. **S.R. Hage:** None. **M. Siegel:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.11/HH14

Topic: E.04. Voluntary Movements

Support: NIH Grant U01NS117836

Title: Human intracranial subthalamic nucleus and globus pallidus local field potential beta and high-gamma dynamics during sentence repetition

Authors: *L. BULLOCK[1], A. BUSH[2], F. H. GUENTHER[3], M. RICHARDSON[2];
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Abstract: The basal ganglia's (BG) specific role in motor control is contentious, with different models foregrounding different aspects of motor control: action selection, sequencing, automation, vigor, and learning, to name a few. The BG's role in speech motor control is even more poorly understood. Intracranial recordings of the BG are rare, but are indicated in specific clinical settings like deep brain stimulation implant surgery. These recordings can provide valuable glimpses of BG speech physiology. Previous studies have reported local field potentials (LFPs) and single-units during simple speech-like tasks, like vowel production, CVC production, and repetition of nonword syllables. Single-unit data reveal that generally around half of motor STN units are task-responsive. Most of the task-responsive units increase in firing rate at speech onset. LFPs from STN show robust beta-band (12-30 Hz) suppression before and during articulation. In this project, we report simultaneous LFP and single-units in STN and globus pallidus internus (GPi) recordings from Parkinson's and dystonia patients during cued sentence production to determine if previous single-word production findings generalize to phrasal speech. Preliminary analyses reveal that beta power is indeed suppressed during the entirety of the utterance in STN and GPi. In contrast to previous studies, we found STN recordings rarely revealed HG modulation. HG activity in GPi increased coincident with speech onset. Planned analyses will differentiate how HG fluctuates at the single-trial level in GPi; we hypothesize that HG power will be tuned to either the rising or falling edge of the speech acoustic envelope, indexing the release or completion of speech motor commands, respectively.

Disclosures: L. Bullock: None. **A. Bush:** None. **F.H. Guenther:** None. **M. Richardson:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.12/HH16

Topic: E.04. Voluntary Movements

Support: NIH/NIDCD Grant R01-DC018523

Title: Neural impairment of speech sensorimotor coordination network for auditory feedback control in post-stroke aphasia: an fMRI study

Authors: *R. BEHROOZMAND[1], K. SARMUKADAM[2];
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Abstract: Emerging evidence suggests the role of a dual speech coordination model, including a dorsal pre-central speech area (dPCSA) and ventral pre-central speech area (vPCSA), to process and regulate human speech. This model is especially promising for delineating characteristics associated with speech disorders as it may allow for more precise diagnosis and treatment in clinical populations with aphasia, which is a common disorder of expressive and receptive language function resulting from left-hemisphere stroke. However, the direct impact and underlying neural mechanisms of the dPCSA and vPCSA associated with speech perception and production still need to be investigated. The aim of the present study was to investigate neural activation in sensorimotor networks associated with the dual coordination model and speech sensorimotor impairment in aphasia compared with neurotypical controls. Functional magnetic resonance imaging (fMRI) and speech data were collected from 15 aphasia subjects with chronic left-hemisphere stroke (5 female; mean age: 63.94) and 16 neurologically intact controls (10 female; mean age: 59.82) while they performed speech vowel productions or silently listened to the playback of their own productions in randomized order under normal auditory feedback (NAF) and pitch-shifted AAF conditions at ±200 cents magnitude (±2 semitone). Analysis of covariate (ANCOVA) was performed to investigate the effects of group and task on neural activity while controlling for subjects' hearing thresholds. Results indicated significantly reduced neural activity in the left inferior frontal gyrus (IFG) pars opercularis and triangularis, dorsal precentral gyrus (dPCG), superior temporal gyrus (STG), planum temporale, supramarginal gyrus (SMG), and putamen as well as the right anterior cingulate gyrus (ACC), angular gyrus, thalamus, and cerebellum in aphasia compared with controls ($p<0.05$, *FWE-corrected*). In addition, speech production was associated with significantly increased neural activity in bilateral anterior cingulate cortex (ACC), ventral precentral gyrus (vPCG), dorsal precentral gyrus (dPCG), and thalamus as well as the right angular gyrus and putamen compared with silent listening ($p<0.05$, *FWE-corrected*). These findings reveal network-wide deficits in sensorimotor coordination for speech auditory feedback control in post-stroke aphasia compared with controls consistent with data from previous studies. Our results provide translational synergy to inform the theoretical models while having clinical applications for diagnosis and treatment of communication disabilities in neurological conditions.

Disclosures: R. Behroozmand: A. Employment/Salary (full or part-time):; University of South Carolina. 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/NIDCD R01-DC018523. **K. Sarmukadam:** A. Employment/Salary (full or part-time):; University of South Carolina.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.13/HH17

Topic: E.04. Voluntary Movements

Support: DFG
Stiftung Polytechnische Gesellschaft

Title: Flexible control of vocal timing in an echolocating bat

Authors: *A. KIAI[1], J. CLEMENS[3], M. KÖSSL[2], D. POEPPEL[4], J. C. HECHAVARRIA[1]; [1]Dept. of Biol. Sci., Goethe Univ. Frankfurt, Frankfurt am Main, Germany; [2]Goethe Univ. Frankfurt, Goethe Univ. Frankfurt, Frankfurt/M., Germany; [3]Princeont Neurosci. Inst., European Neurosci. Inst., Göttingen, Germany; [4]New York Univ., New York Univ., New York, NY

Abstract: In natural environments, background noise can easily degrade the integrity of acoustic signals, posing a problem for animals that rely on their vocalizations for communication and navigation. A simple behavioral strategy to combat acoustic interference would be to restrict call emissions to periods of low-amplitude or no noise. Using audio playback and computational tools for the automated detection of vocalizations, we show that bats (*Carollia perspicillata*) can dynamically adapt the timing of their calls to avoid acoustic jamming in both predictably and unpredictably patterned noise, demonstrating that bats spontaneously seek out temporal windows of opportunity for vocalizing in acoustically crowded environments. We probe the neural underpinnings of this flexible vocal-motor output to elucidate the mechanism for efficient echolocation and communication in cluttered acoustic landscapes.

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Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.14/HH18

Topic: E.04. Voluntary Movements

Support: R01AG069227 National Institute on Aging
R01DE027236 National Institute of Dental & Craniofacial Research

Title: 3d directional tuning in the orofacial sensorimotor cortex during natural feeding and drinking

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Abstract: Motor and somatosensory cortical neurons are known to exhibit directional tuning during reaching tasks. While this phenomenon has been well-studied in the arm region of the sensorimotor cortex, little is known about how 3D tongue direction is encoded in the orofacial region during natural feeding and drinking behaviors. Specifically, directional tongue movements are essential for behaviors such as feeding and speech. Thus, understanding how tongue direction is represented in the brain may help improve therapies and rehabilitation for people with sensorimotor dysfunctions. To investigate how changes in the direction of tongue movement are encoded in the sensorimotor cortex, we recorded both kinematic and neural information from non-human primates (*Macaca mulatta*) during feeding and drinking behaviors. 3D positional data was recorded using high-resolution biplanar video-radiography to track the implanted tantalum beads in the tongue, and spiking activity was simultaneously recorded using chronically implanted microelectrode arrays in the orofacial primary motor (MIo) and somatosensory (SIo) cortices. In some sessions, these behavioral tasks were preceded by bilateral nerve block injections to the sensory branches of the trigeminal nerve to examine how they were affected by the loss of sensation. Modulation to the direction of tongue movement in 3D was found in >70% of neurons in MIo during feeding. This was not the case in SIo neurons, where <40% of neurons displayed directional tuning. During drinking both MIo and SIo displayed modulation to the direction of tongue protrusion in >60% of neurons. With nerve block, the proportion of MIo neurons that displayed directional tuning remained similar. However, in both feeding and drinking tasks, the proportion of directionally-tuned SIo neurons decreased by 20% with sensory loss. Significant shifts in the distribution of preferred directions were also observed in these populations of neurons following sensory loss in both feeding and drinking tasks (Mann Whitney U-test, $p<0.05$), though the changes differed across cortical areas. Overall, our preliminary results show that MIo and SIo neurons exhibit modulation to 3D tongue direction that vary with behavioral tasks, and that sensory loss causes a significant decrease in the directional tuning of SIo neurons as well as a shift in preferred direction. These findings enhance our understanding of directional tuning properties of the orofacial somatosensory cortex during feeding and drinking behaviors and the effects of sensory loss, which have important implications on advancing the treatment of conditions that impair orolingual function.

Disclosures: V. Hosack: None. **F.I. Arce-McShane:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.15/Web Only

Topic: E.04. Voluntary Movements

Support: R01AG069227 National Institute on Aging
R01DE027236 National Institute of Dental & Craniofacial Research
Dr. Douglass L. Morell Dentistry Research Fund

Title: Loss of tactile sensation affects functional connectivity in orofacial sensorimotor cortex

Authors: *W. C. MCLELLAND[1], F. I. ARCE-MCSHANE[2,3];
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Abstract: Oral somatosensory awareness involves the integration of multiple somatosensory modalities (e.g., tactile and proprioception) from multiple oral structures (e.g., tongue, teeth, and palate). Synchronized activity across cortical networks may mediate this process. However, the dynamics of cortico-cortical interactions across neuronal networks in the orofacial sensorimotor cortex during feeding is mostly unexplored. This knowledge is critical in understanding chewing and swallowing dysfunctions common in neurological diseases. Here, we evaluated cortico-cortical interactions across multiple regions of the orofacial sensorimotor cortex and how absent tactile sensation impacted such interactions. Two rhesus macaques (Macaca mulatta) were implanted with tantalum beads in the tongue and jaw to capture their motion during feeding by using hi-resolution biplanar video-radiography. Simultaneously, neural data was recorded from multielectrode arrays in the orofacial sensorimotor cortex (areas 1/2, 3a/3b, rostral and caudal areas of primary motor (MIo) cortex). On some days, animals engaged in the feeding task following bilateral injections of local anesthesia to specific sensory branches of the trigeminal nerve. We found that spike-spike coherence during chew cycles was significantly modulated, with increases and decreases around minimum gape, and was more prominent between motor regions than between somatosensory regions. Across animals, the theta band had the largest neural network and greatest magnitude of coherence in both control and nerve block conditions. Absent tactile sensation caused a change in coherence; one subject had a compensatory increase in coherent activity, while the other had an overall decrease in all areas. Neuronal pairs whose peak coherences were significantly higher than noise in the control condition either ceased to exhibit significant coherence or had a reduced coherence in the nerve block. The time of peak coherence was also shifted from the control to the nerve block. Overall, our findings suggest that cortical-cortical interactions across multiple regions of the orofacial cortex may mediate the integration of motor and somatosensory information during feeding and that these interactions are disrupted by the loss of tactile sensation.

Disclosures: W.C. McLelland: None. **F.I. Arce-McShane:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.16/HH19

Topic: E.04. Voluntary Movements

Support: National Institute on Aging Grant R01AG069227
National Institute of Dental & Craniofacial Research Grant R01DE027236

Title: Encoding of touch and proprioception in the orofacial sensorimotor cortex

Authors: *M. M. L. GRIESKAMP[1], H. XU[2], F. ARCE-MCSHANE[3,4];
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Abstract: Proprioception and tactile sensation of the tongue is essential for maneuvering the tongue effectively without injury during mastication. However, the role of the orofacial primary motor (MIo) and somatosensory (SIo) cortices in encoding proprioception and tactile sensation during natural feeding is still unclear. The aim of this study is to examine how the representation of proprioception and tactile sensation differs across individual neurons and cortical regions in rhesus macaques (*Macaca mulatta*). We recorded electromyographic (EMG) activity of extrinsic and intrinsic tongue muscles using intramuscular fine-wire electrodes and neural activity using chronically implanted microelectrode arrays in MIo and SIo. 3D tongue and jaw kinematics were simultaneously acquired using high-resolution biplanar videoradiography to track radiopaque markers implanted in the tongue and jaw. One network of markers implanted on the superficial layer of the tongue was used as a proxy for tactile information and another network implanted on deeper levels of the tongue for proprioceptive information. Indeed, deep marker kinematics were more correlated with extrinsic muscles at all lead and lag times, consistent with the function of these muscles to position the tongue inside the oral cavity. In contrast, superficial marker kinematics were more correlated to intrinsic tongue muscles when EMG activity led the markers. The ability of marker networks to predict spiking activity of individual neurons during chew cycles was then evaluated using generalized linear models to determine the encoding properties of neurons. The spiking activity of 15% of neurons in each cortical region were better predicted using a model that included either the position of deep or superficial markers (Paired Wilcoxon sign rank test, $p<0.05$). For SIo neurons, deep marker kinematics had a higher predictive power whereas for MIo neurons, superficial marker kinematics showed slightly stronger predictive power. The findings suggest that majority of neurons in MIo and SIo carry both tactile and proprioceptive information. Further application of this method applied to similar data under local anesthesia may reveal how encoding of proprioceptive versus tactile information changes with loss of tactile sensation.

Disclosures: M.M.L. Grieskamp: None. **H. Xu:** None. **F. Arce-McShane:** None.

Poster

PSTR217. Oral and Vocal Motor Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR217.17/HH20

Topic: E.04. Voluntary Movements

Support: NIH Grant F32MH120873
NIH Grant R01NS089652
Kavli NDI Distinguished Postdoctoral Fellowship

Title: Jaw muscle spindle afferents as multiplexed channels for sensing and guiding orofacial movement

Authors: *W. P. OLSON[1], V. B. CHOKSHI[1], J. KIM[1], M. VASS[1], N. J. COWAN[2], D. H. O'CONNOR[1];
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Abstract: Muscle spindle afferents (MSAs) are muscle stretch sensors that provide critical real-time feedback to the nervous system about body position and movement. While classic models proposed that MSAs are 'kinematic encoder' sensory neurons, more recent models highlight the dynamic tuning of these neurons and propose they instead serve task-specific motor control functions. Here, we record from MSAs innervating the jaw musculature (located in the mesencephalic trigeminal nucleus, MEV) in behaving mice to test these competing hypotheses. In our task, head fixed mice lick a moving 'port' through an arc of seven locations surrounding the mouse's face to receive a water reward. MSA ensemble activity is complex, evolving over single lick cycles as well as over entire licking sequences. While a large component of MSA ensemble activity is correlated with jaw kinematics, major components of the activity show clear decoupling from the kinematics. We find that (1) encoding of kinematics varies across the MSA ensemble, with a small fraction of single MSAs showing strong encoding in a manner consistent with classic models, (2) MSAs innervating different jaw synergist muscles show distinct activity, with MSAs from one muscle (temporalis) showing the strongest kinematic encoding, and (3) comparison of activity during active licking vs. passive movement under anesthesia reveals that MSA kinematic tuning is actively set by the awake animal. Ongoing research is aimed at analyzing the complex activity seen in MSAs during active licking to develop a holistic model of the sensory and/or motor control functions of this important class of proprioceptors.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.01/HH21

Topic: E.06. Posture and Gait

Support: Ontario Neuro-trauma Foundation (2016-RHI-PREV-1019)
NSERC Discovery Grant (DG RGPIN-2017-06790)

Title: Three-link analysis of standing posture in individuals with incomplete spinal cord injury

Authors: *B. GUI[1,4], J. W. LEE[1,4], K. CHAN[4], J. UNGER[5], K. E. MUSSELMAN[4,2,3], K. MASANI[4,1];
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Abstract: Individuals with incomplete spinal cord injury (iSCI) commonly experience lower-limb sensorimotor impairment, leading to compromised balance control and increased fall risk. Although previous studies have focused on the effects of spinal cord injuries on ankle and hip joint control during quiet standing, it has been demonstrated in able-bodied individuals that the knee joint also plays a role in maintaining balance. Therefore, the purpose of our study was to investigate the kinematics of quiet standing using a three-link model (ankle, knee, and hip) in individuals with iSCI.

Data from our previous study, involving thirteen individuals with iSCI (53 ±14 years old, 11 female) and thirteen able-bodied (AB) individuals (55 ±13 years old, 10 female) were used for this study. The participant performed 150-s quiet standing tasks with their eyes open and closed. Kinematic and kinetic data were recorded using a motion capture system and a force place, respectively. We compared joint angles and joint angular acceleration from the ankle, knee, and hip joints and investigated the coordination between the three joints using induced acceleration and uncontrolled manifold analyses.

We found that individuals with iSCI had greater postural sway compared to AB, indicated by increased centre of mass (CoM) acceleration. In addition, they showed significantly higher angular displacement at each joint (ankle, knee, and hip). In eyes closed standing, iSCI individuals also showed significantly higher angular acceleration at each joint. The knee joint was found to show significant movement, exhibiting greater angular acceleration than the ankle for both groups.

Using induced acceleration analysis, we found that at each joint, the angular acceleration induced by the net torque from the ankle and hip joints was opposed to that induced by the knee joint. This phase relationship resulted in a decrease in the total acceleration of each joint. This relationship, as measured by correlation, was not reduced in iSCI compared to AB individuals. In addition, we used uncontrolled manifold analysis to investigate the ratio between joint movements that increased and did not increase CoM acceleration, a measure of interjoint coordination, and found no significant difference between iSCI and AB participants.

Our study demonstrated that the knee joint plays a role in quiet standing in both AB and iSCI individuals, acting to reduce joint acceleration induced by the ankle and hip joints. We show that individuals with iSCI exhibit intact coordination between joints in a three-link model of quiet standing, but show increased joint acceleration, resulting in higher postural sway.

Disclosures: B. Gui: None. **J.W. Lee:** None. **K. Chan:** None. **J. Unger:** None. **K.E. Musselman:** None. **K. Masani:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.02/HH22

Topic: E.06. Posture and Gait

Title: Oscillations from 0-1 Hz in postural sway scale differentially with sensory weighting and are of prognostic value in Huntington's Disease

Authors: *J. F. SWINFORD[1], M. MEYER-VEGA[1], D. J. GOBLE[2], P. E. GILBERT[3], J. COREY-BLOOM[4], N. BAWEJA[1], H. S. BAWEJA[1];
[1]Kinesiology, Auburn Univ., Auburn, AL; [2]Exercise Sci., Univ. of Oakland, Rochester, MI; [3]Psychology, San Diego State Univ., San Diego, CA; [4]Neurosci., UC San Diego, San Diego, CA

Abstract: The clinical markers of disease conversion from pre-manifest to manifest Huntington's Disease (HD) remain unknown. Static and dynamic posturography are effective tests to distinguish the movement dysfunction between pre-manifest and manifest HD. For balance to be maintained, the sensory integration of vision, proprioception and vestibular information is necessary. Furthermore, postural sway measurements during sensory information manipulations are of great value in understanding disease progression. Therefore, the purpose of our study was to determine if markers in postural sway vary with disease severity in pre-manifest and manifest HD. 105 individuals (33 HD, 28 Androgen Receptor Positive (ARP), 30 Androgen Receptor Negative (ARN), and 14 aged-matched controls) were recruited to perform 6 consecutive (3 eyes-open and 3 eyes-closed) 10-second balance trials using the Balance Tracking System™ (BTrackS, San Diego, CA). Postural sway was quantified as total, antero-posterior, and medio-lateral center of pressure (COP) displacements. Additionally, a continuous wavelet transform was used to understand frequency modulation from 0-1 Hz in the postural sway signal. It is suggested that specific frequency bands between 0-1 Hz indicate functional contributions from the visual (0-0.1Hz), vestibular (0.1-0.5 Hz), and proprioceptive (0.5-1Hz) systems. We find that postural sway is greatest in individuals with HD>ARP>ARN>HC, especially with eyes closed. Our findings support and extend previous reports that the spectral density of postural sway is predominantly present between 0-1 Hz even in pre-manifest and manifest HD. We also found that pre-manifest HD individuals exhibit greater postural sway with eyes closed when compared with ARN and age-matched control groups. Despite this, individuals with pre-manifest HD exhibit no differences in postural sway with their eyes open. This greater postural sway with eyes closed is indicative of an increased risk for falls. It is also associated with a decrease in deterministic oscillations from 0-1 Hz as disease progresses (HD>ARP>ARN). This suggests that as disease progresses, individuals with pre-manifest HD rely heavily on visual input to maintain balance. Therefore, the differential modulation of postural sway from 0-1 Hz frequency bands is of value in detecting postural sway changes and could be an early indicator of HD conversion. Thus, the understanding of these oscillatory signatures is important and could be used as a prognostic tool in aiding with earlier diagnosis and intervention in HD.

Disclosures: J.F. Swinford: None. **M. Meyer-Vega:** None. **D.J. Goble:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding

diversified mutual funds); Balance Tracking Systems. **P.E. Gilbert:** None. **J. Corey-Bloom:** None. **N. Baweja:** None. **H.S. Baweja:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.03/HH23

Topic: E.06. Posture and Gait

Title: The Perception of Limb Speed and Stepping Responses in Early Parkinson's Disease: Insights from the 'On' Medication State

Authors: *C. L. BRANDMEIR[1], E. HERRICK[2], S. YAKOVENKO[1];
[1]Human Performance, [2]Chem. and Biomed. Engin., West Virginia Univ., Morgantown, WV

Abstract: The cortical processing of sensory feedback may change intentions and modulate execution, for example, walking over a slippery surface would increase the gain of proprioceptive pathways. Cognitive evaluation of movement sensation (or kinesthesia) is affected by both amplitude and timing of movement-related sensory feedback (proprioception). The disruptions in cortical processing due to neurological conditions are likely to disrupt kinesthesia, with consequences for the regulation of movement. However, this link remains sparsely studied. In this pilot study, we tested the limb kinesthesia and postural responses of two participants with early Parkinson's Disease (PD) in the medication 'on' state. We assessed the success rate of detecting limb speed differences during walking on a split-belt treadmill. Additionally, anterior and posterior reactive balance was assessed from stance to determine the preferred stepping limb and the success rate for balance recovery during rapid acceleration and deceleration of the support surface. Participants walked at their preferred speed (PS) and reported on detecting the imposed interlimb speed differences of 0, ±2, ±10, ±20, and ±30% of PS. Logistic fit into the success rate function was used to evaluate just noticeable difference at 75% performance (JND75). We found the JND75 was lateralized and correlated with the affected side showing the decreased detection of turns toward the most symptomatic leg. Furthermore, the reactive balance test indicated a first-step preference for stepping with the least affected limb posteriorly during backward perturbations and conversely, the more affected limb during anterior perturbations. This suggests that individuals with early-stage PD may rely on the least affected limb to compensate for perturbations with a stepping strategy to ensure balance stability. These findings suggest that bradykinesia and rigidity, common symptoms of PD, may already impact stepping responses in the early stages of the disease, despite being in the 'on' medication state. This study highlights the importance of understanding sensorimotor feedback and the perception of limb speed among individuals with PD for the development of goal-directed gait and balance therapies.

Disclosures: C.L. Brandmeir: None. **E. Herrick:** None. **S. Yakovenko:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.04/Web Only

Topic: E.06. Posture and Gait

Title: Effect of home-based balance training with intermittent visual obstruction on balance and gait performance in people with incomplete spinal cord injury

Authors: R. BRAVI[1], S. GUARDUCCI[1], G. PANCONI[1], M. SICHER[1], V. SORGENTE[1], G. LUCCHESI[2], *D. MINCIACCHI[1];
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Abstract: Background. Following an incomplete spinal cord injury (iSCI), despite most of individuals regain standing balance and walking abilities after participation in a rehabilitation program, post-lesion performance continues to be significantly affected resulting in a major challenge for injured people during the activities of daily living.As suggested by prior research, balance and walking deficits in individuals with iSCI can be caused by proprioceptive impairments occurring after the SCI. Additionally, intact visual system seems to dominate over the altered somatosensory and proprioceptive systems, with an increased reliance on visual inputs by people with SCI when maintaining balance.Recently, advanced technology conceived special eyewear with liquid-crystal technology in the lenses to produce intermittent vision obstruction. This special eyewear allows individuals to perform dynamic, functional tasks that, otherwise, could be not performed under a complete visual obstruction. The purpose of this study was to assess the effect of the intermittent visual deprivation integrated to a home-based balance training in influencing balance and gait performance in individuals with iSCI. **Methods.** Ten individuals (7 men and 3 women) with chronic iSCI, ASIA level D, were enrolled for this study. Individuals were randomly assigned to an experimental group (n = 5) that underwent balance training with visual deprivation, and a placebo control group (n = 5) that underwent the same balance exercises program with no visual deprivation (non-active glasses). Balance training program was performed at home and lasted 6 weeks, 3 times per week. Before and after the training, balance and gait were assessed. **Results.** After completing the training program, balance and gait performance improved significantly in both experimental and placebo control groups. Post-test balance and gait performances improved more in the stroboscopic visual deprivation group than in the placebo control group. **Conclusions.** Stroboscopic visual deprivation when integrated to balance training can potentiate the recovery of balance and gait in people with incomplete spinal cord injury (iSCI).

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.05/HH24

Topic: E.06. Posture and Gait

Title: Coherence of chewing and stepping patterns indicate innate coordination between coupled oscillators

Authors: *B. SAMULSKI[1], J. PREBOR[3], S. MORRISON[2];
[1]Rehabil. Sci., [2]Old Dominion Univ., Norfolk, VA; [3]Old Dominion Universtiy, Norfolk, VA

Abstract: Chewing is a repetitive motor task that is central to processing nutrition necessary to sustain life. Stepping during gait is similarly patterned for an entirely different purpose of locomotion. Execution of two simultaneous tasks is generally associated with impaired performance of one or both tasks. Despite their unrelated nature, chewing and walking patterns appear to couple when performed together. The aim of this study was to investigate the relatedness of chewing and stepping frequencies when they are performed individually, together, and initiated prior to one another. Fifteen healthy young (age 18-40) and fifteen older adults (age 60-75) completed the study. Individuals were asked to chew at three different speeds: 1. preferred, 2. self-selected slow, and 3. self-selected fast. Chewing rates were measured using surface EMG recordings from the masseter muscles. Baseline chewing was evaluated while standing still, whereas all other chewing conditions were performed while walking. Each person's typical gait was assessed at a self-selected pace without chewing. Chewing was introduced either prior to the beginning of the walk or mid-way through the walking trial. Participants self-selected the walking speed for all trials while simultaneously chewing. Stepping rates were collected using wireless accelerometers on the legs, trunk, and head. When performed independently, chewing, and stepping frequencies do not appear to be related. When chewing and walking are performed concurrently, the coherence of the two signals increases indicating that the signals are more related. The results indicate that the timing of chewing initiation (i.e., prior to or during walking) does not alter the coherence of the chewing and stepping patterns when performed together. The stepping frequency shifts to match the chewing frequency regardless of when chewing is initiated. The persistent coupling of chewing and walking may indicate an inherent coordination of neural mechanisms for timing of motor tasks performed by the mouth and legs. The coupling emerges when the two are performed simultaneously. The exact mechanisms underlying the dynamic coaction remain unclear but appear to support an intrinsic timing which unites multiple neural circuits.

Disclosures: **B. Samulski:** A. Employment/Salary (full or part-time):; Old Dominion 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.; Optima Health. **J. Prebor:** None. **S. Morrison:** A. Employment/Salary (full or part-time):; Old Dominion 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.; Optima Health.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.06/HH25

Topic: E.06. Posture and Gait

Support: NIH T32-NS082128-06
R21NS119849

Title: Deep brain stimulation improves obstacle clearance in essential tremor by reducing axial tremor

Authors: *Y. CHOI[1], S. DELMAS[2], B. YACOUBI[2], J. KIM[2], J. HUBBARD[2], M. OKUN[3], E. CHRISTOU[2,3];
[2]Dept. of Applied Physiol. and Kinesiology, [3]Departments of Neurol. and Neurosurg., [1]Univ. of Florida, Gainesville, FL

Abstract: Essential tremor (ET) is one of the most prevalent movement disorders with the cardinal symptom being a tremor in both upper limbs. Those with ET experience falls twice as much as healthy age-matched cohorts, making it a significant health concern. The falls often occur due to tripping when navigating obstacles, however, it remains unknown how ET patients clear an obstacle and if neurostimulation alters their obstacle-crossing strategy. Here, we investigate the behavior of clearing an obstacle in ET relative to healthy matched controls (HC) and determine if the behavior changes with deep brain stimulation (DBS). Fourteen ET patients (64.6±6.9 yrs; F=4) who underwent DBS surgery targeting the ventral intermediate (VIM) nucleus of the thalamus and 10 healthy older adults (OA; 63.3 ± 6.1yrs; F=6) volunteered in the study. Each participant completed 3 trials of 10-meter overground walk while clearing a foam block obstacle (height 6.35 x length 13 x width 10 cm). ET patients performed the task twice: once with DBS OFF, and once with DBS ON. We quantified (1) average foot elevation height above the obstacle for the leading and trailing foot using Kalman filter algorithm, (2) tremor during obstacle clearance as the 4-8 Hz power of the filtered acceleration signal in different body locations (axial, head, upper limb, lower limb) from wearable sensors ($APDM^{Inc}$, $Delsys^{Inc}$) using wavelet analysis, (3) EMG peak delay among Soleus ($_{Lead}$SOL)and Rectus Femoris ($_{Lead}$RF) of the leading leg, and Rectus Femoris of the trailing leg ($_{Trail}$RF). ET patients exhibited lower leading and trailing foot clearance than OA, but the differences did not reach statistical significance ($p=0.07$; $p=0.1$). With DBS ON, both leading and trailing foot clearance significantly increased relative to DBS OFF ($p<0.05$). The DBS-induced increase in leading foot clearance associated with the DBS-induced reduction in axial tremor ($R^2=0.5$, $p<0.05$), but not with tremors in other body locations ($p>0.1$). Furthermore, the DBS-induced increase in leading foot clearance associated with the DBS-induced reduction in the EMG peak delay between

$_{Lead}$SOL and $_{Trail}$RF (R^2=0.2, $p<0.05$), which associated with the DBS-induced reduction in axial tremor (R^2=0.37, $p<0.05$). In summary, our research indicates that ET patients generally exhibit less foot clearance when crossing obstacles than older adults without ET. However, neurostimulation appears to effectively increase their foot clearance, likely by mitigating axial tremor.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.07/HH26

Topic: E.06. Posture and Gait

Support: NIH Grant 2T32DC000023
NIH U01-90084554
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NIH NIDCD F31DC021106

Title: Bilateral vestibular loss disrupts gaze stability but not gaze shift timing during macaque locomotion

Authors: *O. R. STANLEY, R. WEI, K. E. CULLEN;
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Abstract: For sighted individuals, stable and accurate gaze - the sum of the head's position and orientation in space with the eyes' orientation in the head - is integral to guiding locomotion. The vestibular system makes critical contributions to both components of gaze stability; the vestibulo-ocular reflex drives the eyes to counteract head movement, while vestibulo-collic and vestibulo-spinal reflexes steady the head in space. To better understand vestibular contributions to visual stabilization during locomotion, we investigate differences in gaze behavior during locomotion between rhesus macaques with and without chronic bilateral vestibular loss (BVL). We recorded eye and head orientation during walking on a treadmill at varied speeds and during traversal of a walkway. Data were captured using single-eye video-oculography via head-mounted camera, a head-mounted 6D inertial measurement unit and retroreflective markers, and markerless feature tracking using high-speed cameras.
We first assessed periods of gaze stability versus redirection. As expected, during compensatory slow phase (i.e., between gaze shifts), which comprised the large majority of the gait cycle for both normal and BVL animals, mean gaze velocity was significantly higher in BVL than in normal animals, indicating reduced gaze stability. We then asked whether the frequency or magnitude of gaze shifts made during locomotion differed for normal versus BVL animals. While all animals made similar numbers of gaze shifts per step, these shifts were larger on

average in BVL. Finally, we analyzed the relationship between gaze shift timing and gait cycle. Gaze shifts were distributed randomly within the gait cycle when all analyzed together. However, when divided by direction - inward toward versus outward from the animal along the support surface - each directional group displayed cyclic modulation with gait. In particular, peaks in gaze shift occurrence were offset between directions. Surprisingly, this pattern held for both intact and BVL monkeys.
Taken together, these results demonstrate a change in gaze control strategy following BVL. With respect to timing of inward versus outward gaze shifts, extravestibular information is sufficient to enable strategic adaptation in chronic BVL. In contrast, the disruption of vestibular input produces challenges to the maintenance of steady gaze throughout the gait cycle that cannot be overcome by behavioral adaptation alone. Further research will be needed both to illuminate the impact of vestibular loss on gaze stability in locomotion during the acute stage and to understand how adaptation to this loss arises at the neural level.

Disclosures: O.R. Stanley: None. **R. Wei:** None. **K.E. Cullen:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.08/HH27

Topic: E.06. Posture and Gait

Support: Presidential Graduate Research Fellowship
Nix Graduate Research Fellowship

Title: Low-frequency Oscillations in Postural Sway May Have Prognostic Value for Fall-risk Assessments in Aging

Authors: *M. V. MEYER VEGA[1], J. F. SWINFORD[1], D. J. GOBLE[2], N. BAWEJA[1], H. S. BAWEJA[1];
[1]Kinesiology, Auburn Univ., Auburn, AL; [2]Exercise Sci., Oakland Univ., Rochester, MI

Abstract: Postural sway is quantified linearly by center of pressure (COP) displacement over time and used as a proxy for fall-risk. Various non-linear measures also quantify COP displacement directionally (anterior-posterior and medial-lateral) in time and frequency domains. Although linear measures provide valuable insights into systemic contributions to postural sway, they are not sensitive to the several timescales that constitute the COP signal. We find that using continuous wavelet transforms to decompose the spectral components of COP are sensitive to changes that accompany aging, disease or task performance that are otherwise undetectable using linear measures. To test this, we examined the effect of a concurrent cognitive task on standing balance in young and older adults. We tested fifty-three participants: 20 young adults and 33 older adults. Older adults were then sub-grouped into high - and low fall-risk based on the Better Balance Test. Each subject then performed 3 specifics tasks: 1) Quiet standing with eyes open -

on force plate (single task); 2) Quiet standing with eyes open and verbal memory encoding task - on force plate (dual task); and 3) Quiet sitting with eyes open and verbal memory encoding task - off force plate (single task). We find that: 1) linear postural sway is greater in older adults at a higher risk for falls. However, the amount of sway was not different across test conditions; 2) postural sway oscillations using continuous wavelet transform exhibit that older adults at a higher risk for falls exhibit differential stability across single and dual-task testing conditions; 3) All older adults exhibit postural imbalance when required to divide their attention on balance and a cognitive task. This difference is exacerbated with fall-risk especially in the medial-lateral direction. Our findings support and extend previous reports that the spectral density is predominantly present between 0-4 Hz. We also find that most oscillatory power in postural sway is present below 0-1 Hz. It is suggested that specific frequency bands between 0-1 Hz indicate functional contributions from the visual(0-0.1Hz), vestibular (0.1-0.5 Hz), and proprioceptive (0.5-1Hz) systems. These findings have significant implications for clinical practice, particularly in the diagnosis and rehabilitation of balance deficits.

Disclosures: **M.V. Meyer Vega:** None. **J.F. Swinford:** None. **D.J. Goble:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Balance Tracking Systems. **N. Baweja:** None. **H.S. Baweja:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.09/HH28

Topic: E.06. Posture and Gait

Title: Individuals with Parkinson's disease (PD) exhibit diminished attenuation of gait-related oscillations due to changes in trunk control during fast walking

Authors: *P. PRUPETKAEW[1], A. GRUNSFELD[2], S. MORRISON[1];
[1]Rehabil. Sci., Old Dominion Univ., Norfolk, VA; [2]Neurol., Sentara Hosp., Charlottesville, VA

Abstract: A major health issue confronting persons with Parkinson’s disease (PD) is the increased likelihood of suffering a fall when performing everyday tasks. The basis for the increased risk of falling is usually associated with declines in postural stability. The loss of stability can be linked to a range of factors including a loss of muscle strength, emergence of freezing of gait, increased trunk rigidity, and slowing of postural reactions. In particular, a decline in trunk control can impact on a person’s gait and balance as the trunk segment plays an important role in regulating and attenuating accelerations from the feet to the head. This attenuation ensures the head is stabilized, allowing for optimal feedback from the visual and vestibular systems. This study was designed to examine the pattern of acceleration for the legs, trunk, neck and head during walking for a cohort of health older adults (n=25) compared to elderly persons with PD (n=25). Participants performed ten trials across a 20ft Protokinetics

pressure sensitive surface while walking at their preferred and faster walking speed. Segmental accelerations were collected using triaxial accelerometers affixed to the head, neck, lower trunk and legs. Comparisons of acceleration amplitude (i.e., root mean square; RMS), attenuation, and regularity (i.e., Sample entropy; SampEn) were performed. Each person's falls risk was measured using the Physiological Profile Assessment. The results revealed that the PD persons were at greater falls risk and walked slower than the healthy controls. Although the PD persons walked slower, the acceleration pattern about the trunk, neck and head segments for this group was more irregular (i.e., higher SampEn) indicating a decline in control. This decline in control was also reflected by increased gain of the acceleration signal from the trunk to the head, indicating that the PD persons had a diminished ability to attenuate gait-related oscillations, particularly during the fast-walking condition. Overall, older adults with PD demonstrated an inability to accommodate and dampen oscillations throughout the body during walking, thereby compromising head stability. As declines in gait speed are often linked with loss of head control, one suggestion is that the inability to appropriately compensate for gait-related fluctuations may, in part, explain why persons with PD walk slower and eventually lead to increased risk of falls.

Disclosures: P. Prupetkaew: None. **A. Grunsfeld:** None. **S. Morrison:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.10/II1

Topic: E.06. Posture and Gait

Support: NIH NICHD 1R01HD095975 (Kesar)

Title: Effects of post-stroke gait training on cortical and spinal circuit excitability - a case series

Authors: *T. M. LEONE[1], J. HOPE[1], J. SPENCER[3], O. ALOBA[2], A. SLUSARENKO[1], C. F. MASON[2], T. M. KESAR[1];
[1]Dept. of Rehabil. Med., [2]Neurosci. Grad. Program, Emory Univ., Decatur, GA; [3]Sch. of Applied Physiol., Georgia Inst. of Technol., Atlanta, GA

Abstract: A stroke induces a cascade of changes in the function of neural pathways across the neuraxis. Stroke-induced neurophysiologic changes such as decreased corticospinal excitability (CSE) and heightened spinal reflex excitability (SRE) contribute to reduced walking function and gait impairments. Fast treadmill walking (Fast) is a high intensity treadmill intervention used to improve walking function post stroke. Fast paired with functional electrical stimulation (FastFES) delivers targeted stimulation to paretic ankle muscles during gait. Both Fast and FastFES interventions have shown promise in targeting gait quality and gait function in post-stroke individuals. However, the neural mechanisms of these gait training interventions remain poorly understood. The objective of this study was to determine the magnitude and time course of changes in CSE and SRE induced by 12 sessions of Fast and FastFES gait training post-

stroke. We hypothesized that individuals who undergo FastFES training will show increased CSE and SRE on the stroke affected leg compared to the Fast intervention.
To date, mechanism-focused clinical case-series has evaluated SRE and CSE in 8 older adult stroke survivors (age 40-90 years, >6 months post stroke) (n=4 Fast, n=4 FastFES). CSE and SRE were measured on the lesioned hemisphere prior to gait training (pre), during training (Post 3-sessions, Post 6-sessions), post-training (Post 12-sessions), and after a 3-week follow-up period. Peripheral nerve stimulation (PNS) evoked H-reflex and M-wave amplitudes in the paretic lower-limb were used to quantify SRE. Motor evoked potentials (MEPs) elicited in response to transcranial magnetic stimulation were used to measure CSE. Future data analysis will also evaluate paired-pulse TMS measures of intracortical inhibition and facilitation.
Our results to date show no significant difference in spinal reflex excitability within the FastFES and Fast groups at each training time point compared to baseline. However, compared to the Fast group (mean = +6.1% ± 0.26mV), the FastFES group (mean = -29.1% ± 0.23mV) showed a significantly lower Hmax/Mmax ratio at the 6-week follow-up timepoint compared to baseline ($p=0.05$). This decreased H/M ratio in the FastFES group may suggest FastFES training induces more spinal reflex circuit plasticity in post-stroke individuals.
By comparing the effects of 2 clinically-relevant gait training treatments on both CSE and SRE across multiple time points, our ongoing analyses promises to elucidate novel insights regarding the neural mechanisms underlying high intensity post-stroke gait training interventions with and without stimulation.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.11/II2

Topic: E.06. Posture and Gait

Support: Grant from the french society of physical and rehabilitation in medicine

Title: Structural Brain Changes Associated with Dual-Task Gait Impairment in Older Adults with Mild Cognitive Impairment

Authors: *P. ALI[1], M. DINOMAIS[2], F. PERUCCINI-FARIA[3], M. MONTERO-ODASSO[3], R. BARTHA[1], C. ANNWEILER[2];
[1]Robarts Res. Inst., London, ON, Canada; [2]Univ. of Angers, Angers, France; [3]Parkwood Inst., London, ON, Canada

Abstract: Introduction: Individuals with mild cognitive impairment (MCI) demonstrate poorer dual-task gait performance compared to cognitively healthy individuals (CHI). Dual-task gait involves simultaneously performing a motor task and an attention-demanding task. Dual-task

gait aids in distinguish between MCI subtypes, which some authors refer to as the “Motor Signature of MCI”. However, the neural subtrates of dual-task gait control across the cognitive spectrum remains poorly explored. Objective: This study aimed to investigate the structural morphometric changes underlying dual-task gait in older adults, with a specific focus on individuals with MCI. Additionally, we sought to examine the structural brain changes associated with different types of tasks during gait. Methods: A cross-sectional study was conducted, recruiting 337 participants from the French GAIT (Gait and Alzheimer Interactions Tracking) cohort, including 122 CHI, 168 MCI, and 47 individuals with dementia. Voxel-based morphometry was employed to measure brain gray matter volume (GMv). Gait speed was assessed under two dual-task conditions: counting backward and naming animals. Linear regression models, adjusted for confounding factors, were utilized to evaluate the relationship between gait performance and GMv. Results: The results revealed a positive relationship between GMv and gait speed during counting backward in the bilateral medial frontal gyri across the entire population. However, no association was observed between GMv and gait speed in either dual-task condition for CHI or those with dementia. In the MCI group, a significant cluster of GMv in the prefrontal cortex showed a positive association with gait speed in both dual-task conditions. Furthermore, gray matter atrophy in the bilateral temporal region was positively associated with slower gait speed during the naming animal task. Conclusion: These findings suggest that individuals with MCI exhibit specific patterns involving the prefrontal cortex under dual-task conditions. The naming animal task poses a more complex cognitive challenge for these individuals and is associated with a broader range of brain regions. Overall, this study enhances our understanding of the "Motor-Cortical Signature of MCI." We emphasize the potentially affected areas and the fundamental importance of interventions combining dual-task cognition and mobility training to prevent progression to dementia.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.12/II3

Topic: E.06. Posture and Gait

Title: Temporal coordination of head, trunk, and feet during 360 degree turning in Parkinson’s Disease

Authors: *P. BURGOS[1], F. B. HORAK[3], C. BATISTA[5], P. CARLSON-KUHTA[4], A. RAGOTHAMAN[1], V. V. SHAH[2], M. MANCINI[2];
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Abstract: Background: Most people with Parkinson's Disease (PD) have their first fall, due to altered postural control, within the first three years 3. The activity where people with PD have the most falls is during turning, and Turning is involved in about 40% of the daily steps. However, turning is less studied than straight-ahead gait or balance. There is disagreement about temporal coordination of the head, trunk, and legs during turning in healthy controls and people with PD because of methodological differences in the degree of turning, the cues used to start the turns, and subject characteristics. The purpose of this study is to describe different strategies of temporal coordination of the head, trunk, and feet in people with PD. **Methods:** 47 individuals with idiopathic PD (age=67±7 years, disease duration=8±5 years, MDS-UPDRS-III=32±10 score, MoCA=26±2 score) were assessed in the ON-medication state. The task was to turn 360 degrees for 1 minute, alternating right and left turnings with steps.The turning strategies were measured with 5 inertial sensors (head, sternum, lumbar, right foot, and left foot). Using gyroscope signals, particularly the axis of body rotation in the transverse plane, we first estimated the period of motion of each segment using a threshold of the angular velocity of 0.6 rad/seg. Second, we selected when the head, sternum, and lumbar segments rested (combined resting). Third we computed the onsets of all segments after the combined resting periods (Figure 1). Finally, we estimated descriptive statistics for turning with simultaneous onsets of the head, sternum, and lumbar segments ("en bloc" turning, with onsets less than 40 ms for each segment), and for the axial segment that was leading turnings without "en-bloc" strategy. **Results:** Only 13/47 subjects did not have "en-bloc" turning. The other 34 participants averaged 46% of their trials with an "en-bloc". The Head led the turning motion in 16 people, the Lumbar segment led in 11, the Sternum in 2, and another 5 did not have a clear preference. On average, the percentage of trial duration segments led was, 36±20%, 24±13%, and 37±18% for the Head, Sternum, and Lumbar segments, respectively, revealing a high variability. The onset of left versus right foot stepping was symmetrical (<60%) in 19 participants; 12 preferred starting with the Right foot and 16 with the Left. **Conclusions:** Most participants with PD used an "en-bloc" turning strategy and preferred to start turning with one foot. The cephalo-caudal or cauda-cephalic strategy varied among the participants, presenting a similar proportion of head versus lumbar leading the 360-degree turns.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.13/Web Only

Topic: E.06. Posture and Gait

Support: PRSS-FRSQ

Title: Aging increases the attentional demand of proprioceptive processing in sedentary seniors

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Abstract: Evidence suggests that postural instabilities and falls in seniors are associated with both decline in proprioceptive and cognitive functions. This study aims to explore the interaction between ankle joint proprioception and attentional resources in sedentary seniors. Old (n=40) and young (n=19) sedentary adults were tested in a dual-task paradigm involving a proprioceptive position-matching and a cognitive-attentional subtraction task (n-3). These tasks were performed both alone (single task) and simultaneously (dual-task). Participants reproduced four angles encoded via a passive ankle reference movement in dorsiflexion or plantarflexion (10^0 and 15^0) with their ipsilateral ankle in absence of vision. In the dual-task condition, the cognitive task was simultaneously performed during the reference movement. A motion analysis system (Polhemus, *Innovation in Motion™*) combined with an automatized software allowed the presentation of target angles as well as the recording of the matching performance. Seniors showed larger absolute matching errors compared to young adults in both single and dual-task conditions in the dorsiflexion ($p<0.05$), but not plantarflexion direction. This result is likely due to reduced maximal range of motion in seniors. Importantly, the between group difference in absolutes errors made in dorsiflexion was larger in the dual-task condition ($p<0.05$). Hence, while seniors significantly degraded their ankle proprioceptive accuracy when simultaneously performing the cognitive task ($p<0.05$), the proprioceptive performance of young adults remained unaffected. Furthermore, although the cognitive performance of old and young adults was similar in the single task condition, the cognitive performance of seniors significantly deteriorated during dual-tasking ($p<0.05$). Our findings indicated that increasing the attentional demand during an ankle proprioceptive task compromises both the proprioceptive accuracy as well as the ability of seniors to perform an easy cognitive task. Such results demonstrate that the sensorimotor and cognitive processes involved in this ankle position-matching task are highly sensitive to the aging process. Understanding the interplay between ankle proprioception and cognitive-attentional resources is crucial to design interventions aimed at improving mobility and reducing fall risk in seniors.

Disclosures: M.J. Vermette: None. **E. Paré:** None. **J.Y. Messier:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

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Topic: E.06. Posture and Gait

Support: NIH NICHD F31HD108988 (Spencer)
NIH NICHD 1R01HD095975 (Kesar)

Title: Evaluation of Acute Training Induced Improvements in Gait Biomechanics and Sensorimotor Pathway Excitability in Individuals Post-stroke

Authors: J. SPENCER, T. LEONE, A. LOPEZ, A. SLUSARENKO, A. GREWAL, *T. KESAR;
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Abstract: Two-thirds of stroke survivors experience long term residual gait deficits due in part to variation in individual treatment response, such that even effective interventions with positive net impact have a large proportion of non-responders. Fast treadmill training (Fast) is one such evidence-supported post-stroke gait intervention. Preliminary research has indicated that the combination of Fast and functional electrical stimulation (FastFES) may be a more targeted and effective treatment than Fast alone, but FastFES may not suitable for all post-stroke individuals. Here, our premise was that measurement of acute changes in neuromotor excitability immediately following Fast and FastFES will elucidate the neural mechanisms involved in locomotor learning for each treatment, allowing for more targeted and personalized intervention selection for post-stroke individuals. Here, we compared the short-term effects of 1 session of Fast versus FastFES on the excitability of two key motor pathways: descending corticomotor pathways using transcranial magnetic stimulation (TMS) induced motor evoked potentials (MEPs), and the spinal reflex arc using Hoffman's (H-) reflexes. We hypothesized that changes in MEP and H-reflex will be greater in the FastFES group than the Fast Group, and that baseline values and acute training-induced modulation of cortical and spinal excitability will be related to training-induced locomotor learning of improved gait biomechanics.Preliminary data to date from 3 participants randomized to Fast and 1 participant randomized to FastFES demonstrate a reduction in MEP amplitude immediately following 1 session of Fast training(mean= -0.26±0.18mV), and a negligible change following FastFES. Additionally, participants in the Fast training group displayed acute increases in (Hmax/Mmax ratio) following training (mean= 6.2±0.14%). Additional data-collection and analysis is ongoing. By combining these acute training-induced changes with longer-term data (12 training sessions) from this cohort, the resultant insights can inform more personalized and precise intervention selection and enhance treatment effectiveness.

Disclosures: J. Spencer: None. **T. Leone:** None. **A. Lopez:** None. **A. Slusarenko:** None. **A. Grewal:** None. **T. Kesar:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.15/II5

Topic: E.06. Posture and Gait

Title: Balance mechanisms used during visual perturbations change with aging

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Abstract: Falls during walking are a leading cause of injury for older adults. In order to send the appropriate efferent signals to control balance, the brain integrates sensory cues from three systems: visual, vestibular, and proprioceptive. After a sensory perturbation, the body engages a combination of mechanisms (i.e., lateral ankle roll, step placement, and push off) to maintain balance while walking. Previous studies indicate that older adults rely more on their visual system than younger controls to maintain balance. In this study, we aimed to understand how a visual perturbation may affect the contributions of the three balance mechanisms to the center of mass (CoM) trajectory in older adults (OA) compared to younger adults (YA). We hypothesized that all three balance mechanisms would contribute to a larger CoM excursion following a visual stimulus. Fifteen older adults (OA) and fifteen younger adults (YA) with no history of neurological or orthopedic impairments walked through a virtual reality environment on an instrumented treadmill for ten 2-minute walking trials. Perceived falls were induced by rotating the visual field 45°/sec^2 for 600ms at randomized heel strikes. Kinetic and kinematic data was collected using motion capture and an instrumented treadmill. Surface electromyography sensors were placed on the muscles of the lower limbs. All data was processed using a custom MATLAB script. The 8 steps following each perturbation were used in this analysis. As expected, the OA group had larger CoM displacement following the visual perturbation than the YA group. This larger response for older adults was primarily modulated through a larger ankle roll response and larger mediolateral step placement. Increased plantar flexion and gastrocnemius activation were also observed. These results suggest that a visual disturbance leads to an increased response in 2 of the 3 balance mechanisms in older adults, resulting in a larger CoM excursion. The 3rd mechanism (i.e., push-off) does not play a role.

Disclosures: J. Gray: None. **M. Arcodia:** None. **A. Sansare:** None. **H. Reimann:** None. **J.J. Jeka:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.16/II6

Topic: E.06. Posture and Gait

Title: Evaluating the Effect of Stochastic Resonance on Gait, Balance, and Proprioception: A Feasibility Trial

Authors: H. IKENBERRY[1], O. ROLIN[2], ***V. CHU**[1];
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Abstract: Stochastic Resonance (SR) is defined as the addition of random noise to enhance a system's sensitivity to weak stimuli. It is theorized that the presence of a low level of noise can

facilitate weak sensory input to be detected. Cerebral Palsy (CP) is caused by a nonprogressive neurological lesion to the developing brain, affecting sensorimotor function. The purpose of this study is to evaluate the feasibility of using SR to improve gait, balance, and proprioception in individuals with CP. The first objective is to determine the optimal position and level of SR stimulation (SRS). The second objective is to explore the use of wearable SRS for improving gait, balance, and proprioception.14 participants (mean = 17 years; 4 - 54 years) were recruited from VCU Children's Hospital of Richmond. Each participant wore 3 SR devices (Accelera, Inc.), 1 in the lower back (sacrum), and 2 on the lower limbs. The location of the lower limb devices was determined using gait analysis by their physician. Vibration detection threshold at each SR location was determined. Timed Up and Go (TUG) test was performed at sub- and supra-threshold SRS (60%, 90%, 120%). The SRS level with the best TUG performance was used as the SRS level for the other gait, balance, and proprioception outcomes. Each participant completed 3 trials (baseline, SRS, sham, random order) of One Minute Walk test, Lower Extremity Kinesthesia test, and Static Load Perception test.All participants completed TUG trials to determine optimal SRS level. Half of the participants (n = 7) had lower limb SR device placement on bilateral ankles, 4 participants on bilateral knees, and 3 participants ipsilateral knee and ankle. The majority of the participants (10/14) had the best TUG performance with SRS at 90% of threshold, 3 at 60% of threshold, and 1 at 120% of threshold. The average time to complete TUG was 12.18s (baseline) and 11.72s (SRS), though the difference did not reach statistical significance ($p>0.05$). We saw slightly increased walk speed and cadence with SRS, but the difference was not statistically significant. No statistically significant difference in static load perception and lower extremity kinesthesia test was observed.We found preliminary evidence suggesting improvements in gait with SRS. There was little difference in balance and lower extremity proprioception with SRS. Fatigue, attention-span limitations, and SR device being audible may have impacted performance. Accurate determination of SR threshold in young participants (4-5 yo) was also challenging. Future research should consider reducing the auditory effect of the SR device, enrolling a larger sample, and special considerations for younger participants.

Disclosures: H. Ikenberry: None. **O. Rolin:** None. **V. Chu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); SR wearable devices used in the study was provided by Accelera, Inc..

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.17/II7

Topic: E.06. Posture and Gait

Support: NSF Career Award 14847891
Pittsburgh Pepper Center P30AG024827
University of Pittsburgh Momentum Fund 3455

Title: Locomotor flexibility for community mobility of older adults: the role of gait automaticity

Authors: ***S. LIU**[1,5], A. L. ROSSO[2], E. M. BAILLARGEON[3], A. M. WEINSTEIN[4], G. TORRES-OVIEDO[1,5];
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Abstract: Community mobility requires gait automaticity, automatic control of walking without recruiting attentional resources mediated through the prefrontal cortex (PFC), and locomotor flexibility: learning movements when interacting with a new environment, remembering the learned movements, and switching between different walking patterns. Gait automaticity and locomotor flexibility both decline with aging, which may contribute to the increased risk of falls and reduced mobility in older adults. Locomotor adaptation training using split-belt walking (SBW), where the two legs move at different speeds, improves locomotor flexibility in older adults, but the underlying mechanisms of these improvements are unknown. We hypothesized that improvements in locomotor flexibility from training would be positively associated with baseline gait automaticity. To test this, 22 participants (>= 65 y/o) experienced 3 sessions of SBW. Locomotor flexibility was quantified by 1) early perturbation, measured by step length asymmetry (SLA) upon starting SBW, 2) adaptation rate, measured by the number of strides to reach steady state walking on split-belt, and 3) switching, measured by SLA overground after transitioning off the newly learned SBW. Gait automaticity was assessed before SBW sessions via dual-task (DT) walking (walking and reciting every 3 letters of the alphabet) and quantified by the automaticity index (a combination of performance and PFC activation assessed using functional near-infrared spectroscopy). We found that all three parameters of locomotor flexibility (perturbation, rate, switching) improved significantly after training ($p<0.001$). Contrary to our expectation, we found that good automaticity was correlated with less improvements in the early perturbation ($\rho=-0.50$, $p=0.02$), and not correlated with improvements in adaptation rate or switching. Interestingly, more reduction in performance (more slowing down and less correct number of alphabet generated) from single- to dual-task walking was correlated with better switching ($\rho=0.46$, $p=0.03$) at baseline. Contrary to our initial hypothesis, our findings suggest that those with poor gait automaticity may use an attentional strategy and recruit their PFC resources during both normal and challenged walking, potentially facilitating greater learning from training. In sum, repeated exposure to SBW improves locomotor flexibility in older adults, and gait automaticity is inversely related to improvements in early perturbation and baseline switching. These results suggest that gait automaticity and PFC involvement are important neural mechanisms modulating locomotor flexibility.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.18

Topic: E.06. Posture and Gait

Title: Effect of Visual Perturbation Magnitude on Postural Adjustment in the Elderly

Authors: *D. KOCEJA[1], K. KITANO[2];
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Abstract: Upright static postural control has been widely studied. However, postural responses to the outside environment, such as anticipatory postural adjustment, are less clear. Previous studies which have addressed postural response to visual perturbation mostly applied periodical perturbations. The purpose of the current study was to investigate postural responses to a linear visual perturbation among the elderly. Seven participants were tested (mean age was 86.7 yrs.). All subjects were free of neurological movement disorders. Subjects were instructed to quietly stand upright on a force plate which was placed in an enclosed environment in order to limit the subject's visual surround. The size of the enclosure was approximately 70cm x 70 cm x 250 cm. While the subjects were standing (15 - 20 seconds) the walls of the enclosure was moved away from the subjects. Initiation of wall movement timing was randomized, and two types of wall velocities (slow and fast) were tested (velocity: 55.0 mm/sec and 101 mm/sec; distance: 87.6 mm and 153 mm, respectively). Force plate data was collected to capture center-of-pressure trajectories. Alpha level 0.05 was used for statistical tests. All subjects demonstrated forward sway in response to the visual perturbation. Mean latencies of the initial recovery movement were 742 msec for the slow condition and 431 msec for the fast condition, which were statistically significant. However, the response magnitudes (15.1 mm and 12.7 mm in slow and fast conditions, respectively) and velocities (52.8 mm/sec and 26.2 mm/sec in slow and fast conditions, respectively) were not statistically significant. In addition, there was a significant correlation between latency of recovery movement and initial wall velocities. It is, therefore, suggested that initiation of postural recovery movement induced by a visual perturbation is associated with perturbation magnitudes while distance and velocity of recovery movement were not related with magnitude of visual perturbation. Further study is recommended for more description and investigation between visual information processing and postural response.

Disclosures: D. Koceja: None. **K. Kitano:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.19/II8

Topic: E.06. Posture and Gait

Support: K. Lisa Yang Integrative Computational Neuroscience Postdoctoral Fellowship

Title: Locomotor control phenotypes in SHANK-3 mutant marmosets

Authors: *A. DE COMITE, W. MENEGAS, G. FENG, N. SEETHAPATHI;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Autism spectrum disorder (ASD) is a neurological developmental disability that is mainly diagnosed using cognitive and social tasks throughout development. Children with ASD can have a range of symptoms and some may exhibit locomotion characteristics that diverge from the neurotypical population. For instance, some patients have hindered coordination and use very wide steps when walking. Recently, genetic models of ASD across mice and NHPs have enabled mechanistic investigations of ASD, and can help discover treatment options. Here, we investigate whether naturalistic locomotor behavior is sufficient to identify the occurrence of ASD in genetically modified SHANK-3 mutant marmosets (found in ASD patients with Phelan-McDermid syndrome). We designed a task in which wild type (n=35) and SHANK-3 (n=15) marmosets, freely walked along a transparent tunnel while cameras located below captured video recordings of their movements during a 30 minute session. We used computer vision, trained on manually labeled frames, to extract the time series of the positions of their paws, head, and tail. We extracted multiple variables characterizing locomotor control: (i) the relative position and timing of successive contacts, (ii) the spatiotemporal coordination of the different paws, and (iii) the oscillation of the head-tail axis during gait. In total, 26 different variables were extracted for each animal, at different movement velocities to capture speed-dependent effects. We trained a Linear Discriminant Analysis (LDA) classifier to identify an animal group (wild type or SHANK-3) from these movement variables, and used this classifier to determine whether locomotor control contained sufficient information to discriminate the SHANK-3 and wild type populations. The classifier had an accuracy of more than 80% on the left-out dataset, which outperformed a similar classifier built on an artificial neural network trained on the raw marker data. Subsequent analyses revealed that the SHANK-3 mutant animals that had received gene therapy treatment have locomotor behaviors closer to wild type than untreated SHANK-3 animals. We were able to identify a-posteriori which movement variables are of particular interest to the SHANK-3 genotype, which could help investigate the neural underpinnings, and help compare to other mutant animals (e.g. comparison to ataxic mutants which exhibit comparatively gross locomotor deficits). Our results demonstrate that locomotor control can be studied as an ASD phenotype in nonhuman primate models, where social and cognitive phenotypes are more challenging to test and compare to humans.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

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Program #/Poster #: PSTR218.20/II9

Topic: E.06. Posture and Gait

Support: NICHD of NIH # F31HD110254 (Manczurowsky)

Title: Motor adaptation to muscular torque asymmetries during gait induced by closed-loop electrical stimulation during treadmill walking

Authors: *J. R. MANCZUROWSKY[1], T. CLINE[2], C. H. HILLMAN[3], C. J. HASSON[1]; [1]PTMRS, [2]Psychology, [3]PTMRS, Psychology, Northeastern Univ., Boston, MA

Abstract: A person after neurological injury, such as stroke, may seek rehabilitation to address gait impairments related to altered muscle force, decreased economy of ambulation, and concomitant pain. Therefore, it is important to guide clinical practice with a better understanding of locomotor adaptation by investigating how neuromotor impairments impact gait mechanics. This study evaluated how humans adapt their gait kinetics to a temporary locomotor impairment designed to mimic disordered neuromuscular control. Once per step during treadmill walking, 20 healthy college-aged volunteers experienced disrupted neuromuscular coordination and discomfort from dysfunctional electrical stimulation (DFES) applied to the right hamstring as the leg began to swing. We hypothesized that DFES would induce an asymmetry between participants' left and right hip and knee torques, and that the complex nature of the perturbation would prevent complete adaptation within one session. DFES was calibrated to produce 20 degrees of passive right knee flexion in standing and 5/10 discomfort on a visual analog scale. Participants were told to maintain symmetrical gait at their preferred walking speed in counterbalanced blocks with and without stimulation. OpenSim musculoskeletal modeling software was used to perform inverse dynamics to determine net joint torques of the lower extremities during leg swing. Maximum hip and knee flexor and extensor torques during leg swing were identified for each leg. The flexor and extensor torque asymmetry, i.e., the difference between these joint torque maxima for left vs. right leg, was used to assess the effect of DFES on kinetics and adaptation for early and late practice with DFES. The torque asymmetry was then compared to trials without DFES using paired t-tests. Hip torque asymmetry did not change with DFES, but knee asymmetry did. Early in DFES exposure (first 10 steps), the maximum knee extensor torque was reduced in the DFES leg ($p < .05$) as expected, showing that DFES created torque asymmetry. By late DFES exposure (last 10 steps after three minutes of walking), this knee extensor torque asymmetry disappeared, but an asymmetry for maximum knee flexion torque in early swing developed to become larger in the DFES leg compared to without stimulation ($p < .05$). Thus, even after a few minutes of walking with DFES, gait kinetics were still perturbed relative to normal. To conclude, we demonstrated electrical stimulation can induce an asymmetry in gait kinetics that is challenging to overcome, which may be useful for testing new motor adaptation hypotheses and informing future applications in rehabilitation.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.21/II10

Topic: E.06. Posture and Gait

Title: Does hip muscle performance during the weight transfer phase affect voluntary lateral stepping in people with chronic stroke compared to healthy older adults?

Authors: *S. LATEEF, M. B. LANZA, V. L. GRAY;
Physical Therapy & Rehabil. Sci., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Background: People with chronic stroke experience persistent lower limb sensorimotor deficits resulting in asymmetrical weight bearing, with a preference for the nonparetic leg, while standing and walking. Of particular concern is the effect of these asymmetries on hip abductor (ABD) and adductor (ADD) function while maintaining medio-lateral stability needed to prevent a fall. The purpose of this study was to examine the effects of stroke on weight transfer during a voluntary lateral step and whether muscle performance during weight transfer prior to the step influenced subsequent step characteristics. Approach: Twenty community dwelling individuals with chronic stroke (≥ 50 years) and 10 healthy controls were recruited for this study. Participants were instructed to take a voluntary lateral step as quickly as possible in response to a light cue. Performance during weight transfer in preparation for stepping was examined by measuring the ABD and ADD rate of muscle activation (RoA) and time taken to transfer weight prior to the step. Step performance was assessed by the time taken to initiate a step, step length, and step clearance. Statistical Analysis: The Kruskal-Wallis Test with the Dunn test for Pos-Hoc testing was used to examine group differences in outcomes between people with chronic stroke and healthy controls. Spearman Rho Correlations (ρ) were used to define the relationship between performance during the weight transfer phase and step characteristics. Results: People with chronic stroke had a reduced ABD and ADD RoA ($p < 0.01$) in both the paretic and nonparetic legs during weight transfer, spent more time in weight transfer ($p < 0.01$) and were slower to initiate a step ($p < 0.01$) compared to healthy controls. Time taken to initiate a step was strongly and positively correlated with time taken to transfer weight ($\rho = 0.80$, $p < 0.01$) and was moderately and negatively associated with stance leg ABD RoA ($\rho = -0.63$, $p < 0.01$) and ADD RoA ($\rho = -0.56$, $p < 0.01$). Conclusion: Muscle performance during the weight transfer phase influenced step characteristics, particularly the time taken to initiate a voluntary lateral step. People with chronic stroke are slow to initiate a voluntary lateral step compared to healthy controls and this is associated with increased fall risk while carrying out daily activities. Quicker ABD and ADD activation during weight transfer, along with less time spent in transferring weight, allowed participants to initiate a quicker step. These findings may have implications for balance, stability and fall risk and must be considered further when preparing people with chronic stroke for safe community ambulation.

Disclosures: **S. Lateef:** A. Employment/Salary (full or part-time):; University of Maryland, Baltimore. **M.B. Lanza:** A. Employment/Salary (full or part-time):; University of Maryland, Baltimore. **V.L. Gray:** A. Employment/Salary (full or part-time):; University of Maryland, Baltimore.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.22/II11

Topic: E.06. Posture and Gait

Support: ANR (XL)
INSERM (XL)

Title: Early Dopamine-dependent motor symptoms in a mouse model of Alzheimer's disease

Authors: M. C. MEDRANO[1], M. CARRENO-MUNOZ[2], C. MIGUELEZ[3], P. DE DEURWAERDERE[1], ***X. LEINEKUGEL**[4];
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Abstract: Prospective clinical studies have revealed that motor symptoms often precede cognitive symptoms in Alzheimer's disease (AD) by several years, and have been proposed as a complement to cognitive assessment to evaluate the risk of developing AD-related dementia at a later stage. In fact, it has been proposed that executive functions and motor ability are related and both get degraded in parallel during aging and AD. But possibly due to more elaborate compensatory mechanisms for cognition than for motor function, cognitive deficits might remain difficult to detect and diagnoze at the clinical level until a very advanced stage of neuronal impairment. Identifying quantitative motor markers of preclinical dementia is a promising approach that may lead to new insights into early disease stages, improve diagnostic assessment and identify new preventive strategies. In this work, we have investigated the expression of motor functions in mouse models of AD. Using an innovative phenotyping device allowing to detect the finest animal movements in non-invasive behavioural conditions (up to individual heart beats of a resting mouse), we now report that AD mice (3xTgAD, APP-PSS1) express shaking (80-130Hz invisible body movements) as early (P21) motor symptoms, related with a localized deficit of dopamine (DA) in the striatum, identified using HPLC. Causality between DA-deficit and shaking was established by (i) reproducing the symptoms with DA lesions (6-OHDA) in WT mice and (ii) rescuing the symptoms in 3xTg and APP-PS1 mice with L-DOPA. These results suggest that shaking may be used as a very early marker of AD, and that DA-rescue may be an interesting therapeutical strategy against early AD symptoms.

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Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.23/II12

Topic: E.06. Posture and Gait

Title: Relationship between sensorimotor network and visual reliance during gait in Parkinson's disease

Authors: *M. ARCODIA[1], J. GRAY[2], R. G. BURCIU[2], J. J. JEKA[2], H. REIMANN[2]; [2]Kinesiology & Applied Physiol., [1]Univ. of Delaware, Newark, DE

Abstract: Individuals with Parkinson's disease (PD) rely heavily on visual cues during walking. Visual information is crucial for maintaining balance however much is still unknown about quantifying visual reliance during walking in older adults and those with PD and the underlying neural connectivity related to it. Our study aims to 1) quantify visual reliance using frequency response function (FRF) gains, 2) use resting state functional magnetic resonance imaging (rs-fMRI) to understand the relationship between regions within the sensorimotor network and 3) determine the relationship between visual reliance during gait and functional connectivity strength of the sensorimotor network in individuals with PD. We hypothesized that individuals with PD would have; 1) larger FRF gains than older adults without PD, 2) greater functional connectivity strength between regions within the sensorimotor network, and 3) would have a negative association between their FRF gains and functional connectivity z-scores. A pilot cohort of 5 individuals with PD and 5 controls performed rs-fMRI imaging and a treadmill walking assessment. Individuals with PD were tested in their ON medication state. For the walking assessment, participants walked on an instrumented treadmill in a virtual reality environment. Balance was perturbed by a visual stimulus where the virtual environment continuously rotated in the frontal plane in a pseudorandom pattern composed of 10 sinusoids. Participants performed ten trials of 125 s each. We analyzed rs-fMRI data using an independent component analysis followed by dual regression to extract z-scores which represent functional connectivity strength. We chose the sensorimotor network a priori as our network of interest. We analyzed walking data by FRF gains between the visual stimulus and the whole-body center of mass movement in the medial-lateral direction. Interestingly, results show that older adults responded more strongly to the visual stimulus and had higher functional connectivity z-scores between the sensorimotor network and the right superior temporal gyrus (STG) than those with PD. When considering data from both groups, we observed a tendency towards a positive association between FRF gains and the strength of functional connectivity between the sensorimotor network and the right STG. Previous studies show the right STG is involved in integrating visual information and is connected to regions of the basal ganglia, a key region to PD. This study further supports the association between the sensorimotor network and the right STG, as those with PD have reduced coupling between these areas and reduced visual reliance.

Disclosures: M. Arcodia: None. **J. Gray:** None. **R.G. Burciu:** None. **J.J. Jeka:** None. **H. Reimann:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.24/II13

Topic: E.06. Posture and Gait

Support: Mangurian-Fixel-McKnight Grant

Title: Pallidal activity during split-belt walking adaptation in Parkinson's disease with freezing of gait

Authors: *J. CHOI[1], J. K. WONG[1], C. DE HEMPTINNE[1], D. D. WANG[2];
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Abstract: INTRODUCTION: Patients with Parkinson's disease (PD), especially those with freezing of gait (FOG), show walking deficits that worsen in situations requiring gait adaptations (e.g., turning, obstacle crossing). In PD patients treated with deep brain stimulation (DBS) of the globus pallidus (GPi), significant increase in step length and gait velocity, and improvements on clinical symptoms (i.e., postural instability and gait difficulties) have been reported. However, our understanding of the pallidal physiology that underlies gait adaptation in PD with FOG remain limited. METHODS: Here we used a split-belt treadmill to study locomotor adaptation in two PD patients with FOG. Both patients have chronically implanted bilateral GPi DBS with Medtronic Percept PC stimulator. GPi local field potentials (LFPs) were recorded wirelessly from both patients in the DBS OFF condition during walking. Power spectrum of LFP signals were calculated for the following experimental epochs: slow and fast baselines (tied-belts), pre-adaptation (tied-belts slow), split-belt adaptation (right side fast, left side slow), and post-adaptation (tied-slow) walking. RESULTS: Split-belt walking increased spatiotemporal gait asymmetries during adaptation. Step length was adapted toward symmetry, but double support time remained asymmetric during the adaptation period in both patients. Compared to pre-adaptation, theta power (4-8 Hz) decreased, and alpha power (8-12 Hz) increased in the left GPi (contralateral to the 'slow' leg) during post-adaptation. There was also an increase in beta power in the left GPi during adaptation. CONCLUSIONS: The novel findings from this study are that 1) PD patients with FOG who can adapt step length symmetry may still have difficulty adapting gait timing during split-belt treadmill walking, and 2) GPi oscillations across different frequency bands are modulated both during and after split-belt walking adaptation.

Disclosures: J. Choi: None. **J.K. Wong:** None. **C. de Hemptinne:** None. **D.D. Wang:** Other; Consultant for Medtronic, Boston Scientific, Insightec, Iota Bioscienes.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.25/II14

Topic: E.06. Posture and Gait

Support: NSF Grant 2001222

Title: A cognitive dual task suppresses corticospinal modulation during walking adaptation in healthy older adults

Authors: *M. MULVEY[1], J. T. CHOI[2];
[2]Univ. of Florida, [1]Univ. of Florida, Gainesville, FL

Abstract: Introduction: Corticospinal drive plays an important role in adaptive control of locomotion that requires precise muscle activation patterns. In addition, the frontal lobe, which is important for executive function and attention, has been implicated in age-related decline in gait function. However, the extent to which divided attention directly interacts with corticospinal drive during adaptive walking is unknown. We hypothesize that in older adults, a cognitively demanding dual task would 1) decrease corticospinal drive resulting in lower intramuscular coherence in the beta-gamma frequency range (13-60 Hz) during walking adaptation, and 2) decrease kinematic adaptation resulting in greater spatiotemporal gait asymmetries. **Methods:** Healthy older participants (n=9, 71.2 ± 10.4 years) walked on a split-belt treadmill, where 2 separate belts moved at different speeds (i.e. 2:1 speed ratio) with and without a concurrent dual task. For the dual task, participants counted backwards by 7s as quickly and accurately as possible, which requires attention and working memory. Beta-gamma coherence was calculated between electromyography recordings from the proximal and distal ends of the tibialis anterior muscle on each leg. Spatial and temporal walking adaptation were assessed by calculating changes in step length symmetry and double support symmetry, respectively. **Results:** Preliminary data showed a significant increase in beta-gamma coherence in the slow leg during the transition from tied-belt to split-belt walking for the single task but not the dual task (condition*epoch interaction effect, p=0.014). Additionally, the dual task altered both step length symmetry and double support time symmetry during adaptation, as evidenced by a significant effect of condition (p=0.011 and p=0.049, respectively). **Conclusion:** The serial 7s task suppressed corticospinal modulation during split-belt walking adaptation in older adults, inhibiting the typical increase in beta-gamma coherence during early adaptation that is normally seen in the single-task condition (Sato & Choi, 2022). Interestingly, the dual-task condition also caused less asymmetry in both the temporal and spatial kinematics during initial adaptation, indicating that the addition of a dual task caused the older adults to subconsciously adopt a more cautious gait and become less perturbed.

Disclosures: M. Mulvey: None. **J.T. Choi:** None.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.26/II15

Topic: E.05. Brain-Machine Interface

Support: NIH-R01NS130183 (R01)
MJFF-010435 (MJFF)

Title: Neurophysiology of Deep Brain Stimulation for Modulating Gait Functions in Individuals with Parkinson's Disease

Authors: *H. FEKRI AZGOMI, K. LOUIE, J. BATH, J. BALAKID, D. WANG;
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Abstract: Deep brain stimulation (DBS) has shown tremendous potential in mitigating many symptoms of Parkinson's disease (PD), such as tremors and bradykinesia. However, the treatment of advanced gait-related problems is still a complex challenge with variable responses to DBS and a range of parameters that can be adjusted, including stimulation frequency, amplitude, and pulse width. In this research, we investigate the impacts of changes in DBS parameters on the basal ganglia thalamocortical network neurophysiology and analyze their influence on gait functions. We further develop a data-driven approach to derive individually optimized DBS configurations for improving the gait functions. Here, we recorded local field potentials (LFPs) from the globus pallidus internus (GPi), premotor (PM), and motor (M1) cortex of three subjects with PD. They were all implanted with a bidirectional, investigational DBS device (Summit RC+S, Medtronic). Gait kinematic measurements were captured during over-the-ground walking under different stimulation conditions (i.e., gait speed, stride length, double support time, and arm swing amplitudes). We uncovered the stimulation settings that, on average, increased their gait speed by 17.98%. These enhancements in their gait speed were achieved along with a 29.49% increase in their arm swing amplitudes. By conducting a comprehensive analysis of LFP power fluctuations across discrete gait phases, we identified the prominent frequency band powers that are associated with improved gait parameters. These include a 34.36% increase in PM delta amplitude and a 29.34% increase ($p<0.01$) in M1 theta amplitude during the contralateral leg swing period in patients 1 and 2, respectively. In patient 3, the DBS setting parameters associated with faster gait speed resulted in a 13.13% increase ($p<0.01$) in theta amplitude during double support time. Our results support the hypothesis that different stimulation parameters alter pallidal-motor circuit activities associated with the changes in gait kinematics. By employing the Gaussian process regressor, we generated personalized maps of the relationship between DBS settings and gait kinematics for each subject, informing the stimulation settings that would result in optimal gait metrics.

Disclosures: H. Fekri Azgomi: None. **K. Louie:** None. **J. Bath:** None. **J. Balakid:** None. **D. Wang:** F. Consulting Fees (e.g., advisory boards); consultant for Medtronic, Boston Scientific, Insightec, Iota Biosciences.

Poster

PSTR218. Posture and Gait: Aging, Injury, and Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR218.27/II16

Topic: E.06. Posture and Gait

Support: DoD Grant PD200023

Title: Alpha and theta activity in the supplementary motor area correlates with gait benefits from auditory cues in Parkinson's Disease and their healthy counterparts.

Authors: *L. MA, I. TALU, K. A. CROSS;
Neurol., UCLA, Los Angeles, CA

Abstract: Auditory cues such as metronome have been shown to improve PD gait by increasing stride length and gait speed and reducing step variability. However, it is unclear what cortical mechanisms are driving the patient's response to auditory cues, and better insight into these mechanisms will guide individualized treatment strategies for PD. Considering that the supplementary motor area (SMA) is linked to beat maintenance and sensorimotor synchronization, we hypothesized that SMA responses would be related to behavioral gait changes in response to auditory cues. Twenty-five PD patients and nine healthy controls walked overground in a clinic with a mobile 64-channel EEG and eight internal measurement units (IMU) to record cortical and kinematic gait behavior. Subjects walked under two conditions: normal gait on a pre-determined path (uncued) and synchronized steps to auditory metronome-delivered cues on the same path (cued). Mean stride length, gait speed, and cadence variability were extracted from IMU data. Electroencephalography (EEG) data were segmented into individual gait cycles to examine Event-Related Spectral Perturbation (ERSPs) across gait cycles between cued and uncued conditions across 3 to 80Hz. Brain-related independent components (IC) extracted from the EEG channels were repeatedly clustered to identify ICs that are the closest to the SMA. Linear mixed model testing on the kinematics variables with subjects as the random factor revealed significant group and condition effects for stride length and gait speed ($p < 0.001$) and condition effect for cadence variability ($p = 0.005$). No interaction effects were present for the kinematic variables. Permutation testing with cluster correction was performed on the ICs near the SMA, revealing significantly increased 4-13Hz power activity before, during, and after toe-off and decreased 4-13Hz power during the mid to late swing phase from the null ($p < 0.05$). To account for individual differences, the degree of changes between uncued to cued conditions within these time and frequency periods were correlated with the change in stride length, walking speed, and cadence variability across all subjects with FDR correction for the multiple comparisons. Results show higher 4-13Hz gait cycle modulation is significantly correlated with decreased stride length ($p = 0.04$), decreased gait speed ($p < 0.01$), and increased cadence variability ($p = 0.03$). That is, lesser 3-14Hz SMA activity is associated with greater gait improvement with auditory cues across subjects. This suggests that SMA may play a role in modulating gait behavior to auditory cues and that PD's SMA response is similar to their healthy counterparts.

Disclosures: L. Ma: None. **I. Talu:** None. **K.A. Cross:** None.

Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.01/II17

Topic: E.06. Posture and Gait

Support: K01HD100588
1R01HD095975-01A1
5U01NS086607-05
U01NS166655
1U01NS102353-01

Title: Reduced Inhibition From Quadriceps Onto Soleus After Acute Quadriceps Fatigue Suggests Golgi Tendon Organ Contribution To Heteronymous Inhibition

Authors: *C. CUADRA, S. WOLF, M. LYLE;
Emory Univ., atlanta, GA

Abstract: Heteronymous inhibition between lower limb muscles is attributed primarily to recurrent inhibitory circuits, but these effects could also arise from Golgi tendon organs (GTOs).Differentiating between these possibilities is difficult, because in humans the heteronymous effects are elicited by stimulating peripheral nerves or the muscle belly above motor threshold, procedures that can activate both inhibitory pathways. Stimulation elicits recurrent inhibition from antidromic propagation along motor axons acting on Renshaw cells, whereas GTOs are activated mechanically due to muscle contraction and by electrical activation of Ib sensory axons.Here, we used acute muscle fatigue to decouple recurrent from GTO inhibition when evaluating heteronymous inhibition from quadriceps muscle belly stimulation onto ongoing soleus (SOL) EMG. Since acute muscle fatigue from repeated muscle stimulation of quadriceps causes a reduction in twitch evoked force (i.e. decreased GTO activation) when using the same pre-fatigue stimulation current (i.e. same antidromic recurrent inhibition input), we hypothesized that a reduction in heteronymous SOL EMG inhibition after quadriceps fatigue could be attributed to reduced mechanical activation of GTOs.In 15 participants, heteronymous inhibition was quantified as a reduction in ongoing SOL EMG by stimulating the quadriceps muscle belly at several intensities (1.5-2.5x motor threshold) while producing ~20% SOL MVC isometric contraction. The quadriceps stimulation evoked twitch forces (i.e. GTO mechanical activation) were quantified by recording knee torque at each of the stimulation intensities. Then, a quadriceps stimulation fatigue protocol was used until twitch torques became attenuated by ~50%; heteronymous inhibition was quantified with the same pre-fatigue muscle belly stimulation current intensities. Heteronymous inhibition was larger for higher muscle belly stimulation intensities (e.g. 2.5 vs 1.5 x motor threshold). Knee extension torque after the fatigue protocol was reduced, and heteronymous inhibition from quadriceps stimulation onto ongoing SOL EMG was reduced by ~30% across stimulation intensities after fatigue. Our data support the role of GTO feedback as a contributor to heteronymous inhibition. This work provides new insights into the differential contribution of GTO and recurrent inhibitory feedback.

Disclosures: **C. Cuadra:** None. **S. Wolf:** None. **M. Lyle:** None.

Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

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Program #/Poster #: PSTR219.02/II18

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783
VA Merit I01 CX001812
NYS SCIRB C32236GG and C32279GG

Title: Operant Conditioning of the Flexor Carpi Radialis H-Reflex in Healthy Controls

Authors: *J. A. BRANGACCIO[1,2], J. J. S. NORTON[3], D. E. GEMOETS[4], H. MOJTABAVI[5], T. M. VAUGHAN[6], J. CARP[7], J. WOLPAW[8];
[1]Res., NCAN/Stratton VA Med. Ctr., Albany, NY; [2]Natl. Ctr. for Adaptive Neurotechnologies, [3]NCAN/Albany Stratton VAMC, Albany, NY; [4]Albany Stratton VAMC, Albany, NY; [5]Natl. Ctr. for Adaptive Neurotechnologies Stratton VAMC, Albany, NY; [6]Stratton VA Med. Ctr., Stratton VA Med. Ctr., Albany, NY; [7]Natl. Ctr. For Adaptive Neurotechnologies, Albany, NY; [8]Natl. Ctr. for Adaptive Neurotechnologies, Delmar, NY

Abstract: H-reflex operant conditioning is a new targeted-plasticity therapy that can enhance recovery of motor function following neurological illness or injury (Thompson et al., J Neurosci, 2013). The patient is operantly conditioned to decrease (or increase) the size of an abnormally large (or small) H-reflex in a muscle important in a motor skill (e.g., locomotion or reach-and-grasp). Normalizing the reflex can lead to much wider beneficial plasticity that improves the performance of other muscles and enhances functional recovery. H-reflex operant conditioning is non-invasive, has no known adverse side effects, and can complement existing therapies (Norton & Wolpaw, COBS, 2018). Previous investigations of H-reflex operant conditioning have focused on the soleus and rectus femoris muscles in the leg (e.g., Thompson et al., J Neurosci, 2009; Thompson et al., J Neurosci, 2013; Kim et al.,Sci Reports, 2023). To extend this new therapy to the arm, we are studying operant conditioning of the flexor carpi radialis muscle (FCR). Our conditioning protocol is based on Thompson et al. (J Neurosci, 2009). Each participant completes 6 baseline and 24 conditioning sessions (3/wk over ~10 wks). In each session, the participant maintains a predefined level of FCR EMG activity while 225 FCR H-reflexes are elicited by transcutaneous electrical stimulation of the tibial nerve. Stimulus intensity is automatically adjusted to maintain a constant direct muscle response (M wave). During conditioning sessions, the participant receives immediate visual feedback after each H-reflex that indicates whether H-reflex size was below (for down-conditioning) or above (for up-conditioning) a criterion value. Fifteen healthy participants (8 down- and 7 up-conditioned) have completed the protocol. For each, we compared H-reflex size from the 6 baseline sessions to H-reflex size from the last 6 conditioning sessions. In 10 people (4 down, 6 up), the protocol was

successful: H-reflex operant conditioning led to a change in H-reflex size in the rewarded direction (two-sample ratio t-test; $p<0.05$). In the other 5 participants, H-reflex operant conditioning was not successful. The results indicate that FCR H-reflex operant conditioning is possible and is comparable in magnitude to soleus H-reflex conditioning. FCR H-reflex conditioning may extend the therapeutic use of this new therapy to impairments in arm function resulting from spinal cord injury, stroke, or other disorders.

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Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.03/II19

Topic: E.06. Posture and Gait

Support: Lockheed Martin Corporation

Title: Lower leg H-reflexes are modulated supra-spinally during split-belt locomotor adaptation

Authors: *O. REFY[1], B. BLANCHARD[1], A. MILLER-PETERSON[1], A. DALRYMPLE[2], A. ZARIPOVA[1], N. MOTAGHEDI[3], O. MO[1], Y. SHI[1], S. PANTHANGI[1], E. H. BEDOY[4], A. REINHART[1], G. TORRES-OVIEDO[4], H. GEYER[1], D. J. WEBER[2];
[2]Carnegie Mellon Univ., [1]Carnegie Mellon Univ., Pittsburgh, PA; [3]Car, Pittsburgh, PA; [4]Univ. of Pittsburgh, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The central nervous system exhibits a great capacity for acutely or chronically adapting locomotor patterns in novel environments. Split-belt treadmill studies provide a useful paradigm to induce and study adaptations in locomotor control. However, the focus in these studies has been to infer neurological changes from biomechanical parameters. Explicit probing of nerve response changes during split-belt adaptation has not been reported before. In this work, we seek to directly probe spinal reflex adaptation in novel locomotion environments. To this end, we recruited 14 subjects to walk repeatedly on a split-belt treadmill during repetitions of a fixed speed change sequence. We used a motion-capture system to measure the subjects' leg kinematics, a force plate sensor to measure ground reactions forces and a set of EMG electrodes to measure the muscle activity of major leg muscle groups. Stimulation pules (1ms duration) were delivered at mid-stance (around 50 +/- 5% of stance duration) at approximately 10-second intervals to measure H-reflex responses in the soleus and gastrocnemius muscles simultaneously. Participants were divided into three groups where we measured H-reflexes on the leg ipsilateral (ipsi group) and contralateral to the speed change (contra group). We show that H-reflexes are significantly modulated only ipsilaterally to the speed change, and the adaptation pattern correlates strongly with the typical step length asymmetry (SLA) pattern reported widely in the literature. In agreement with prior work on H-reflex conditioning [1-3], our results suggest that

the observed H-reflex modulation is not a byproduct but rather an underlying mechanism of split-belt locomotor adaptation. However, it is unclear whether such mechanism is of spinal or supra-spinal origins. To investigate the origin of such adaptation, we recruited an additional group of 7 participants and measured their H-reflexes under the influence of noisy galvanic vestibular stimulation (nGVS). We show that nGVS slows down the adaptation time of both the H-reflexes and SLA by 58%. Our result suggests that the observed reflex modulation is of supraspinal origin.
[1] Thompson, A. K. and Wolpaw, J. R. (2021). The Journal of Physiology, 599(9):2453-2469.[2] Wolpaw, J. and O'Keefe, J. (1984). The Journal of Neuroscience, 4(11):2718-2724.[3] Chen, Y., Chen, X. Y., Jakeman, L. B., Chen, L., Stokes, B. T., and Wolpaw, J. R. (2006). The Journal of Neuroscience, 26(48):12537-12543

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Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.04/II20

Topic: E.06. Posture and Gait

Title: The modulatory effect of estradiol on descending tracts projections onto spinal motoneurons

Authors: *Y.-C. CHUNG[1], **S. THOMAS**[2], S. SOEDIRDJO[4], L. MOHAMED[5], C. KUMALA[3], Y. DHAHER[6];
[1]UT Southwestern, Dallas, TX; [2]Biomed. Engin., [3]Biol. Sci., Univ. of Texas at Dallas, Dallas, TX; [5]Physical Med. and Rehabil., [4]Univ. of Texas, Southwestern Med. Ctr., Dallas, TX; [6]Physical Med. & Rehabil., UT Southwestern Neurosci. Grad. Program, Dallas, TX

Abstract: BACKGROUND AND AIM: Animal models show that estradiol (E2) receptors are present in the central nervous system, both the cortex (Kritzer, 2002) and spinal cord (VanderHorst, 2009). In humans, direct evidence is lacking, but hormone concentrations have been linked to brain conditions like seizure, cognitive function and emotional states (Toffoletto, 2014; Velíšková & DeSantis, 2013). In this study, we sought to explore the likelihood that E2 would modulate the interaction between descending tracts and the spinal network of interest using trans-spinal magnetic stimulation (TSMS). We hypothesized that the influences of descending tracts on spinal motor circuits would change with different E2

levels.**METHODS:**Twelve young females with regular menstrual cycles were tested at menses and peri-ovulation. TSMS was applied at the T12 spinal segment to condition motoneurons at a spinal segment distal to T12, S1. All three TSMS intensities applied at T12 did not evoke a motor response in muscles innervated by S1 (soleus muscle). We employed a paired-pulse stimulus paradigm, pairing the peripheral nerve stimulation on the posterior tibial nerve with T-12 TSMS. Repeated-measures ANOVA was used to compare the normalized h-reflex amplitudes, the ratio between conditioned and unconditioned-h reflex amplitudes, between menses and peri-ovulation. We focused our analysis on a specific set of inter-stimulus intervals (ISI, 10, 11, 12, 16, and 20 ms). Our choice of these ISIs was based on our earlier reported data suggesting that these ISIs likely correspond to the orthodromic transmission of descending motor tracts with specific inhibitory signatures at the 11 and 12 ms ISIs. **RESULTS:**Data obtained on the 12 young females showed that the suppressed H-reflex at the 11- and 12-ms ISIs did not differ significantly between menses and peri-ovulation. In 2 females, we employed the paired-pulse paradigm with refined ISIs between 10-14 ms with a resolution of 0.2 ms. The data from the 2 females revealed a consistent trend that a higher inhibition of conditioned h-reflex amplitudes was observed among the ISIs between 11-12 ms at peri-ovulation than menses.**CONCLUSIONS:**The preliminary results suggest that E2 may modulate the contribution of descending tracts to the spinal motor circuits associated with the soleus muscle, and the E2 effect may be tract specific. The data also suggest that the E2 effect seems to be expressed at shorted time scale (fraction of a millisecond). The findings are consistent with the reported E2 effect on auditory brainstem responses in females (Elkind-Hirsch, 1992) and needed to be confirmed with a larger sample size when it comes to the motors system at the spinal level.

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Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.05/II21

Topic: E.06. Posture and Gait

Support: NSERC Grant 2017-04504
NSERC Grant 2019-04513

Title: A cautionary note on the use of suprathreshold noisy tendon vibration

Authors: *G. ESCHELMULLER, A. SZARKA, B. GANDOSSI, M. G. CARPENTER, J. INGLIS, R. CHUA;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Noisy tendon vibration (NTV) is a technique that has been used to assess the frequency characteristics of muscle spindle reflex pathways. This technique involves vibrating

the muscle tendon with a noisy vibration, which consists of white noise bandpass filtered between ~10-100 Hz. NTV has been demonstrated to be an effective method to assess the frequency characteristics of muscle spindle reflex pathways during quiet standing without causing any major perturbations to standing balance. Tendon vibration is also known to affect the central nervous system (CNS) in a few different ways that may confound the use of NTV as a tool to assess the muscle spindle reflex pathways. One of the effects that is important to consider is that tendon vibration will suppress the excitability of the Ia afferent-alpha motoneuron synapse, resulting in a smaller reflex amplitude, which could bias the results obtained using NTV. Tendon vibration can also induce the kinesthetic illusion that the vibrated muscle is longer than it actually is, which could impact studies that require participants to hold certain positions. Most of the current research has focused on periodic vibration to assess these affects. Therefore, the purpose of the current work was to compare the effects of periodic and noisy tendon vibration on muscle spindle reflex function and kinesthesia. To assess the effects on the Ia afferent-alpha motoneuron synapse, wrist flexor stretch responses were compared between conditions with no vibration, periodic vibration (20 and 100 Hz), and noisy vibration (~20-100 Hz). Overall, the data indicate that both periodic and noisy vibration suppress the stretch response in a similar way, as both conditions produced a robust suppression of the short and long latency stretch responses. To assess the effects of noisy and periodic vibration on kinesthesia, participants made a series of wrist extension movements to a visual target without vision of the arm with no vibration, periodic vibration (20, 40, 60, 80, 100 Hz), and noisy vibration (~20-100 Hz). The results from this study indicate that both periodic and noisy vibration results in an undershooting of the target when compared to the no vibration condition. This is consistent with the typical effects seen with periodic tendon vibration. Overall, the data from these studies indicate that both periodic and noisy tendon vibration are causing similar effects on reflex function and kinesthesia. These data will be important to consider for the future use of the technique to assess muscle spindle reflex pathways and more work needs to be done to fully understand how these effects may be influencing the data obtained using NTV.

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Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.06/II22

Topic: E.06. Posture and Gait

Support: NSF RES515698//DBI-2015317

Title: Propriospinal commissural neurons of Lamina VIII contribute to postural movements in cats

Authors: *M. CHARDON[1], A. MAHROUS[1], M. JOHNSON[1], D. J. BENNETT[2], C. J. HECKMAN[1];
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Abstract: Little is known about the spinal circuits that control posture, even though they are necessary to initiate locomotion. In recent preliminary work, Bennett and colleagues have unexpectedly found that excitatory propriospinal commissural neurons (PSC neurons) that express the Sim1 transcription factor (V3 neurons) produce robust standing when they are optogenetically activated at 10 Hz, including in mice that are paralyzed after spinal cord injury (SCI). These V3 neurons may be ideally suited to maintaining posture: directly innervating extensor motoneurons throughout the limb and axial muscles, and possessing large persistent inward sodium currents (Na PICs) that prolong their output. Thus, V3 neurons seem to essentially bypass the central pattern generator (CPG) to robustly produce extensor tone. The ventral V3 neurons in particular form descending commissural tracts that innervate motoneurons, much like the previously described lamina VIII neurons in cat, as detailed by Jankowska et al (PSC neurons). Thus, our goal of this work was to examine whether cat lamina VIII PSC neurons are likewise involved in postural control in the decerebrate cat preparation. In the feline preparation an intercollicular decerebration was performed, as it is known to favor extensor tone and exhibit standing posture. Intracellular electrodes were advanced into lamina VIII neurons and identified as descending PSC neurons by antidromic stimulation of the contralateral ventral PSC tracts one segment caudal. We found that these PSC neurons fired steadily in the decerebrate state, and a spike triggered average of the contralateral ventral root demonstrated that they monosynaptically contributed to the extensor motoneuron tone in this cat preparation. Furthermore, PSC neurons were monosynaptically excited by sensory stimulation (tibial or common peroneal) that produced a very long lasting EPSP, with a remarkably similar long time course to that observed in mice V3 neurons. Repeated sensory stimulation (at 20 - 100 Hz) evoked markedly increased steady PSC neuron firing and motoneuron output associated functionally with a crossed extensor reflex. Finally, PSC neurons have axons that appear to bifurcate: sending one axon locally to ipsilateral motonerons and another across the midline, since antidromic activation of the contralateral axon evoked marked EPSPs in ipsilateral motoneurons; this provides a novel way to activate these PSC neurons that may be useful in promoting muscle tone. In summary, PSC neurons in cat fire spontaneously and provide extensive direct excitation to motoneurons, helping to support extensor tone, providing a new target to help restore posture after SCI.

Disclosures: M. Chardon: None. **A. Mahrous:** None. **M. Johnson:** None. **D.J. Bennett:** None. **C.J. Heckman:** None.

Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.07/II23

Topic: E.06. Posture and Gait

Support: K01HD100588
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1U01NS102353-01

Title: Inhibitory heteronymous reflexes circuits from quadriceps onto soleus are modulated by limb loading and task context

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Abstract: Heteronymous reflex circuits from quadriceps can influence lower limb movement coordination by changing the motor output of other muscles such as the soleus (SOL). Importantly, prior work suggests that the strength of spinal reflexes is purposely modulated by the nervous system based on loading, posture, and task context. For instance, compared to sitting, the magnitude of heteronymous excitation and inhibition from quadriceps onto soleus EMG was found to decrease during standing and in an unsupported squat position. However, the independent modulatory influence of limb loading, posture and task context remains unknown for these heteronymous interactions. A better understanding of the factors that influence modulation of heteronymous reflexes would help to clarify their functional role during movement and their potential contribution to movement disorders.In this study, to differentiate the role of limb loading and posture, we evaluated heteronymous feedback from the quadriceps muscles onto soleus (SOL) EMG in a supine recumbent position with and without 50% body weight loading, and while standing with wall support. Additionally, to test the modulatory influence of task-context, we compared heteronymous inhibition while standing with wall support and during an unsupported squat position. We hypothesized that heteronymous inhibition from quadriceps onto SOL will decrease due to limb loading and when tasks require SOL activity to maintain task performance.In 15 participants, we examined heteronymous feedback by stimulating the femoral nerve (FN) at 30% M max, while participants held 20% SOL MVIC in all task conditions (supine loaded/unloaded; standing with wall support and unsupported squat position).We found that heteronymous inhibition decreased when the limb was loaded with 50% body weight in supine when compared to the supine unloaded condition; however, no difference was found between the supine with 50% body weight and standing with wall support conditions. Additionally, decreased heteronymous inhibition from quadriceps onto SOL EMG was found in an unsupported squat position compared to standing with wall support suggesting an additional influence of task context since reduced SOL activation could be destabilizing. Heteronymous inhibition appears to be modulated based on limb loading and task context where functional consequences of increased inhibition could be undesirable and lead to poor performance of the task.

Disclosures: **M. Lyle:** None. **C. Cuadra:** None. **S. Wolf:** None.

Poster

PSTR219. Reflex, Modulation, and Afferent Control

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Program #/Poster #: PSTR219.08/II24

Topic: E.06. Posture and Gait

Support: NSF REU 2050915

Title: Contributions of the intrinsic tail muscles to tail movement and stiffening in the intact, unanesthetized rat

Authors: *L. WENGER, A. B. PEROE, M. W. CHRISTENSEN, C. L. CLELAND;
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Abstract: The nociceptive withdrawal response (NWR) of the tail can be robustly evoked by focused heat stimulation. Previous results from our laboratory have shown that the tail NWR consists of four components - local bend, local bend progression, tail base rotation, and stiffening of the tail. Our recent preliminary electromyography (EMG) has strongly implicated the intrinsic muscles of the tail in stiffening and bend progression. Matched bilateral timing and magnitude of EMG in the dorsal intrinsic muscles throughout the tail can explain stiffening, while rostral-caudal sequencing of EMG timing can explain local bend progression. The specific aim of our study was to determine the contributions of intrinsic tail muscles to tail co-contraction and stiffness during the heat evoked tail NWR in intact, unanesthetized rats using multiple EMG recordings. In adult Sprague-Dawley rats, bipolar fine wire EMG electrodes were bilaterally placed intramuscularly in the dorsolateral intrinsic tail muscles at various rostral-caudal locations under isoflurane anesthesia. EMG was recorded isometrically. The heat-evoked NWR was evoked using a focused 908nm laser diode at various bilateral locations distributed rostral-caudally along the lateral aspect of the tail. Cross-talk between bilateral pairs of electrodes was minimal (<15%). Our preliminary results continue to show that bilateral pairs of intrinsic muscles are activated at the same time and magnitude, implying that their actions will contribute to stiffening rather than movement. EMG was rostral-caudally sequenced (rostral led), potentially explaining bend progression. Overall, variation in stimulus location and intensity did not alter relative co-contraction. Our findings demonstrate that the NWR is functionally distributed across extrinsic and intrinsic muscles, with the intrinsic muscles primarily contributing to tail stiffening that may improve the efficacy of withdrawal response. The similarity of opposing muscle timing and magnitude suggests that muscles synergies may also simplify the control of the tail during the NWR.

Disclosures: L. Wenger: None. **A.B. Peroe:** None. **M.W. Christensen:** None. **C.L. Cleland:** None.

Poster

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Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.09/II25

Topic: E.06. Posture and Gait

Title: The nociceptive withdrawal response in intact, unanesthetized rats exhibits strong dependence on initial posture but weak dependence on stimulus location

Authors: *C. M. LARSON, E. H. RATERMAN, B. L. SIMPSON, G. L. HANSON, C. L. CLELAND;
Biol., James Madison Univ., Harrisonburg, VA

Abstract: Animals adopt numerous survival behaviors in response to aversive stimuli. One of these behaviors, the nociceptive withdrawal response (NWR), consists of the removal of an animal's limb from the affected site when presented with a noxious stimulus. Preliminary studies in our laboratory have identified three distinct components of the NWR - early extension, rapid flexion, and rapid extension. These studies have suggested that posture may be critical in influencing the animal's NWR when the animal is in a weight-bearing stance. Despite these findings, how the NWRs of intact, non-human mammals rely on stimulus location and how this pattern may be influenced by the animal's initial posture and stimulus location remains unclear. The specific aim of our research was to investigate the NWR in intact, unanesthetized rats in a weight-bearing posture when presented with noxious heat stimuli at various locations on the sole of the foot and circumferentially around the leg. We hypothesized that as stimulus location changed, the direction of the NWR direction of the rat's hind limb would depend on both stimulus location and initial posture. To accomplish this, adult Sprague-Dawley rats were anesthetized and marked with 2 mm circles at six locations on the left hind leg, which defined rotation around the toes, ankle, knee and hip. Following recovery, the rats were then presented with localized heat stimuli that targeted specific rostra-caudal locations on the plantar surface of the foot and anterior and posterior surfaces of the lower leg. Hind limb movement was tracked laterally using a high-speed (500 fps) video. Rotation of the toe, ankle, knee, and hip joints was calculated based three adjacent marks. Based on preliminary results, we found that there was limited effect of stimulus location on the three phases of response but a clear dependence on the initial location of the foot prior to stimulation. Overall, the NWR appears designed to preserve postural stability and sufficiently remove the leg from the noxious stimuli, rather than accurately direct withdrawal movements away from the stimuli.

Disclosures: C.M. Larson: None. **E.H. Raterman:** None. **B.L. Simpson:** None. **G.L. Hanson:** None. **C.L. Cleland:** None.

Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.10/II26

Topic: E.06. Posture and Gait

Title: Characterization of the nociceptive withdrawal response in the rat tail based on concurrent electromyographic and high speed video recording

Authors: *M. W. CHRISTENSEN, L. A. FRAYSER, A. S. SAHNI, C. L. CLELAND; Biol., James Madison Univ., Harrisonburg, VA

Abstract: Movement planning by the central nervous system is computationally difficult. To decrease computational load, the CNS must use strategies that simplify computations. Previous kinematic studies from our laboratory have shown that the movement of the hyper-redundant 28 segment tail can be largely explained by four components - tail base rotation, local bend, bend progression, and whole tail stiffing. Based on isometric electromyography (EMG), we preliminarily determined that the patterns of extrinsic and intrinsic muscle activity are consistent with the four components of movement. However, our studies were conducted in absence of tail movement. Consequently, a more definitive evaluation should incorporate a trial-to-trial analysis of both EMG and the resulting nociceptive withdrawal response (NWR) movement. The specific aim of our study was to determine the contributions of extrinsic and intrinsic tail muscles of the tail to movement during a heat-evoked NWR by utilizing concurrent EMG recordings and tail kinematics based on high speed video (500 fps) records. In adult, Sprague-Dawley rats, eight bipolar fine-wire EMG electrodes were inserted percutaneously and bilaterally, four of which targeted the extrinsic dorsal lateral muscle in the pelvis, and four of which targeted the dorsal intrinsic muscles in the tail. Placement of electrode location in target muscles was verified by electrical stimulation and postmortem dissection. The NWR was evoked by lateral noxious heat (980 nm infrared laser diode) at five stimulus locations on both sides of the tail. Lateral tail movement was quantified by software tracking of overhead high-speed video (325 fps). Based on a preliminary results, we have identified 3 correlations connecting EMG and tail movement. Magnitude and duration of the extrinsic agonist muscle EMG showed a positive correlation with tail movement. EMG also showed that agonist activation preceded antagonist activation, and the time delay was broadly correlated with lateral tail deceleration. Finally, intrinsic muscle activity, which followed extrinsic muscle activity, was temporally correlated with stiffening of the distal tail. Our preliminary results suggest that the extrinsic muscles contribute to tail movement and braking, while intrinsic muscles, possibly through synergies, contribute to tail stiffening.

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Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.11/II27

Topic: E.06. Posture and Gait

Title: Interdependence of the components of the nociceptive withdrawal response in intact rats

Authors: *E. RATERMAN, E. L. HESLEP, C. M. LARSON, C. L. CLELAND;
Biol., James Madison Univ., Harrisonburg, VA

Abstract: Animals depend on withdrawal responses to noxious stimuli for their survival, such as the nociceptive withdrawal response (NWR), which can be evoked in rats by noxious stimulation of the skin. The NWR has been primarily thought of as one singular movement with the sole purpose of protection, but recent studies emphasize that the NWR consists of multiple components that may contribute to posture and locomotion. Our laboratory preliminarily found that in response to a noxious stimulus in intact rats, there were three distinct sequential phases of movement: early extension, rapid flexion, and rapid extension; however, little is known about their interdependence. The goal of this study was to characterize the three phases of NWR movement and to determine the extent to which they are interdependent. The NWR in male Sprague-Dawley rats (n=8, 10-30 weeks) was evoked using localized noxious heat stimulation (980 nm infrared laser) delivered to the left hind limb and foot, either briefly (0.5 s) and near threshold, or continuous at various intensities. The two-dimensional response was captured using high-speed video (500 fps). The iliac crest, trochanter major, knee joint, lateral malleolus, fifth metatarsophalangeal joint, and distal phalanx of the 2nd toe were marked and tracked over time, to provide the magnitude and direction of movement for each anatomical landmark and allow for calculation of hip, knee, ankle, and toe joint angles. In response to brief stimuli near threshold, rats moved the stimulated hind limb (37%) or failed to respond entirely (63%), consistent with stimulating near threshold. Importantly, there were no occurrences of the early extension phase without the succession of the rapid flexion phase, and the rapid flexion phase rarely (3%) occurred without the early extension phase. Further, the third rapid extension phase always followed the rapid flexion phase. In response to continuous stimulation, there was no significant correlation between latency and time difference ($p=0.3$) for the first two phases, suggesting that they cannot be temporally dissociated. Additionally, there was a strong, positive correlation ($p<0.001$) between the magnitudes of these phases, further suggesting their association. Preliminary results demonstrate that the three phases of the NWR could not be disassociated, indicating that these phases are not independent but rather integrated into a singular response. Our results support a more complex understanding of the NWR, suggesting that the NWR is a multiphasic response that incorporates postural adjustments, preparatory responses, and withdrawal from the noxious stimulus.

Disclosures: E. Raterman: None. **E.L. Heslep:** None. **C.M. Larson:** None. **C.L. Cleland:** None.

Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.12/II28

Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant 2020-06603 (SDP)

Title: The effect of reduced plantar cutaneous sensation on gait and muscular reflex patterns

Authors: K. A. ROBB, ***S. D. PERRY**;
Kinesiology & Physical Educ., Wilfrid Laurier Universtiy, Waterloo, ON, Canada

Abstract: Cutaneous mechanoreceptors from the plantar sole are important contributors to balance control and the detection of pressure changes under the foot. When the transmission of afferent sensory signals to the central nervous system is deprived, modeling what occurs in individuals with peripheral neuropathies and aging, these investigations can provide insight into balance reactions during gait in those with sensory deprivation. This study attenuated cutaneous afference via hypothermic anesthesia from the foot's plantar-surface of 10 healthy adults (19.8±0.42years) and while attempting gait across an uneven terrain. Kinematic (13 linked-segment model from 21 infrared light-emitting diode markers, 2 Optotrak Cameras, 100Hz) and kinetic (3 force plates, AMTI OR6, 500Hz) data were recorded while completing 5 level walking and 5 uneven terrain walking trials across 3 different platform orientations. Surface electromyography (EMG) data (Bortec AMT-8, 500Hz) were recorded from 4 muscles bilaterally (tibialis anterior, peroneus longus, rectus femoris, and medial hamstring). Raw EMG was full-wave rectified, filtered, and normalized over 100% of the gait cycle. Reduce sensation EMG signals were then subtracted from normal EMG signals to differentiate between sensory facilitation and inhibition of muscle activity. Statistical differences between sensory and walking conditions were evaluated with repeated-measures analysis of variance (significance set at p=0.05). Results of this study demonstrate that compared to level walking, participants significantly ($F_{3,79}=3.74$, $p=.0116$) reduce walking velocity over the uneven terrain when sensation from the plantar soles is deprived (normal: 1.32±0.12m/s; deprived: 1.26±0.18m/s). Most commonly, cutaneous deprivation inhibited participant's muscle activity when walking on the level surface. The wedged conditions, which created a greater challenge to the balance control system, reduced EMG amplitude, shifted the timing of muscle bursts, and occasionally increased muscle activity throughout the gait cycle. These results demonstrate the inter-subject variability in response to cutaneous attenuation and highlights that as the demands of the walking task increases, the detrimental effects of sensory deprivation also increase. These insights are valuable in understanding which locomotor tasks pose the greatest fluctuations in gait, and larger gait variability increases the risk of falls.

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Poster

PSTR219. Reflex, Modulation, and Afferent Control

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR219.13/JJ1

Topic: E.06. Posture and Gait

Title: Spinal sensory feedback contribution to soleus EMG during stair ascent in able bodied humans

Authors: A. B. ANDERSEN, Jr[1], A. J. T. STEVENSON, Senior[1], J. B. NIELSEN[3], ***T. SINKJÆR**[2];
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Abstract: The involvement of sensory feedback during complex motor tasks like stair climbing is not yet known. At Aalborg University, we have recently developed an experimental staircase where one of the stair steps can be moved slightly up, down, or rotated when the subject is about to step on it. In this way we may compare the behavioral and neural consequences of correct and incorrect prediction of changed shape and time of ground contact during an unconstrained natural stair walking task. In the present study, we investigated the involvement of spinal afferent feedback contributions to motoneuronal drive and corrective stretch reflexes during stair climbing. Subjects climbed a seven-step perturbation stair apparatus unrestrained. On random trials and just prior to foot contact, the fourth step was either rotated slightly downward (study 1) or moved 5 cm downward causing a "drop" of the stair (study 2). All data presented were recorded from the left leg. Nine subjects were instrumented with bipolar surface electromyography (EMG) electrodes over the soleus (SOL) and tibialis anterior (TA) muscles of the left leg. The EMG signals were amplified and band-pass filtered (10-1 kHz) using an analog filter and custom-built amplifiers. Foot-strike was recorded using a force sensitive resistor placed under the sole of the left shoe. Left ankle angle was recorded using a surface mounted electrogoniometer.The changes in the SOL EMG at the short M1 and medium M2 stretch reflex latencies as function of imposed ankle joint velocity were analyzed. In study 1 the perturbation caused a small extra dorsiflexion which was not sensed by subjects when asked. The M1 and M2 increased non-significantly with the velocity of the ankle joint angle. No changes were observed in TA EMG. During the 50 cm "drop" of the stair step in study 2, a significant M1 response was measured. M2 increased non-significantly, with no changes observed in TA EMG.We speculate that during stair climbing, the distinct M1 during "stair-drop" is a Ia-afferent mediated corrective response, whereas the small increase in SOL EMG around the time of M1 and M2, during the small rotation of the step, may be caused by similar afferent contributions as seen during unperturbed floor walking.

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Poster

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Program #/Poster #: PSTR219.14/JJ2

Topic: E.06. Posture and Gait

Title: Good proprioception and stretch induced hypermobility can increase sway in young adults

Authors: T. M. GAUSS[1], J. B. MORALAS[1], R. M. LORMAND[1], M. A. YEOMANS[2], *J. HONDZINSKI[1];
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Abstract: Impact of static stretching on postural sway and proprioception remains controversial despite the stretch-related improvements in joint range of motion and flexibility. Stretching different muscles and length of muscle stretching times differed across studies to possibly contribute to the varied results. We wanted to determine if stretching opposing ankle muscles (oppose) or single sided, non-opposing ankle muscles of the posterior compartment of the lower limb (nonoppose) at different stretch times changed standing postural sway and proprioception compared to a pre-stretched (control) condition and each other. Twenty-four barefoot young adults (16 F/8 M; Age 22.13 +/- 3.53 years) performed 3 trials of static stance and proprioception tasks in each condition on 2 days separated by at least 1 week. In one visit we emphasized muscle stretch times (control, 15 s, 30 s, 45 s), while in the other visit we emphasized stretched muscle locations (control, oppose, non-oppose). Weekly order was counterbalanced across participants, while conditions were counterbalanced after completing control trials. Participants received passive stretching to discomfort in oppose (plantar- and dorsi-flexion) and nonoppose (dorsi-flexion) conditions. Participants stood as still as possible with eyes closed and heels 7.5 cm apart for 45 s on an ATMI force plate (100 Hz) to determine postural sway (center of pressure—COP) variables of interest. We used a hand-held goniometer to measure ankle proprioception, in which participants actively matched remembered ankle angles. We previously reported data for stretched muscle locations which revealed decreased time series complexity and increased variability in the COP mediolateral (M-L) direction for oppose compared to pre-stretched and/or nonoppose conditions. Unlike stretched muscle locations, repeated measures ANOVAs for muscle stretch times showed no significant differences in time series complexity or COP variables across conditions. Stretching muscles with varied actions produced greater impact on postural sway than stretch time. Results also indicated that stretching ankle muscles for different time periods or muscle locations did not influence proprioception. However, associations between proprioception errors and postural sway variables revealed greater M-L sway for those with less errors in proprioception only for the visit in which stretched muscle location varied, resulting in a relatively large range of proprioception errors. The ability to actively reproduce ankle positioning well with only proprioception may encourage an internal focus of attention on movement which does not improve postural control.

Disclosures: T.M. Gauss: None. **J.B. Moralas:** None. **R.M. Lormand:** None. **M.A. Yeomans:** None. **J. Hondzinski:** None.

Poster

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Topic: E.06. Posture and Gait

Support: CIHR PJT-162357
R01 NS110550
NSF 2024414

Title: Role of proprioceptive feedback from muscle spindles in lateral postural stability during mouse locomotion

Authors: *T. AKAY[1], A. N. KLISHKO[2], B. I. PRILUTSKY[3];
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Abstract: Efficient locomotion in natural environments requires robust postural stability. Natural environments where animals typically move around are unpredictable, making somatosensory feedback potentially crucial for maintaining stability. Previous research has demonstrated the importance of mechanosensory cutaneous information in maintaining lateral stability (Park et al., 2019; J Exp Biol 222:jeb198648), but the role of proprioception remains obscure. To address the role of proprioceptive feedback from the muscle spindles in the control of lateral stability during locomotion, we determined measures of lateral stability during treadmill locomotion at different speeds in wild-type (WT) mice and in mice that lack proprioceptive muscle spindle feedback, the *Egr3 knock out* mice (*Egr3-KO*, Tourtellotte and Milbrandt, 1998; Nat Genet 20: 87). The measures of lateral stability were calculated using the positions of the center of mass (CoM), labeled with a white marker, and paws recorded by a high-speed video system while animals were locomoting on a transparent treadmill with a mirror underneath. The CoM and the four paw positions were tracked frame by frame using DeepLabCut (Mathis et al., 2018; Nat. Neurosci. 21, 1281). The following measures of lateral stability were calculated: (i) the forepaw and hindpaw step width, (ii) the lateral distance between the CoM and each paw, and (iii) the lateral distance between the extrapolated CoM (Hof et al., 2005, J Biomech 38:1-8) and each paw. We show that mice without muscle spindle feedback assume a lower body posture. Moreover, the CoM lateral position does not fluctuate in *Egr3-KO* mice as much as in WT. Furthermore, on average, the hindlimb step width is lower in *Eg3-KO* mice than in WT, whereas the forelimb step width is similar. Changes in the height of the body, step width, and lateral CoM fluctuation led to similar measures of lateral stability in WT and *Egr3-KO* mice during locomotion. We also found correlation between the lateral CoM shifts in individual walking cycles with the change in lateral position of the ipsilateral paws regardless of the presence of muscle spindle feedback. In accordance with this, we also show that changes in the lateral stability measures in response to sudden and random lateral displacement perturbations in WT and *Egr3-KO* mice were very similar. These observations indicate that although muscle spindle feedback is not involved in step-by-step regulation of lateral stability, lateral stability seems still affected by feedback from the muscle spindles because their removal leads to locomotor adaptations to maintain stability, i.e. lowering body height.

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Poster

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Program #/Poster #: PSTR219.16/JJ4

Topic: E.06. Posture and Gait

Support: NSF CRCNS 2113028

Title: Force sensing in very small animals: monitoring the effects of motor actions

Authors: *S. ZILL[1], S. CHAUDHRY[1], H. CHAUDHRY[1], N. S. SZCZECINSKI[2];
[1]J.C. Edwards Sch. Med., Huntington, WV; [2]Dept. of Mechanical and Aerospace Engin., West Virginia Univ., Morgantown, WV

Abstract: As part of a larger study in neuro-robotics, we have characterized and modeled force detecting sense organs (campaniform sensilla) of insect legs. These receptors can detect the magnitude and dynamics of forces and body loads in the legs of larger insects. However, it has been unclear whether small insects, with very low body weights, require equivalent sensory information about forces. In the present study, we have adapted extracellular recording techniques developed in larger insects to characterize the activities of the hindleg tibial group of sensilla in Calliphora flies. After ablation of the tarsus, forces applied to the tibia (with joint movement resisted) elicited vigorous afferent discharges that were eliminated by subsequent ablation of the cuticular caps of the tibial sensilla. Tests using forces applied as ramp and hold functions showed that discharges were strongly phasicotonic, as firing frequency both reflected the force magnitude and rate change of forces. In addition, tests using transient perturbations imposed within increasing exponential functions demonstrated that, at low sustained force levels, receptor discharges showed hysteresis and were largely inhibited by small phasic force decreases. These results were also tested in a mathematical model of force encoding by campaniform sensilla (Szczecinski et al. 2020, 2021) which calculates the receptor discharge frequency as the sum of adaptive and tonic functions. After tuning using data on amplitude and rate sensitivities of fly campaniform sensilla, the model successfully reproduced receptor responses to exponential waveform stimuli and perturbations (with no parameter retuning). These findings support the idea that force detection scales to body weight and that monitoring force magnitude and dynamics may be necessary even in very small animals with minimal body weight. Our working hypothesis is that animals of all sizes benefit from force receptors that detect resisted muscle contractions (and show no or minimal discharge to unresisted movements): these afferents can signal that the legs have made contact with the substrate that is sufficient to allow the appendage to move the animal in terrestrial locomotion. The hysteresis in sensory discharges to sudden decreases in forces can effectively alert the system to leg slipping and other destabilizing perturbations. Last, our results suggest that these characteristics of force sensing can be incorporated in the control of robotic legs and may also be applied with similar advantage to machines that emulate walking in small insects.

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Poster

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Topic: E.06. Posture and Gait

Support: Aspirant Research Fellow

Title: Central Proprioceptive Processing during Postural Control is Related to Peripheral Proprioceptive Acuity

Authors: *S. J. MONGOLD, C. GEORGIEV, L. ABI-KHALIL, G. NAEIJE, M. VANDER GHINST, M. BOURGUIGNON;
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Abstract: Proprioception, defined here as the sense of bodily kinematics, is crucial for movement control. Yet, the neural processing of sensory mechano-afferents that give rise to proprioception has received little attention, which is surprising given its implication in such basic behaviors as standing. A promising tool to fill this gap is corticokinematic coherence (CKC), which is the coupling between cortical activity and limb kinematics during repetitive movements, and is thought to specifically assess the cortical processing of somatosensory afferences. Importantly, peripheral tests of proprioception have not been compared to CKC, which would shed light onto whether this method represents sensory processing. We collected center of pressure (COP) data from 63 participants (32 female, age range: 22-85), who were equipped with EEG and stood on a force plate. Standing balance was assessed across 4 condition types, either with eyes open or closed and on either a hard or foam surface. For each condition, we quantified 1) instability as the standard deviation of COP fluctuations restricted to 0.1-10 Hz and 2) CKC as the coherence between EEG and the velocity of the COP. Joint position sense (JPS) was evaluated through the joint position reproduction test. Two linear mixed model analyses were employed to determine whether postural instability (first model) and CKC (second model) depend on condition type and JPS. Across participants, CKC peaked at frequencies between 2 and 8 Hz at electrodes proximal to Cz. The first model revealed a main effect of condition ($\chi^2(3) = 251$, $p < 0.0001$) and JPS ($\chi^2(1) = 5.57$, $p = 0.018$) on instability, but no interaction ($\chi^2(3) = 2.48$, $p = 0.48$). Across all conditions, worse JPS was correlated with instability ($r = 0.24$ - 0.28, $p = 0.026$ - 0.06). The second model resulted in a main effect of condition ($\chi^2(3) = 60.9$, $p < 0.0001$) and JPS ($\chi^2(1) = 6.70$, $p = 0.0096$) on CKC, but no interaction ($\chi^2(3) = 5.23$, $p = 0.16$). Across all conditions, worse JPS was correlated with CKC ($r = 0.21$ - 0.37, $p = 0.003$ - 0.099). Our findings show that CKC computed between cortical activity and the velocity of COP resembles that of previous literature using other kinematic signals. Expectedly, we verified that condition type influenced stability. The inclusion of JPS for CKC prediction improved our model, signifying that CKC is related to the ability to detect joint position. Furthermore, stronger CKC coincided with increased relative error on the JPS test. Together these findings suggest that CKC represents a neural marker of proprioception, and its magnitude may be indicative of the quantity of cortical resources allocated for sensory processing, tentatively, as a compensation mechanism.

Disclosures: **S.J. Mongold:** None. **C. Georgiev:** None. **L. Abi-Khalil:** None. **G. Naeije:** None. **M. Vander Ghinst:** None. **M. Bourguignon:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.01/JJ6

Topic: E.09. Motor Neurons and Muscle

Support: NSF CAREER Award 2002261

Title: Modeling Ankle Torque using an Ultrasonography-based Tissue Displacement Tracking Algorithm

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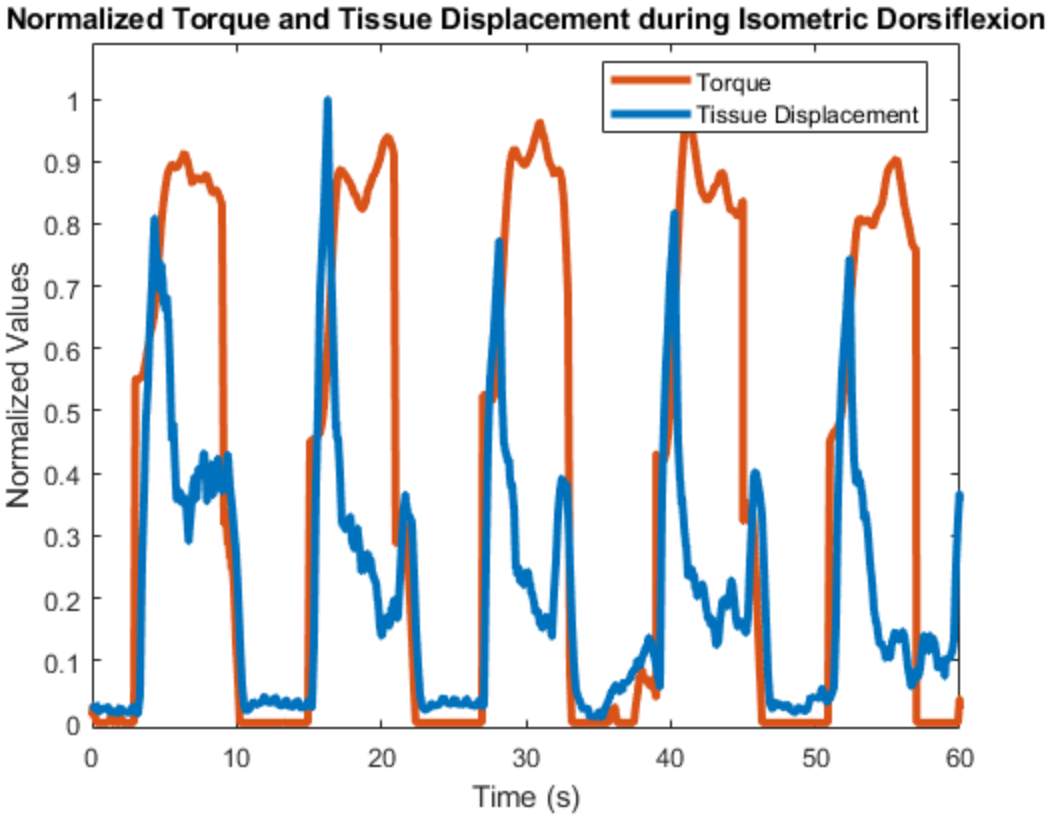
[1]Joint Dept. of Biomed. Engin., [2]Dept. of Electrical Engin., North Carolina State Univ., Raleigh, NC; [3]Joint Dept. of Biomed. Engin., North Carolina State Univ., Cary, NC

Abstract: Determining the amount of assistance that should be provided for motor learning following neurological injury is a major challenge. With the rising prevalence and healthcare costs of spinal cord injury (SCI), effective design of therapy devices is vital. In rehabilitative exoskeletons, control design entails the use of active-assist techniques that must anticipate a user's volitional capabilities. Thus, seamless human-machine interfacing is reliant on the accuracy of the predictive dynamic model for limb forces.
Ultrasonography can non-invasively provide visual feedback of skeletal muscles. Measurement of one-dimensional architectural parameters like tissue displacement are useful in estimating muscle activity. To account for these changes in the dynamic models of rehab devices, ultrasound (US) analysis algorithms must yield relatively higher processing speeds. One approach to real-time processing is adaptive correlation-based speckle tracking (CBST), which was originally described by Lubinski et al. (1999). Previous work by Sheng et al. (2022) demonstrated the ability to use real-time CBST for muscle fatigue. This led us to explore the possibilities of CBST use in modeling. We aim to analyze the feasibility of CBST for joint torque estimation, which can be used to create more accurate predictive models for the controls of rehab devices. To achieve this, we present a CBST algorithm with the means to measure tissue displacement, validated through the development of a Koopman operator-based data-driven model to predict ankle torque for people with SCI.
Initial results utilize US images of the tibialis anterior of an able-bodied individual during seated, isometric dorsiflexion. The correlation between the normalized tissue displacement and torque was 0.5501. To apply the results to gait rehab exoskeletons, we intend to collect US sequences and ground reaction forces during different tasks, such as treadmill walking, for one SCI subject. This data will be used for real-time torque estimation which can be further used in assistance design for rehab exoskeletons.



Disclosures: **N. Hakam:** None. **M. Singh:** None. **N. Sharma:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.02/JJ7

Topic: E.09. Motor Neurons and Muscle

Support: NIH

Title: Investigating a link between motoneuronal dysfunction and cognitive impairment in alzheimer's disease

Authors: ***A. ROSHANI DASHTMIAN**[1], F. BABAEI DARVISHI[1], P. J. BOBBILI[2], R. RODRIGO[2], H. HARRIS[2], W. ARNOLD[1];
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Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder that causes progressive loss of cognitive function as a primary feature. Importantly, loss of mobility and falls are major

contributors to AD morbidity, and there is increasing evidence suggesting that loss of motor function might precede the onset of overt cognitive impairment. Yet, the relationships between motor and cognitive dysfunction in AD are not well understood. We aimed to investigate the association between motor dysfunction and cognitive impairment and to explore motoneuronal dysfunction as a possible link in the 5XFAD mouse model of AD and age-matched wildtype controls (C57BL/6J background). Both groups underwent a longitudinal in-vivo study where electrophysiological approaches were applied to the gastrocnemius muscle including motor unit number estimation (MUNE) and motor evoked potentials (MEP: to assess spinal excitability and conduction). Additionally, single molecule array immunoassay technique was applied to measure serum neurofilament light chain (NFL) as a biomarker of axonal degeneration. Our results suggest that MUNE shows progressive decline after ~4 months of age ($p=0.0045$) paralleling finding of increased NFL ($p=0.0001$). Importantly, there was an inverse correlation between NFL and MUNE at the age of 6 months 5XFAD mice. These results are indicative of axonal loss and neurodegeneration in an AD phenotype. In contrast, MEP amplitude increased by ~180% in 5XFAD mice compared with controls suggesting potential compensatory increases of spinal excitability. Our results suggest a clear decline in the motoneuronal function that may be pathophysiologically linked to the onset of AD. Future experiments will determine whether motor function decline precedes, tracks with, or follows progression of cognitive defects. We propose that motoneuronal and motor function decline may be accurate indicators of the progression of AD and that better understanding the temporal and pathophysiological relationship between motor and cognitive decline may aid to improve diagnosis and management of AD. Our ongoing study will help us to explore the molecular and cellular mechanisms of motoneuronal function in the context of age-related cognitive impairment and neurodegeneration.

Disclosures: **A. Roshani Dashtmian:** None. **F. Babaei Darvishi:** None. **P. J. Bobbili:** None. **R. Rodrigo:** None. **H. Harris:** None. **W. Arnold:** 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.; NMD Pharma, Avidity Biosciences. F. Consulting Fees (e.g., advisory boards); Dyne Therapeutics, Novartis, La Hoffmann Roche, Genentech, Design Therapeutics, Cadent Therapeutics, Catalyst Pharmaceuticals.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.03/JJ8

Topic: E.09. Motor Neurons and Muscle

Support: DGAPA Posdoc Fellowship

Title: Estrogen and neurotrophic-like agonists regulate the functional expression of SNAT2 on C2C12 culture cells simulating metabolic damage

Authors: *A. G. RODRIGUEZ-CAMPUZANO[1], J. AGUILERA[2], A. ORTEGA[3], F. CASTELÁN[4];
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Abstract: Pelvic floor dysfunctions are an increasing health problem in young adult and middle-aged women. This structure can be weakened by pregnancy, childbirth, obesity and chronic constipation, resulting in urinary incontinence, fecal incontinence and in a more advance problem, pelvic organ prolapse in older women. The nature of this problems is related to age and decreasing in the hormonal levels. The estrogenic actions that modulate the recovery of muscular lesions in the pelvic floor have been exhaustively studied, however, given the nature of secondary effects in this type of therapy, the need for search for preventive and palliative treatments that emulate the given results of estrogens has been urgent. A recombinant carboxyl terminal from the tetanus toxin (XT1) has been studied, having neurotrophic and estrogenic actions in the recovery of neuromuscular lesions in the pelvic floor. In the recovery of the muscular lesion the need for energy and building blocks substrates is imperative, the sodium-couple neutral amino acid transporter 2 (SNAT2) is highly expressed in skeletal muscles and has a transceptor activity modulating intracellular signaling pathways needed for cell differentiation and proliferation. In this study we characterized estrogenic and neurotrophic actions of XT1 and estradiol in the recovery of muscular lesions simulated by metabolic damage in an in vitro model using C2C12 cells at two differentiation stages, myoblasts and myotubes. We measured the activity of SNAT2 by uptake assays using ^{3}H-L-Glumatine, the most abundant substrate of SNAT2 in the extracellular space. We found that XT1 and estradiol independently increase SNAT2 activity at both differentiation stages, and using pharmacological approaches we discarded other amino acids transporters. XT1 and estradiol also modified the levels of SNAT2 mRNA and protein levels after metabolic damage. In order to understand the metabolic couple needed for the recovery of muscular lesions, we also determined the activity and expression of glucose transporter 4 (GLUT4) when cells were pre-treated with XT1 and estradiol, and dissected part of the signaling pathway that can be activated by XT1, an interesting treatment alternative to estrogenic therapies.

Disclosures: A.G. Rodriguez-campuzano: None. **J. Aguilera:** None. **A. Ortega:** None. **F. Castelán:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

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Program #/Poster #: PSTR220.04/JJ9

Topic: E.09. Motor Neurons and Muscle

Support: PAPIIT UNAM: IN211720
CONACyT : ERB1007567

Title: Pelvic floor neuromuscular damage and regeneration indicators due to multiparity at different times postpartum in female rabbits.

Authors: *E. RODRIGUEZ-BENITEZ[1,3], N. XELHUANTZI-ARREGUIN[4], D. CORONA-QUINTANILLA[2], F. CASTELÁN[5], M. MARTINEZ-GOMEZ[5];
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Abstract: In women, multiparity, or age, are considered factors associated with from the prevalence of urinary incontinence. During labor, pelvic nerves and muscles are damaged and to produce histological changes in both structures of the pelvic floor. Some studies in animal models have shown that the multiparity in rats and rabbits can affect the pelvic floor muscles. However, studies are needed that determine the mechanisms of neuromuscular regeneration of the pelvic floor, due to the difficulty in obtaining the samples. The aim of this study was to analyze the damage and regeneration of pubococcygeus (Pc) and bulbospongiosus (Bs) nerves and muscles. Nulliparous (N, n=6) and multiparous (M, n=6) Chinchilla-breed rabbits from 3 and 20 days postpartum (n=6) were used; the nerves and muscles were dissected and measured. Both muscles were transversely cut at 7 µm. The sections were subjected to immunohistochemistry against Pax7 and TGF-B, and hematoxylin-eosin staining was performed for central myonuclei count. The length of the nerves was measured, they passed the Kolmogorov-Smirnov normality test and only significant differences were obtained with one-way Anova between N and M rabbits at 3 days postpartum for both nerves (Pc, P= 0.0035; and Bs P= 0.0068). The qualitative results to Pc muscle with satellite cell labeling with Pax 7 and TGF-B. The Pc muscle showed a greater labeling of positive cells in M rabbits at 20 days postpartum. Also, in M at 3 and 20 postpartum days, the Pc (P= 0.0159) and Bs (P= 0.0475) muscles showed the central nuclei count of the myofibers in conclusion, the increase in the length of the nerves at 3 postpartum days represents a stretching nerve during the parturition, while the labeling of Pax 7, TGF-B and the central nuclei of the myofibers is indicative of regeneration at 20 days postpartum.

Disclosures: E. Rodriguez-Benitez: None. **N. Xelhuantzi-Arreguin:** None. **D. Corona-Quintanilla:** None. **F. Castelán:** None. **M. Martinez-Gomez:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.05/Web Only

Topic: E.09. Motor Neurons and Muscle

Title: Generation of Novel, Orally Active Selective Macrocyclic Peptide Inhibitors of Myostatin

Authors: *H. KITAMURA;
PeptiDream Inc., Kawasaki, Kanagawa, Japan

Abstract: Myostatin is a major negative regulator of muscle growth via ActRIIB and ALK4/5/7 receptors. In past, myostatin inhibitors with several different mechanisms including non-selective neutralizing antibody of myostatin and GDF-11, or ActRIIB neutralizing antibody, have been clinically developed for various neuro-muscular diseases (NMDs) including Duchenne muscular dystrophy (DMD), disuse muscle atrophy, sarcopenia and inclusion body myositis (IBM). However, these first generation myostatin blockers did not achieve clinical benefit in muscular function improvement in impaired muscle condition even though muscle mass was increased in targeted diseases. Although the reason for these failures is not fully understood, inhibition of GDF11, a hypothetical juvenilizing factor with very highly conserved amino acid sequence to myostatin, might be a cause of insufficient muscle function improvement of these drugs. Minimal blockade of autocrine/paracrine signaling of myostatin by anti-myostatin antibodies due to very limited distribution into muscle could be another potential reason. Recently, selective myostatin neutralizing antibodies are being investigated in pivotal studies for spinal muscular atrophy (SMA) in combination with genetic therapy, suggesting selective myostatin blockade is a promising approach to improve muscle function in NMDs. Therefore, we applied PDPS (Peptide Discovery Platform System), a proprietary technology of PeptiDream Inc., which rapidly generates and displays high diverse peptide libraries ($>10^{13}$) to discover macrocyclic peptides with selective myostatin inhibition and muscle penetrating properties. Macrocyclic peptides were successfully identified with potent inhibition against myostatin with >100 fold IC_{50} selectivity over GDF-11 in a cell assay system, and a lead compound, 99m, showed distribution into muscle (muscle/plasma=3). Weekly subcutaneous administration of 99m improved grip strength in DBA/2 mdx mouse superior to an anti-latent myostatin antibody. Surprisingly 99m suppressed myofiber necrosis in diaphragm in DBA/2 mdx mouse, whereas anti-latent myostatin antibody was not able to demonstrate. Impressively, 99m improved grip strength in B10 mdx mouse by weekly oral administration with comparative grip strength improvement to SC dosing. Taken together, 99m is a potential novel, best-in-class drug to treat DMDs via convenient oral dosing and provide consistent muscle integrity to counteract irreversible muscle dysfunction in DMD. Selective myostatin inhibitor, 99m, could improve muscle function in other NMDs including SMA or amyotrophic lateral sclerosis with standard treatments.

Disclosures: H. Kitamura: None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.06/JJ10

Topic: E.09. Motor Neurons and Muscle

Support: ALS Canada
Healthy Brains, Healthy Lives initiative at McGill University
CQDM FACS and Quantum Leap Program

Title: Scalable human NMJ-on-a-chip™ model: a breakthrough for neuromuscular research and drug discovery

Authors: *M. AUROUSSEAU[1], M. J. CASTELLANOS-MONTIEL[2], E. FAURE[1], L. MOELLER[1], A. FRANCO-FLORES[2], C. X. CHEN[2], T. DURCAN[2], H. MCGUIRE[1]; [1]eNUVIO Inc., Montreal, QC, Canada; [2]EDDU, The Neuro, McGill Univ., Montreal, QC, Canada

Abstract: The neuromuscular junction (NMJ) is a specialized structure underlying neuromuscular synaptic connectivity that is central for muscle control by the nervous system. Disruption to the formation or maintenance of the NMJ is involved in the pathophysiology of various neuromuscular disorders (NMDs) such as amyotrophic lateral sclerosis (ALS) and myasthenia gravis (MG). As such, the development of physiologically-relevant, scalable, human NMJ models are important to elucidate pathological mechanisms as well as to screen for therapeutics to treat NMDs.
Here we present a commercially-available compartmentalized microfabricated device from eNUVIO called the OMEGA-NMJ that provides the basis for a human *in vitro* NMJ-on-a-Chip™ model. Contrary to previous models, this device provides a scalable, structural environment to support the co-culture of self-assembled 3D neuronal and 3D skeletal muscle microtissues to best reproduce the cellular and physiological environment of the *in vivo* neuromuscular system.
Utilizing this model, we co-cultured human induced pluripotent stem cell (iPSC)-derived motor neurons and primary human skeletal muscle to produce functionally-coupled 2D/3D and 3D/3D co-cultures. Morphological characterization of the NMJ was achieved by identifying and quantifying the co-localization of neuronal neurofilament-H, synaptic vesicle glycoprotein (SV2), and alpha-bungarotoxin signals using confocal fluorescence microscopy. The functional coupling of live co-cultures was assessed using a novel optical method to measure the contraction of 3D skeletal muscle microtissue during motor neuron stimulation.
Owing to the unique open-top device design, we also scaled this device to a standardized 96-well microplate format to suit high-throughput screening modalities based on high-content imaging and automated liquid handling systems. Together, the NMJ-on-a-Chip™ model provides a powerful and accessible tool for both the investigation of human NMJ physiology as well as for the identification and development of novel therapies for NMDs.

Disclosures: **M. Aurousseau:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); eNUVIO Inc.. **M.J. Castellanos-Montiel:** None. **E. Faure:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); eNUVIO Inc. **L. Moeller:** A. Employment/Salary (full or part-time):; eNUVIO Inc.. **A. Franco-Flores:** None. **C.X. Chen:** None. **T. Durcan:** None. **H. McGuire:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); eNUVIO Inc..

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.07/JJ11

Topic: E.09. Motor Neurons and Muscle

Support: NIH grant R01NS099210
NIH grant R01NS112942

Title: Improving movement in chronic stroke survivors through home-based myoelectric interface neurorehabilitation conditioning

Authors: *A. KHORASANI, J. HULSIZER, V. PAUL, P. PRAKASH, Y. Y. DHAHER, M. W. SLUTZKY;
Northwestern Univ., Chicago, IL

Abstract: After a stroke, abnormal muscle co-activation significantly contributes to movement impairment in both the arm and leg. Here, we present interim findings from an ongoing randomized controlled trial that utilizes a wearable version of the myoelectric computer interface (MyoCI) called MINT. MINT uses EMGs from the abnormally co-activating muscles to control cursor movement in customized computer games. Our research aims to assess the effectiveness of MINT in reducing muscle co-activation and enhancing movement in chronic stroke survivors. For the arm, participants with moderate to severe impairment engaged in 90-minute daily training sessions, six days a week, for a duration of six weeks. Three training paradigms involving training two or three abnormally co-activating muscles at a time were implemented, compared to a sham control group that trained with a single muscle at a time. Out of the 51 participants who completed the training, all experimental groups combined exhibited a promising trend of greater improvement on the Wolf Motor Function Test compared to the sham group (mean -3.7 s vs. -1.4 s, timed portion). The improvement exceeded the minimal clinically important difference (MCID) of 1.5 s. This positive trend continued even four weeks after the training concluded (mean -6.7 s vs. -3.3 s, timed portion). Participants training with three muscles at a time showed nearly significant improvement at six weeks compared to the sham group (-6.1 s vs. -1.4 s, p=0.06, t-test) and significantly improved at four weeks after training stopped (-9.6 s vs -3.3 s, p=0.03). Arm reaching kinematics also improved in the experimental groups more than in the sham. In addition to the arm, we performed a pilot study of MINT conditioning in the leg. Previous research conducted by our group indicated that co-activation of hip adductors and knee extensors contributes to gait impairment following stroke. Nine participants with moderately impaired gait underwent six hours of MINT conditioning either in a lab setting or at home. The participants trained while standing with the paretic leg in the near toe-off position, associated with increased co-activation. Participants achieved an average reduction of 70% in co-activation compared to baseline. No safety issues were encountered by participants using MINT at home. Moreover, significant improvements were observed, including an increase in gait speed by 0.16 m/s, exceeding the MCID, an increase in knee flexion angle, and a substantial decrease in hip circumduction. These findings suggest that MINT conditioning holds

promise in enhancing arm and leg kinematics, as well as overall function, in chronic stroke survivors.

Disclosures: A. Khorasani: None. **J. Hulsizer:** None. **V. Paul:** None. **P. Prakash:** None. **Y.Y. Dhaher:** None. **M.W. Slutzky:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.08/JJ12

Topic: E.09. Motor Neurons and Muscle

Title: Molecular alterations underlying hypotonia in SHANK3 deficiency

Authors: *B. YILDIZ[1,2], M. MULAW[3], A.-K. LUTZ[1], T. BOECKERS[1,4];
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Abstract: Phelan-McDermid syndrome (PMDS) is a syndromic form of Autism Spectrum Disorders (ASD) classified as a rare genetic developmental disease featuring global developmental delay, absent or delayed speech, ASD-like-behaviour and neonatal skeletal muscle hypotonia. PMDS is caused by *SHANK3* mutations or a heterozygous deletion of the distal end of chromosome 22q13.3, that in most cases affects the *SHANK3* gene. As previously seen in adult *Shank3Δ11(-/-)* mice, we found the same ultrastructural alterations of the sarcoplasmic reticulum in newborn *Shank3Δ11(-/-)* animals, indicative of a Shank3-dependent change directly present at birth. We therefore thoroughly analyzed transcriptional differences by RNA-sequencing of muscle tissue of neonatal *Shank3Δ11(-/-)* mice and compared those to *Shank3(+/+)* controls. We found significant differences in gene expression of ion channels crucial for muscle contraction and for molecules involved in calcium ion regulation. In addition, calcium storage- (i.e. Calsequestrin (CSQ)), calcium secretion- and calcium- related signaling-proteins were found to be affected. By immunostainings and Western blot analyses we could confirm these findings both in *Shank3Δ11(-/-)* mice and PMDS patient muscle tissue. Our results emphasize that SHANK3 levels directly or indirectly regulate calcium homeostasis in a cell autonomous manner causing -at least in part- muscular hypotonia especially seen in the newborn.

Disclosures: B. Yildiz: None. **M. Mulaw:** None. **A. Lutz:** None. **T. Boeckers:** None.

Poster

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Program #/Poster #: PSTR220.09/JJ13

Topic: E.09. Motor Neurons and Muscle

Support: DFG, German Research Foundation – EXC-2049 – 390688087
Humboldt-Universität zu Berlin

Title: Trunk Tip Muscle Architecture and Innervation in African and Asian Elephants

Authors: *A. SHUBITIDZE[1], L. EIGEN[1], L. L. LONGREN[1], T. HILDEBRANDT[2], M. BRECHT[1,3];
[1]Bernstein Ctr. For Computat. Neuroscinece Berlin, Berlin, Germany; [2]Leibniz Inst. for Zoo and Wildlife Res., Berlin, Germany; [3]NeuroCure Cluster of Excellence, Humboldt-Universität zu Berlin, Berlin, Germany

Abstract: Elephants are the largest terrestrial mammals and heavily rely on their muscular trunk for survival. The trunk is a fascinating grasping organ that operates as a muscular hydrostat, enabling elephants to perform a diverse range of tasks including grasping, socializing, showering, or moving obstacles. The trunk tip differs between African, *Loxodonta africana*, and Asian, *Elephas maximus* elephants. While Asian elephants wrap with one finger, African elephants can pinch with two fingers. We performed dense muscle fascicle reconstructions of the hemi-trunk tip of an Asian baby elephant and an adult African elephant utilizing high-resolution microfocus tomography (microCT) scans. In both species we found that (i) the trunk tip contains thousands of muscle fascicles; (ii) each muscle has its own insertion points, i.e., can in principle operate independently; (iii) tip and in particular finger fascicles are very small, pointing to a role of muscle miniaturization in elephant dexterity; (iv) radial muscle fascicles predominate in the trunk tip. Our data also indicate that compared to the bulbous ventral trunk tip lip of the Asian elephant, there might be more muscle fascicles in the ventral finger of the African elephant. We reckon this difference relates to the pinching (African elephants) versus wrapping (Asian elephants) strategies. Currently, we apply high resolution histological techniques to resolve muscle innervation patterns in the elephant trunk and relate them to our earlier work (Kaufmann et al., 2022) on the elephant facial nucleus. Based on our results, we can confidently state that the muscular apparatus in the tips of elephant trunks is the most complicated and sophisticated of any known organism on Earth.

Disclosures: A. Shubitidze: None. **L. Eigen:** None. **L.L. Longren:** None. **T. Hildebrandt:** None. **M. Brecht:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.10/JJ14

Topic: E.09. Motor Neurons and Muscle

Support: KAKENHI 23H03256
Yamaha Motor Foundation for Sports

Title: Neuromuscular junction degeneration due to immobilization impairs neuromuscular transmission and alters myofiber composition

Authors: *T. YAMAGUCHI[1,2], K. SASAKI[1], K. NAKAZATO[3];
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Abstract: Physical inactivity leads to a decrease in muscle strength more markedly than in muscle mass. Recent studies suggest that the degeneration of the neuromuscular junction (NMJ) during inactivity may contribute to muscle weakness, although the detailed mechanisms remain unclear. In this study, we aimed to clarify how the NMJ morphology and neuromuscular transmission are altered during physical inactivity in mice. Male C57BL/6J mice (12-13 weeks old) were divided into control (CON) and immobilization groups (IM, n = 10 for each group). IM mice were subjected to a combination of cast immobilization and hindlimb suspension for 20 days. After the intervention period, neuromuscular transmission index (the ratio of evoked ankle plantar flexion torques between supramaximal nerve and muscle stimulations) was measured in anesthetized mice. Subsequently, mice were dissected to measure the wet weight of the lower hindlimb muscles. NMJ morphology and myofiber composition in these muscles were analyzed with immunohistochemistry. We found that the wet weights of the gastrocnemius, plantaris, and soleus muscles were significantly smaller in IM than in CON mice. There was no significant difference in the plantar flexion torque normalized by the wet weight of the lower hindlimb muscles between the groups. However, the neuromuscular transmission index at 50-, 100-, and 200-Hz stimulation was significantly lower in IM mice than in CON mice. IM mice had a significantly higher proportion of denervated myofibers in the plantaris muscle than CON mice. The proportion of type I fibers in the plantaris was significantly higher in IM than in CON mice. These results suggest that physical inactivity increases the number of denervated myofibers and impairs signal transmission at NMJ. The increase in the number of denervated myofibers may be related to the shift in myofiber composition toward a slower phenotype, also contributing to the muscle weakness.

Disclosures: T. Yamaguchi: None. **K. Sasaki:** None. **K. Nakazato:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.11/Web Only

Topic: E.09. Motor Neurons and Muscle

Title: Retrospective analysis of postviral dysphagia using FEES

Authors: C. FOREK[1], M. STEINBACH[2], B. AL KADAH[3], *D. M. WEINERT[4], H. BENNEFELD[1];
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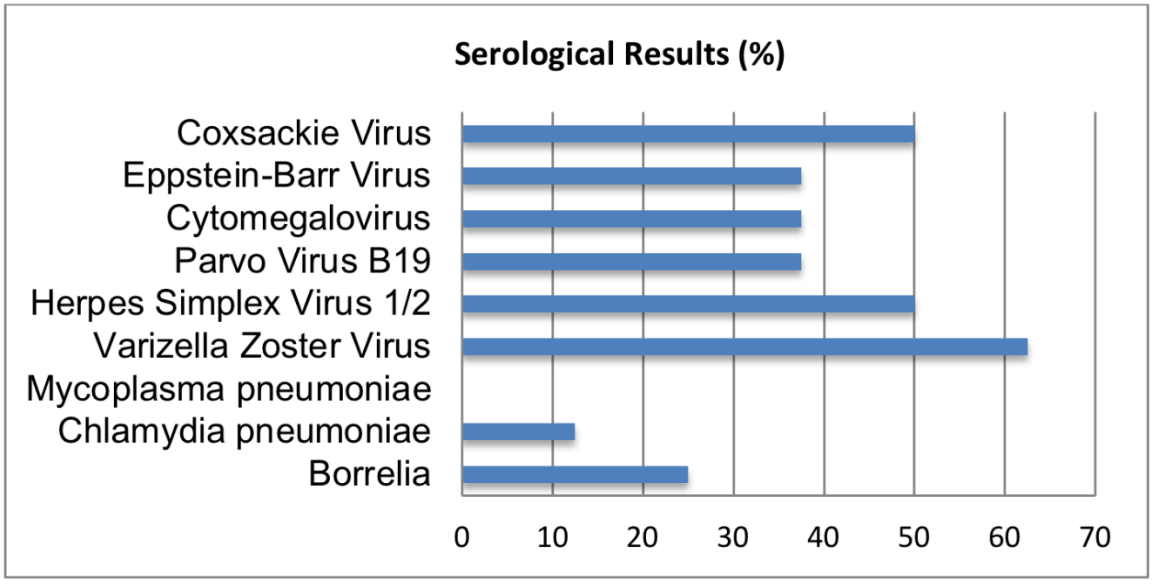
Abstract: How to proceed in the treatment of a dysphagia patient? Swallowing disorders can have multiple causes: neurological conditions (e.g. a stroke), injuries or esophageal diverticula but also bacterial and viral infections. Nowadays, the prevalent ENT examination method employed in patients with swallowing disorders as well as neurodegenerative and neuromuscular conditions is the flexible endoscopic evaluation of swallowing (FEES). It has also been utilized in the present study in order to determine a correlation between bacterial and viral infections and dysphagia. Patients with unclear dysphagia and no therapeutic success by previous treatment approaches were admitted to the early neurological rehabilitation clinic. The clinical symptoms were evaluated and viral serological laboratory tests were done and a swallowing diagnosis via FEES was successfully performed. 20 patients (10 male/10 female) with detected infective viral or bacterial agents (Fig.) received both an antiviral infusion and a functional logopedic dysphagia therapy. Patients underwent a final clinical evaluation and 14 of them were reevaluated with a FEES examination later. All participants had positive serological results with high antibody titers against viruses such as Varizella zoster, Herpes simplex and Coxsackie, as a sign of reactivated infection.



Evaluation of the results was done with the Bogenhausen dysphagia score (BoDS). Before therapy and medication, the test revealed the following severity of the score: 43% (light), 21% (moderate) and 36% (severe). After a 2-weeks treatment the results showed 36% (no), 43% (light), 7% (moderate), 14% (severe), respectively.
Determining the cause is crucial to the success of the treatment performed. The final FEES examination showed noticeable improvement in patients owing to the functional dysphagia

therapy. The study observes a significant correlation between postviral infections and neurological swallowing disorders. Only patients with viral infections improved significantly.

Disclosures: C. Forek: None. **M. Steinbach:** None. **B. Al Kadah:** None. **D.M. Weinert:** None. **H. Bennefeld:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.12/JJ15

Topic: E.09. Motor Neurons and Muscle

Support: JSPS KAKENHI Grant Number 22KK0139
JSPS KAKENHI Grant Number 20KK0353

Title: Activation of motor neurons of posterior belly of digastric muscle during swallowing.

Authors: *Y. TSUTSUI, T. TSUJIMURA, K. PIRIYAPRASATH, T. CHOTIRUNGSAN, J. MAGARA, K. OKAMOTO, K. YAMAMURA, M. INOUE;
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Abstract: Backgrounds and aims Although suprahyoid muscles are known to be activated during swallowing, functional role of posterior suprahyoid muscles such as posterior belly of digastric muscle (PostDig) or stylohyoid muscle has not yet been understood. The aim of this study was to clarify the location of PostDig motor neurons in the brain and their activities during swallowing. **Methods** Experiments were carried out on Sprague-Dawley male rats. The first experiment was designed to characterize the neurons in the accessory facial nucleus (Acs7) associated with swallowing reflex shown below: (1) c-Fos immunoreactivities evoked by repetitive mechanical stimulation of the larynx: (2) immunoreactivities of retrograde tracer, Fluoro-Gold (FG), administered into the PostDig muscle: (3) quantification of the number of both c-Fos and FG positive cells: (4) immunoreactivities of choline acetyltransferase (ChAT). In the second experiment, electromyographic (EMG) activity in PostDig and thyrohyoid muscles were recorded during swallowing evoked by mechanical stimuli to the vocal folds. In the third experiment, extracellular neural recordings in the Acs7 was conducted by following procedures: (1) detection of bursts of the trigeminal motor nucleus (Vmo) by jaw stretching as a reference point: (2) identifying of Acs7 activities responded to antidromic stimulation of PostDig branch of facial nerve: (3) investigating the Acs7 activities during swallowing evoked by electrical stimulation of the superior laryngeal nerve (SLN), topical administration of distill water (DW) or capsaicin to the pharynx. **Results** A substantial level of c-Fos expression was found in rostral rather than caudal Acs7 after swallowing. Quantitative analysis revealed the number of c-Fos cells was significantly greater than that of sham rats. Further, c-Fos positive cells were also found in the nucleus tract solitarius and nucleus ambiguous. ChAT immunoreactivity was identified in the Acs7. FG labeled cells were clearly found in the Acs7, and several c-Fos/FG

double-labeled cells were identified in the rostral Acs7. EMG bursts were observed in the Post-Dig and thyrohyoid muscles during swallowing, while neural activities in the Acs7 were increased during swallowing. **Conclusions** Our current results indicate Acs7 and PostDig muscle were activated during swallowing.

Disclosures: **Y. Tsutsui:** None. **T. Tsujimura:** None. **K. Piriyaprasath:** None. **T. Chotirungsan:** None. **J. Magara:** None. **K. Okamoto:** None. **K. Yamamura:** None. **M. Inoue:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.13/JJ16

Topic: E.09. Motor Neurons and Muscle

Support: CONACyT Grant 1311312 to DLCQ

Title: Electrical activity of the external urethral sphincter muscle in response to mechanical stimulation of the rabbit urogenital tract: effect of ventral L6-S2 root avulsion

Authors: *O. SANCHEZ GARCIA[1], A. FLORES-HERNÁNDEZ[2], F.-L. ZAMANTHA[1], R. ZEMPOALTECA[1], I. JIMÉNEZ-ESTRADA[3], M. MARTÍNEZ-GÓMEZ[1,4], D. CORONA-QUINTANILLA[1];
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Abstract: The spinal cord is a center specialized in receiving and sending information. The lumbosacral level (L6-S2), it integrates excitation-inhibition reflexes to regulate different functions of the lower urinary tract and pelvic floor musculature. Nerve roots that emerge from the ventral horn of the spinal cord and these have a certain fragility. The axons carry information to effectors, such as viscera and striated pelvic floor muscles. However, ventral roots are susceptible to a variety of mechanical damage, e.g, their stretching or rupture (avulsion) interrupts the transmission of bioelectrical signals between the central nervous system and effectors. One of the main striated muscles is to maintenance of urinary continence, as is the external urethral sphincter (EUS). In female rabbits, the EUS is activated during storage and inhibited during voiding. This function is similar to occurs in women without spinal cord injury or peripheral nerve level injury. In fact, in various species, sensory stimulation has been shown to trigger reflexes that influence pelvic viscera function in neonates and adulthood. This work aimed to determine the activity of the urethral external sphincter during stimulation of the urogenital tract of the rabbit and the effect of L6-S2 ventral root avulsion. For this purpose, the female rabbits were anesthetized and to made electromyogram recordings of EUS in response to

mechanical stimulation of the bladder, urethra (proximal, medial, and distal), as well as the perigenital, perineal vagina, pelvic and abdominal skin. In this study, we demonstrated that stimulation of both the proximal urethra and pelvic vagina triggers a higher frequency of EUS activity compared with another regions of the urogenital tract (urinary bladder, medial and distal urethra). After L6-S2 ventral root lesion, the pelvic vagina stimulation produces only a few bursts of EUS. Possibly, at the level of the proximal urethra as well as pelvic vagina more sensory receptors are located that allow EUS activity reflex to exert a greater force at this level for the maintenance of urethral closure, and to avoid episodes of involuntary urine expulsion.

Disclosures: O. Sanchez Garcia: None. **A. Flores-Hernández:** None. **F. Zamantha:** None. **R. Zempoalteca:** None. **I. Jiménez-Estrada:** None. **M. Martínez-Gómez:** None. **D. Corona-Quintanilla:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.14/JJ17

Topic: E.09. Motor Neurons and Muscle

Support: NIH

Title: Investigating heterogeneity of neuromuscular resiliency in aging wildtype C57BL/6 mice

Authors: *F. B. DARVISHI[1], A. ROSHANI DASHTMIAN[1], P. J. BOBBILI[2], D. CHUGH[2], C. IYER[2], K. RICH[2], H. HARRIS[2], W. ARNOLD[1];
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Abstract: The world-wide population over the age of 60 years is expected to double by 2050. Chronological aging is a major risk factor for accumulation of adverse health conditions, but cumulative effects of aging (biological age) are heterogenous across individuals. Chronological age often receives greater research focus as compared with biological age, particularly in preclinical studies. Here we aimed to investigate the heterogeneous effects of chronological aging on neuromuscular function in aging wild type mice. We retrospectively examined a total of 303 wildtype C57BL/6 mice from 4-30 months, and divided them into six age cohorts: 4-6, 11-13, 19-21, 22-21, 25,27, and 28-3 months. A comprehensive set of electrophysiological assessments were performed in gastrocnemius muscle included muscle excitation (compound muscle action potential or CMAP), motor unit number estimation (MUNE), average single motor unit potential size (SMUP), and neuromuscular junction transmission (single fiber electromyography jitter and blocking). Additionally, in vivo plantarflexion muscle contractility was assessed. We then applied a K-cluster analysis for stratifying resilient and frail groups and compared muscle bulk transcriptome profiles between groups. Our data demonstrates a chronological age effect on the neuromuscular function with notable heterogeneity of biological age accumulation. We observed a significant reduction of MUNE at the 19 months through 27

months compared with younger mice (4-6 months) ($p<0.0001$). Muscle excitation (CMAP) showed a significant decline at 25-27 months ($p<0.0001$). An initial increase in the SMUP at 19-21 months ($p<0.0001$) but a reduction later at 28-30 months suggests collateral sprouting as an initial compensatory mechanism for the loss of motor neurons that is lost in very old animals. Plantar flexion torque showed a significant decline starting at 11-13 months ($p=0.03$) through 28-30 months. NMJ transmission failure occurred later from 25-27 months indicated by significant increase in the Jitter (variability of NMJ transmission, $p<0.0001$) and blocking (failure of NMJ transmission, $p<0.0001$). Altogether, our studies reveal a remarkable decline in motor output, MUNE, and muscle contractility with age. Importantly, our data show remarkable heterogeneity during aging. Transcriptomic signatures demonstrate significant differential gene expression in the resilient versus frail mice (at similar chronological age) that could be targeted to potentially mitigate frailty.

Disclosures: F. B. Darvishi: None. **A. Roshani Dashtmian:** None. **P. J. Bobbili:** None. **D. Chugh:** None. **C. Iyer:** None. **K. Rich:** None. **H. Harris:** None. **W. Arnold:** 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.; NMD Pharma, Avidity Biosciences. F. Consulting Fees (e.g., advisory boards); Dyne Therapeutics, La Hoffmann Roche, Novartis, Genentech, Design Therapeutics, Cadent Therapeutics, Catalyst Pharmaceuticals.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.15/JJ18

Topic: E.09. Motor Neurons and Muscle

Support: The Nakatomi Foundation Grant

Title: Abnormal Mechanical Force with Cerebral Palsy Causes Muscle and Tendon Degeneration in Postnatal Phase in Mice

Authors: *Y. USAMI[1], T. KOKUBUN[2];
[1]Saitama Prefectural Univ., Saitama, Japan; [2]Grad. Sch. of Health, Medicine, and Welfare, Saitama Prefectural Univ., Koshigaya City, Saitama, Japan

Abstract: INTRODUCTION: Joint contracture is a common problem in patients with cerebral palsy (CP). Generally, joint contracture is caused by the shortening of muscles, tendons, ligaments, and joint capsules or by heterotopic ossification, regardless of paralysis type, both rigid and flaccid paralysis. However, in CP patients, the developmental change also affects all the tissues, so the mechanism of contracture is complicated. Therefore, there are no effective intervention strategies for muscle-tendon degeneration in CP to prevent and treat contracture. Because of the developmental phase, abnormal muscle tonus in CP induces delaying rolling over

and gait initiation. These also cause a reduction of tensile stress applied to the muscles and tendons with joint mobility depending on weight bearing. To reveal the pathological mechanism of joint contracture, we developed a novel rodent model to inhibit joint movement with internal fixation. Here, we aimed to investigate the pathogenesis of abnormal muscle tendon development in flaccid paralysis models to reveal the effect of muscle contraction.**METHODS:** All experiment procedures were approved by the Animal Experimental Committee. Postnatal day seven wildtype C57BL/6 mice were divided into three different models; Sham, sciatic nerve denervation model (DN) as Flaccid paralysis, and both DN and Ankle Arthrodesis Mode (D/A) to limit joint movement. We evaluated at the 7 and 14 days after the intervention. Histological imaging was stained with Alcian blue, Hematoxylin, and Eosin. Immunohistochemistry (IF) and imaging were incubated in primary antibodies Collagen type I and My32 and secondary antibodies Dylight588 and Cy3 secondary detection, removing unwanted fluorescence. We also stained with DAPI for counterstaining to visualize cell nuclei.**RESULTS:** DN and D/A muscle showed apparent atrophy in gross observation and histological analysis. The tendon width of the D/A group at d14 was significantly narrower than the Sham group. Histological evaluation showed collagen type I, the main component of the tendon, decreased in the D/A model.**DISCUSSION:** Our results showed that decreasing muscle contraction and tensile stress due to weight-bearing induced muscle-tendon degeneration like a CP patient. This indicates that even CP patients who have difficulty controlling muscle contraction might be able to prevent muscle-tendon degeneration through joint movement with appropriate weight-bearing induction. These results suggest that rehabilitation can prevent muscle-tendon degeneration and joint contractures by compensating for these mechanical stresses.

Disclosures: **Y. Usami:** None. **T. Kokubun:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.16/JJ19

Topic: E.09. Motor Neurons and Muscle

Support: NSF CBET 1932192

Title: Neuroactive Biologics Secreted from Neuromuscular Engineered Tissue Mimics

Authors: *K.-Y. HUANG[1], G. UPADHYAY[1], Y. AHN[1], M. SAKAKURA[1], G. PAGAN DIAZ[1], Y.-H. CHO[2], C. HUANG[1], A. WEISS[1], M. U. GILLETTE[3], J. SWEEDLER[1], S. IM[2], R. BASHIR[1], G. POPESCU[1], M. GAZZOLA[1], H. KONG[1];
[1]Univ. of Illinois, Urbana-Champaign, Urbana, IL; [2]Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [3]Dept. of Cell & Developmental Biol., Univ. of Illinois, Urbana-Champaign Neurosci. Program, Urbana, IL

Abstract: Physical activity has been shown to be advantageous to brain health, improving cognitive function and reducing the risk of neurological disorders. One of the important factors is the endocrine capacity of skeletal muscle. Skeletal muscles can secrete cytokines and small proteins, so-called myokines, in response to the contraction. Irisin and brain-derived neurotrophic factor (BDNF) derived from muscles promote brain activity and neurogenesis. Besides, skeletal muscle-derived exosomes containing proteins and micro-RNAs (miRNA) regulate brain function.
The neuromuscular junction (NMJ) is a chemical synapse between a motor neuron and a muscle fiber. At the junction site, neurons transmit a stimulatory or inhibitory signal to the muscle to control muscular contraction. Patients with muscular dystrophy and neuromuscular disorders often encounter multi-organ dysfunction and cognitive dysfunction. These complications are likely due to the abnormal level of muscle-secreting factors mediated by neural impulses.
In this work, we reproduced the neuron-innervated muscles to address the extent to which neural innervation affects myokine secretion and subsequently modulates the physiological activities of muscles and other organs. The muscles were cultured on a grooved substrate with a myofibril-like pattern and co-cultured with neural stem cell-derived motor neurons to recreate the high-functional NMJs. We examined the change in myokine gene expression and secretion between neuron-innervated muscles and neuron-free muscles, as well as exosome secretion. The neuron-innervated muscles showed more contracting areas in response to glutamate stimulation and secreted higher levels of irisin and exosome than the neuron-free muscle. Several miRNA cargos in the neuron-innervated muscle-derived exosomes regulated the nerve system development. The collective biologics from neuron-innervated muscle increase the neuron-to-glia ratio, axonal transport activity, and electrobiological activity of in vitro hippocampal neuron culture. The results of this study will be useful for better understanding the crosstalk between muscles and neurons and exploring future therapy for neural disorders.

Disclosures: **K. Huang:** None. **G. Upadhyay:** None. **Y. Ahn:** None. **M. Sakakura:** None. **G. Pagan Diaz:** None. **Y. Cho:** None. **C. Huang:** None. **A. Weiss:** None. **M.U. Gillette:** None. **J. Sweedler:** None. **S. Im:** None. **R. Bashir:** None. **G. Popescu:** None. **M. Gazzola:** None. **H. Kong:** None.

Poster

PSTR220. Muscle Physiology and Biochemistry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR220.17/JJ20

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant R01DK125543

Title: Urothelium-derived prostaglandins mediate phasic contractions and afferent nerve activity during urinary bladder filling in the mouse.

Authors: H. J. FALLON[1], E. M. BEAULIEU[1], G. W. HENNIG[1], T. J. HEPPNER[1], M. T. NELSON[1], *G. M. HERRERA[2];
[1]Pharmacol., [2]Univ. of Vermont, Burlington, VT

Abstract: The transitional epithelial cells (urothelium) that line the lumen of the urinary bladder form a barrier between potentially harmful pathogens, toxins, and other urine contents and the remaining layers of the bladder wall. The urothelium, however, is not simply a passive barrier, but also produces signaling factors, such as ATP, nitric oxide, and prostaglandins, that can modulate bladder function. We sought to test the hypothesis that the urothelium could modulate the contractility of the underlying urinary bladder smooth muscle, independent of the effects on nerves. Force was measured in isolated strips of mouse urinary bladder in the presence or absence of urothelium. Bladder strips developed spontaneous tone and phasic contractions. In urothelium-intact strips, basal tone, as well as the frequency and amplitude of phasic contractions, were 25%, 32%, and 338% higher than in urothelium-denuded strips, respectively. Basal tone and phasic contractility in urothelium-intact bladder strips were abolished by the cyclooxygenase inhibitor indomethacin (10 μM) or the voltage-dependent Ca^{2+} channel blocker diltiazem (50 μM), whereas blocking neuronal sodium channels with tetrodotoxin (1 μM) had no effect. These results suggest that prostaglandins produced in the urothelium enhance smooth muscle tone and phasic contractions through pathways involving voltage-dependent Ca^{2+} channels in the adjacent bladder smooth muscle. Furthermore, indomethacin greatly attenuated the afferent nerve activity during bladder filling, suggesting that urothelial prostaglandins may play a role in sensory nerve signaling. This work was supported by National Institutes of Health Grant R01DK125543.

Disclosures: H.J. Fallon: None. **E.M. Beaulieu:** None. **G.W. Hennig:** None. **T.J. Heppner:** None. **M.T. Nelson:** None. **G.M. Herrera:** None.

Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.01/JJ21

Topic: F.01. Neuroethology

Support: ERC CoG 726280
ANR-10-LABX-54 MEMO LIFE
ANR-11-IDEX-0001-02 PSL
École des Neurosciences de Paris Ile-de-France

Title: Radial astrocyte synchronization modulatesthe visual system during behavioral state transitions

Authors: *A. URIBE[1], G. SUMBRE[2];
[1]Ecole normale superieure, Paris, France; [2]Neurosciences, Ecole Normale Superieure, Paris, France

Abstract: Glial cells support the function of neurons. Recent evidence show that astrocytes are also involved in brain computations. To explore whether and how their excitable nature affect brain computations and motor behaviors, we used two-photon Ca2+ imaging of zebrafish larvae expressing GCaMP in both neurons and Radial Astrocytes (RAs). We found that in the optic tectum, RAs synchronize their Ca2+ transients immediately after the end of an escape behavior. Using optogenetics and ablations, we observed that RA synchronous Ca2+ events are mediated by the locus-coeruleus-norepinephrine circuit. RAs synchronization modulated the direction selectivity of tectal neurons and their long-distance functional correlations. This mechanism may support freezing behavior following a switch to an alerted state and improve visual detection. These results show that LC-mediated neuro-glia interactions modulate the visual system during transitions between behavioral states.

Disclosures: A. Uribe: None. **G. Sumbre:** None.

Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.02/JJ22

Topic: F.01. Neuroethology

Support: HORIZON-MSCA-2021-PF-01: 101066743

Title: Neural circuit and architecture underlying sensory processing and learning in the zebrafish pallium.

Authors: *A.-T. TRINH[1], I. DEL CASTILLO BERGES[1,2], B. SERNEELS[1], A. M. OSTENRATH[1], E. YAKSI[1];
[1]Kavli Inst. for Systems Neurosci., Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [2]Univ. of Maastricht, Maastricht, Netherlands

Abstract: Sensory information is first processed by various thalamic nuclei before reaching the pallium in amniotes. In teleost fish, the structure that is homologous to the amniote thalamus, the dorsal thalamic nuclei projects primarily to the subpallium. In contrast, the major sensory inputs (for example, visual inputs) from the teleost diencephalon to the pallium originate from the preglomerular nucleus (PG). The PG in teleost fish has thus been suggested to perform analogous functions to thalamocortical computations in amniotes. To examine this, we first mapped the anatomical connections from PG to palium using local tracer injections, confirming the PG ipsilateral projections to the dorsal lateral (DL) and dorsal medial (DM) pallium of juvenile zebrafish which are homologous to the mammalian hippocampus and amygdala respectively. Next, we tested the function of these PG inputs, by stimulating PG neurons and

imaging calcium signals in the entire juvenile zebrafish forebrain. PG microstimulations caused spatially restricted activity in DL, DM and the dorsal anterior regions of the telencephalon (DA). Notably, the DM, DL and DA responses exhibited different temporal dynamics, outlasting the transient PG microstimulation responses. These "persistent" neural responses were also found in the same brain regions when head-fixed juvenile zebrafish were presented with various sensory stimuli. Finally, the presentation of different sensory stimuli elicits different responses in the PG of these fish suggesting that this nucleus may encode multimodal information that would then activate distinct cell groups in the pallium that are homologous to the amniote hippocampus and amygdala. We are currently analyzing the temporal features of these forebrain ensembles upon PG, DL and DM microstimulations and salient sensory stimulation to see if distinct temporal sequences of forebrain neural responses are triggered and whether experience-dependent processes can facilitate them.

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Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.03/JJ23

Topic: F.01. Neuroethology

Support: CIHR

Title: Vision loss significantly alters how mice perform everyday tasks

Authors: *N. ARNOLD[1], M. WAN[1], A. VILLEMAIN[2], S. TRENHOLM[3];
[1]IPN, [2]McGill Univ., Montréal, QC, Canada; [3]McGill Univ., Montreal, QC, Canada

Abstract: Background: Mice are a leading animal model in visual neuroscience. However, relatively little remains known about the extent to which lab mice use vision during their day-to-day life. To address this issue, we performed a diverse set of behavioral tests that span the scope of 'natural' behaviors for a lab mouse, undertook detailed analyses of their behaviors, and compared results between sighted and blind mice. **Methods**: The behavioral tests include open-field and novel object exploration tests, mating and aggression assays, and a screen of maternal behavioral (i.e. pup-retrieval test). All behaviors were recorded with an overhead camera. Mice were then tracked using DeepLabCut and their behaviors classified using simBA. Detailed statistical analyses were then performed on the simBA outputs to define differences in behaviors between sighted and blind (and male and female) mice. **Results and Conclusions**: Preliminary results indicate significant differences in many aspects of each behavioral test studied, both between male and females, and blind and sighted mice. These results indicate the importance of vision in every aspect of a lab mouse's daily life, and provide insights into novel 'naturalistic'

visual behavioral paradigms that could be further investigated at the cellular, circuits and systems levels.

Disclosures: **N. Arnold:** None. **M. Wan:** None. **A. Villemain:** None. **S. Trenholm:** None.

Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.04/JJ24

Topic: F.01. Neuroethology

Support: HHMI

Title: Optimizing virtual reality for visual behavior experiments in tethered walking flies

Authors: *O. WADHWA[1,2,3], F. LOESCHE[2], M. B. REISER[2];
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Abstract: When shown a rotating grating pattern, *Drosophila melanogaster* moves in the direction of the pattern. This is known as the optomotor response. It is a robust response and has been observed over multiple experimental conditions. Here, I obtain replicable data of the robust optomotor response of flies on an inexpensive fly-on-ball setup, thus validating the setup. I also characterize the intensity of the stimuli at which flies show optomotor responses on the setup. Characterizing the setup increases the replicability of experiments by having multiple fly-on-ball setups using a low budget. Experimenters have used virtual reality environments to solve neuroscience problems. LED arenas and projectors have been used to show virtual reality environments to study behavior. Here, I used a single tablet to stimulate flies visually and study their responses on the inexpensive setup. I attempted to optimize the virtual reality of two tablets to replicate optomotor experiments on the inexpensive fly-on-ball setup. This increases the field of view of the stimuli on the fly eye and opens up a repertoire of experiments that researchers can do on inexpensive setups to study visual circuitry. In the single tablet setup, I obtained symmetric responses in the case when I placed the tablet in the front and asymmetric responses when I placed it at the side of the fly. When the tablet was placed in the front of the fly, the stimuli were lateral to the fly, while when the tablet was at the side, the stimuli had a component along the anteroposterior axis of the fly. The result of asymmetric responses corroborates previous literature, where it has been shown that flies have a greater optomotor response to stimuli moving from the front-to-back than those moving from the back-to-front. In the two-tablet setup, I obtained unexpected results. I obtained asymmetric responses where I expected symmetric responses and symmetric responses where I expected asymmetric responses. Three plausible factors of the cause of these unexpected results are reflections between the two tablets, differences in brightness levels between the tablets, and a larger distance of the stimulus in the two-tablet case than in the one-tablet case. After minimizing reflections and standardizing

brightness levels in both the tablets, I still obtained a few unexpected responses of low magnitude. In order to see if distance of the tablets affected the optomotor responses, I changed the distance between the tablet and the fly in the single tablet case when it is placed in the front. This experiment revealed that optomotor responses decreased when the tablet is placed farther away from the fly.

Disclosures: O. Wadhwa: None. **F. Loesche:** None. **M.B. Reiser:** None.

Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.05/JJ25

Topic: F.01. Neuroethology

Support: NSF IOS-1656714

Title: Neural correlates of visual object classification in the jumping spider brain

Authors: *A. M. WINSOR[1], L. REMAGE-HEALEY[2], R. R. HOY[3], E. M. JAKOB[4]; [2]Dept. of Psychological and Brain Sci., [1]Univ. of Massachusetts, Amherst, MA; [3]Dept. of Neurobio. and Behavior, Cornell Univ., Ithaca, NY; [4]Dept. of Biol., Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: Many animals use visual information to classify objects into appropriate categories such as food, predators, or social partners. Important questions include whether animals use particular features or combinations of features to identify objects, and where in the brain features are processed. We explore these questions in the modular yet highly integrated visual system of jumping spiders (Araneae: Salticidae). Salticids have six secondary eyes that serve as motion detectors with wide fields of view, and a forward-facing pair of principal eyes capable of high spatial resolution and color vision. The principal-eye retinas have narrow visual fields, but reside at the back of mobile tubes that enable saccades and scanning motions to inspect objects. Together, the two eye types are functionally analogous to our peripheral and foveal vision. In *Phidippus audax*, we monitored gaze direction using our custom-built eyetracker while simultaneously collecting the first reported dual extracellular recordings of lower and higher-order optic neuropils. We presented spiders with a randomized stimulus set of ecologically relevant holistic objects, including insect prey species, conspecifics, heterospecific salticids, predators, and unpalatable insects, as well as exemplars with stepwise complexity reductions that distill objects into their elemental components, to parse whether spiders respond to local features, global structure, or categories of objects. Our results across nine subjects show that multi-unit activity within both the lower-order (putative principal-eye lamina) and higher-order (putative arcuate body) brain regions are strongly driven by images of palatable prey and suppressed by images of unpalatable prey, even though stimuli within these categories varied widely in appearance. We have begun sorting higher-order single units and detected a ~68% neuronal

activation when the spider viewed palatable prey and a ~72% neuronal suppression when the spider viewed unpalatable prey, relative to the baseline firing rate. Moreover, neural responses to reduced images of flies suggest that simple features are extracted for recognition. Ongoing analyses examine responses within stimulus categories and to their simpler components, and correlations between retinal exploration of images (using DeepLabCut) and neural activity. We are now conducting behavioral trials to test if we can predict spider behavioral responses from their neural responses, and we are mapping connectivity of the optic tracts using dextran injections and synapsin immunostaining. In summary, our work reveals neural correlates of prey categorization in an invertebrate predator.

Disclosures: A.M. Winsor: None. **L. Remage-Healey:** None. **R.R. Hoy:** None. **E.M. Jakob:** None.

Poster

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Program #/Poster #: PSTR221.06/KK1

Topic: F.01. Neuroethology

Support: NSF DBI 2021795
NSF IOS-2212750

Title: To jump or not to jump: Comparing effects of phenotypic plasticity on visual responses and behavior between desert locusts & grasshoppers

Authors: *S. MITRA[1], M. EISENBRANDT[1], B. HALDER[1], R. B. DEWELL[1], F. GABBIANI[2]; [1]Baylor Col. of Med., Houston, TX; [2]Baylor Col. Med., Baylor Col. Med., Houston, TX

Abstract: To jump or not to jump: Comparing effects of phenotypic plasticity on the visual responses and behavior between desert locusts(*Schistocerca gregaria*) & grasshoppers (*S. americana*)**AuthorsSoumi Mitra**, , Margaret Eisenbrandt, Bidisha Halder, Richard Dewell Fabrizio Gabbiani, Dept. of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77054
keywords: DCMD, insect, looming, dendrite, locust, LGMD
AbstractLocusts exhibit remarkable phenotypic plasticity switching between solitary grasshoppers and gregarious locusts changing their appearance and behavior when population density increases. These changes include morphological differences in the size and shape of brain regions, but very little is known about changes within individual neurons and non-swarming behaviors. We examined looming escape behavior and the properties of a well-studied collision-detection neuron in isolated and crowded reared animals of two closely related species, the desert locust and the American bird grasshopper. In this collision-detection neuron, the lobula giant detector neuron (LGMD) we examined dendritic morphology, membrane properties and looming responses. Crowded reared animals reliably jump in response to looming stimuli,

but surprisingly isolated desert locusts did not produce escape jumps. These isolated animals also had smaller LGMD dendrites which we analyzed after intracellular injection of Alexa fluorophore. These behavioral, anatomical and physiological analyses are being conducted in parallel with transcriptomics and biophysical modeling and will ultimately lead to a much more detailed understanding of both the role of neuronal processing in escape behavior and the phenotypic plasticity of neuronal properties.

Disclosures: S. Mitra: None. **M. Eisenbrandt:** None. **B. Halder:** None. **R.B. Dewell:** None. **F. Gabbiani:** None.

Poster

PSTR221. Sensory Control of Behavior

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Program #/Poster #: PSTR221.07/KK2

Topic: F.01. Neuroethology

Support: NSF DBI-2021795
NIH R01NS130917
NSF IOS-2212750

Title: Single-cell transcriptomics of an identified looming-detection neuron in locusts identifies membrane ion channels critical for collision-avoidance behaviors

Authors: *R. B. DEWELL[1], J. LUO[2], D. BELLINI[3], C. ZONG[2], F. GABBIANI[1];
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Abstract: To determine the role of voltage-gated ion channels in neuronal computation related to collision-avoidance behaviors, detailed electrophysiological studies have been conducted on an identified looming-detecting neuron in grasshoppers. These studies have been hindered by a lack of genetic information, making channel identification harder and preventing use of modern genetic techniques for extrinsic neural modulation. Recent high quality genomes of the desert locust and the American bird grasshopper have opened new avenues for expanded multi-omic research in these animals. We have removed the somas of the identified lobula giant movement detector (LGMD) neuron from both species, including from desert locusts in both solitarious and gregarious phases, for single-cell RNA sequencing. The transcriptome data confirms the presence of voltage-gated ion channels previously characterized using electrophysiology and pharmacology, in addition to the identification of channel types. Comparison between the solitarious and gregarious phases of the desert locust allows us to explore the differential gene expression involved in their newly-discovered differences in escape behavior. Further, this transcriptomics data will allow us to develop new techniques for interrogating neural processing in these species. This will further expand our understanding of the role of voltage-gated ion channels in neuronal computation and the role of differential gene expression in phenotypic plasticity.

Disclosures: **R.B. Dewell:** None. **J. Luo:** None. **D. Bellini:** None. **C. Zong:** None. **F. Gabbiani:** None.

Poster

PSTR221. Sensory Control of Behavior

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Support: London Interdisciplinary Doctoral Programme (M.A.)
Biotechnology and Biological Sciences Research Council (BB/P007201/1, J.F.L.)

Title: Mice got rhythm: sound-evoked whisker, nose and pinna movements in the awake mouse and their relationship to auditory cortical activity

Authors: M. AKRITAS[1], ***S. S. SRIRANGA**[1], A. G. ARMSTRONG[1], J. M. LEBERT[1], A. F. MEYER[2], J. F. LINDEN[1,3];
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Abstract: The auditory system has often been described as an "early warning" system for the brain, optimised for fast detection of sound events occurring far away or outside the focus of attention. A possible behavioural correlate of this “early warning” in awake mice is sound-evoked whisker twitches (Meyer et al. 2018 Neuron) and other sound-evoked body movements (Bimbard et al. 2023 Nat Neurosci). What is the nature of these sound-evoked movements, and how do they relate to auditory cortical activity? We used sequences of noise bursts varying in sound intensity, predictability, and duration to analyse sound-evoked whisker, nose, and pinna movements in 7 awake head-fixed mice. In 4 of the animals, we also investigated the relationship between the sound-evoked movements and simultaneously recorded single-unit and multi-unit activity in the auditory cortex.Sound-evoked whisker, nose, and pinna movements did not resemble startle responses. The movements occurred even for quiet sounds and grew gradually with increasing sound intensity (25-70 dB SPL). Moreover, the movements were minimally affected by stimulus predictability or expectation. In fact, facial movements evoked by 65 dB SPL noise bursts were either similar for regularly and irregularly timed noise bursts (1s versus jittered 0.8-1.2s inter-onset intervals), or slightly but significantly larger for the rhythmic sounds. Further experiments involving long, variable-duration noise bursts (0.4-1.6s) showed that while sound onsets evoked robust increases in whisker, nose, and pinna movements, sound offsets only evoked increases in pinna movement. Finally, analysis of auditory cortical recordings revealed a small but significant subset of units (15% for whisker and pinna, 7% for nose) in which trial-to-trial variation in sound-evoked response magnitudes correlated (or anti-correlated) with trial-to-trial variation in sound-evoked movement magnitude.We conclude that sound onsets reliably evoke whisker, nose, and pinna movements in awake head-fixed mice, and sound offsets can also

evoke pinna movements. Moreover, trial-to-trial variation in the magnitude of sound-evoked movements is related to trial-to-trial variation in sound-evoked firing rates for a small but significant fraction of neurons in the auditory cortex.

Disclosures: M. Akritas: None. **S.S. Sriranga:** None. **A.G. Armstrong:** None. **J.M. Lebert:** None. **A.F. Meyer:** None. **J.F. Linden:** None.

Poster

PSTR221. Sensory Control of Behavior

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Program #/Poster #: PSTR221.09/KK4

Topic: F.01. Neuroethology

Support: AU ORCS FRG 11-2011-75-201160

Title: Protein kinase modulation of the neurocircuitry underlying phonotaxis in the cricket Acheta domesticus

Authors: *L. KIM, L. WIDDICOMBE, D. MBUNGU, B. NAVIA;
Dept. of Biol., Andrews Univ., Berrien Springs, MI

Abstract: Juvenile hormone is a key regulator in the development, physiology and behavior of insects, but little has been reported about the mechanisms of action of this hormone in the cricket model system. In the species *Acheta domesticus,* young, virgin females with elevated levels of juvenile hormone, respond selectively to computer-generated calls which mimic the calls of conspecific males. As these females age, they become increasingly less selective, responding to a wider range of calls. This reduction in their ability to discriminate attractive calls has been correlated with reduced levels of juvenile hormone. Similarly, a decline in juvenile hormone levels has been demonstrated to influence the response of the L3 prothoracic auditory neuron in *A. domesticus.* As these crickets age, both the quantity and pattern of response in L3 changes. L3 is a morphological and functional homolog of the Ascending Neuron 2 (AN2) described in other cricket species. It is presumably a key constituent of the prothoracic auditory network responsible for encoding and processing information which it then sends to the brain. In *A. domesticus,* this neuron has been posited to play a role in selective phonotaxis as demonstrated by experiments where inactivation of L3 in previously selective females caused them to behave unselectively. Although the details of the precise molecular targets of juvenile hormone in the cricket nervous system remain to be discovered, we know that one of the ways in which juvenile hormone elicits its actions in other insects is through a protein kinase C (PKC) signaling pathway. Using a pharmacological approach, we have tested the effect of manipulating PKC activity on L3's spiking and on the phonotactic behavior of *A. domesticus.* Bath application of the synthetic biomolecule 1-(5-isoquinolinyl-sulfonyl)-2- methylpiperazine (H7), which has been shown to inhibit PKC, resulted in the inhibition of L3's spiking activity. This suppression was reversed upon removal of H7. Further, intra-ganglionic nano injection of H7 to selective crickets

reduced their phonotactic responsiveness across the full range of syllable periods presented, which contrasted with the control group which remained phonotactically selective following nano injection of saline. Taken together, the findings of the H7-induced decline in L3 activity, the H7-induced decline in phonotactic responsiveness and the already demonstrated juvenile hormone associated regulation of phonotaxis, point to L3 being part of the neural network under modulation by a complex interplay of regulatory agents including second messenger signaling cascade.

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Poster

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Title: The Anterior Cingulate Cortex Top-down Modulates Auditory Responses in the Auditory Cortex through Direct and Indirect Pathways in Mice

Authors: *Y. LIANG[1,2], J. LI[1], Y. TIAN[1], P. TANG[1,2], J. HE[1,3], X. CHEN[1,3];
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Abstract: The anterior cingulate cortex (ACC) is a crucial cognitive center that regulates neuronal activity in the brain. Our previous study showed that direct activation of ACC projections in the auditory cortex (ACx) enhances auditory responses and flight behavior in mice. This study discovered that the ACC promotes the long-term enhancement of auditory responses in the ACx through the lateral rhinal cortex (lRCx). Activation of ACC projections in lRCx facilitated sound-evoked flight responses in mice even after an hour. Additionally, we identified two distinct groups of neurons in the anterior ACC that separately project to the ACx and lRCx.Furthermore, the ACC was found to encode different types of information, such as air-puff and noise, and played a role in mice's auditory go-no-go tasks with sustained activity in the delay period. These results suggest that the ACC modulates the ACx with both direct and indirect strategies, contributing to higher-level sensory perception and cognition. Our findings provide critical insights into the ACC's top-down modulation of ACx processing, paving the way for further exploration of its diverse functions in the brain.

Disclosures: **Y. Liang:** None. **J. Li:** None. **Y. Tian:** None. **P. Tang:** None. **J. He:** None. **X. Chen:** None.

Poster

PSTR221. Sensory Control of Behavior

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Topic: F.01. Neuroethology

Support: JSPS KAKENHI 19K06762 to HA
17K19195 to TN and HA
17H06406 to TN

Title: Toxic pufferfish detect a nontoxic TTX analog, 5,6,11-trideoxyTTX, as an odorant.

Authors: *T. SUZUKI[1], Y. NOGUCHI[1], K. MATSUTANI[1], R. SAKAKIBARA[2], R. NAKAHIGASHI[2], M. ADACHI[2], T. NISHIKAWA[2], H. ABE[1];
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Abstract: Toxic pufferfish are widely known for their neurotoxin, tetrodotoxin (TTX), which has been reported to cause severe poisoning symptoms by blocking voltage-gated sodium channels when consumed. These toxic pufferfish accumulate TTX as a defensive substance, which they acquire through the food chain by preferentially feeding on TTX-bearing organisms. Several studies have reported that TTX attracts toxic pufferfish from the genus *Takifugu* via their olfaction. Why do these toxic pufferfish smell such toxins?
In this study, we demonstrated that toxic pufferfish do not detect TTX but rather perceive a nontoxic TTX analog, 5,6,11-trideoxyTTX (TDT), which is present alongside TTX in various

toxic pufferfish and their prey. Using electroolfactogram and behavioral experiments, we demonstrated that grass puffers (*Takifugu alboplumbeus*), a commonly found toxic pufferfish in the northwest Pacific Ocean, responded to TDT as an olfactory chemoattractant. Intriguingly, a similar attraction to TDT was observed in the green spotted puffer (*Dichotomyctere nigroviridis*), a phylogenetically and geographically distinct species from the grass puffer inhabiting brackish water in south and southeast Asia.
To identify the olfactory sensory neurons (OSNs) responsive to TDT detection, we performed double immunohistochemistry using antibodies against phosphorylated ribosomal protein S6 (pS6) as a neuronal activity marker and S100 as a crypt OSN marker. We found these antibodies labeled oval cells with apical invagination on the olfactory epithelium surface when exposed to TDT. To confirm that these oval OSNs respond to TDT odor, we transiently introduced GCaMP6s, a genetically encoded calcium indicator, into OSNs by electroporation. Calcium imaging demonstrated that the oval OSNs in the olfactory epithelium surface, similar to those labeled with S100 antibody, respond to TDT but not to amino acids mixture (AAs). AAs-responsive OSNs did not respond to TDT and displayed different morphology from TDT-responsive OSNs. Additionally, immunohistochemistry using the pS6 antibody indicated that the TDT-responsive cells are specifically localized within the dorsomedial region of the caudal olfactory bulb.
In summary, our findings suggest that TDT acts as an olfactory chemoattractant detected by crypt OSNs, and the attraction to TDT odor appears to be a characteristic feature among toxic pufferfish. These results provide valuable insights into the olfactory mechanism of toxic pufferfish, contributing to our understanding of their chemical communication, toxin accumulation (toxification), and evolutionary adaptations.

Disclosures: **T. Suzuki:** None. **Y. Noguchi:** None. **K. Matsutani:** None. **R. Sakakibara:** None. **R. Nakahigashi:** None. **M. Adachi:** None. **T. Nishikawa:** None. **H. Abe:** None.

Poster

PSTR221. Sensory Control of Behavior

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Program #/Poster #: PSTR221.12/KK7

Topic: F.01. Neuroethology

Support: AU ORCS FRG 11-2011-75-201160

Title: Pheromones as modulators of phonotaxis in female cricket Acheta domesticus

Authors: M. SUKUMARAN[1], D. MBUNGU[1], *B. NAVIA[2];
[1]Dept. of Biol., [2]Andrews Univ., Berrien Springs, MI

Abstract: Using crickets as model organisms in the investigation of communication and mating systems, researchers have gained insight relative to the vibration mechanisms of male crickets, signal transduction systems and signaling cascades in conspecific females. Insects, possess

capacity for multimodal signaling and there is evidence that at least in *Gryllus bimaculatus*, pheromones play an important role in mate choice. We have previously shown that young, virgin female *Acheta domesticus*, exhibits selective phonotaxis to attractive calls of conspecific males and their ability to discriminate between calls correlated with age of the female, syllable period of the call and environmental temperature. In this study, we have tested possible involvement of pheromones as modulators of phonotactic behavior in female *A. domesticus.* Following the imaginal molt, virgin females were immediately placed in one side of a double chamber, isolated from physical contact with males which were placed in the other adjoined chamber. Tiny holes in the partition wall allowed airflow between the two sides. After 6 - 7 days of exposure to males, females were tested for phonotaxis using a non-compensating treadmill. Our preliminary results showed that 57% of the crickets tested exhibited positive phonotaxis to at least one syllable period. Of this group, 5% responded to all syllable periods, while 95% responded selectively to 3 or less syllable periods that are within the most attractive range (50 - 70 ms). Analysis of the vectors and polar orientation diagrams of the non-phonotactic females, revealed that total distances walked were comparable to distances walked by those that responded with positive phonotaxis, but lacked particular directionality. These results are consistent with the view that phonotaxis is a complex behavior that can be modulated by multiple intrinsic and extrinsic factors, with sound being a principal contributor.

Disclosures: **M. Sukumaran:** None. **D. Mbungu:** None. **B. Navia:** None.

Poster

PSTR221. Sensory Control of Behavior

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Program #/Poster #: PSTR221.13/KK8

Topic: F.01. Neuroethology

Support: NIH Grant 1R01NS118406-01

Title: Multisensory contributions to social behavior in developing zebrafish

Authors: *S. J. STEDNITZ[1], A. LESAK[2], L. MAZZUCATO[3], P. E. WASHBOURNE[4], E. K. SCOTT[1];
[1]Anat. & Physiol., Univ. of Melbourne, Melbourne, Australia; [2]Physics, [3]Inst. of Neurosci., Univ. of Oregon, Eugene, OR; [4]Inst. of Neuroscience, Univ. of Oregon, Eugene, OR

Abstract: Social behavior is governed by multimodal sensory input and past experience, and ranges from simple pairwise interactions to thousands of individuals coordinating goal-directed movements. Regardless of the scale, the reciprocal nature of social interaction requires animals to actively attend to cues and respond appropriately for the context. We leveraged the zebrafish, a highly social and experimentally tractable model organism, to study freely-behaving pairwise interactions across development. Using unsupervised modeling we characterized the ontogeny of distinct reciprocal social behaviors over a narrow developmental window. We tested the

contributions of visual and mechanosensory input to specific behaviors, and measured morphological features to predict sociality. Finally, we utilize these techniques to understand how early developmental and genetic perturbations can alter the brain-wide sensory processing underlying social behavior. Altogether, we showed that reciprocal social interactions are reliably elicited in juvenile zebrafish early in development, and that specific social behaviors rely on different sensory modalities and distinct developmental milestones.

Disclosures: S.J. Stednitz: None. **A. Lesak:** None. **L. Mazzucato:** None. **P.E. Washbourne:** None. **E.K. Scott:** None.

Poster

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Program #/Poster #: PSTR221.14/KK9

Topic: F.01. Neuroethology

Support: Owens Family Foundation

Title: Chemoreception and Complex Social Behaviors in Drosophila Larvae

Authors: P. YEE[1], *B. CONDRON[2];
[1]Neurosci. Undergraduate Program, [2]Biol., Univ. of Virgina, Charlottesvle, VA

Abstract: Complex social behaviors are highly valued for their benefits in improving individual and group outcomes. Drosophila larvae exhibit social behaviors by forming cooperative clustering groups to optimize food digestion. The specific neural mechanisms underlying the decision to cooperate and participate in clustering behavior is unknown. With the current availability of a fully mapped connectome and experimental access to the majority of neurons, mapping circuits governing social behavior becomes a feasible prospect. Previous research has established vision as an important regulator in larval cooperation, but we propose that chemoreception also plays a significant role. In this study, we aim to investigate nutritional cues for cooperation and identify the neural circuits involved in evaluating this decision. By manipulating the concentrations of ingredients in larval food, such as agar, starch, sucrose, fructose, and protein sources, we created a medium that effectively promotes social behavior. Clustering was measured by the percent of larvae in clusters in a 2D assay during a 24-hour period. Preliminary results showed that the absence of yeast in the media leads to cannibalistic behaviors, emphasizing the importance of protein for both cooperation and group survival. Building on these findings, we have explored different protein and amino acid sources and determined their influence on larval behavior and adult fitness. Along with this, we have examined primary taste receptor neurons and identified components that appear to detect proteins. Drosophila larvae exhibit various social behaviors relevant to other animals, making the identification of critical neuronal circuits in this model organism valuable for understanding behavioral mechanisms across species. Ultimately, identifying the chemoreceptive circuits that

govern social behavior can pave the way for the development of a neural circuit-based model, shedding light on the decision-making processes underlying social behaviors in various animal species.

Disclosures: P. Yee: None. **B. Condron:** None.

Poster

PSTR221. Sensory Control of Behavior

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Program #/Poster #: PSTR221.15/KK10

Topic: F.01. Neuroethology

Support: National Science and Technology Council (MOST) Grant/ 111-2320-B-001 -008 -MY3

Title: Hypothalamic SF1-expressing neurons encode a conspecific-tuned, investigation-driving behavioral state

Authors: *S. LIN[1,2], H.-J. LEE[3], S.-B. YANG[1];
[1]Inst. of Biomed. Sci., Academia Sinica, Taipei, Taiwan; [2]Grad. Inst. of Mind and Brain Sci., Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; [3]Dept. of Biotech. and Animal Sci., Natl. Ilan Univ., Ilan, Taiwan

Abstract: The ventromedial hypothalamic nucleus (VMH) is a vital hub for various innate behaviors. Among various neuronal subtypes within the VMH, the estrogen-receptor-1-expressing neurons in the ventrolateral division (VMHvl) are well-known for their involvements in consummatory social behaviors. Another non-overlapping neuronal subtypes expresses steroidogenic factor-1 (SF1), and occupies the dorsomedial VMH (VMHdm). The VMHdm^{SF1} neurons are well-studied for their essential involvements in driving predator-orientated defensive state. Nevertheless, neuroanatomical evidences suggested that VMHdm^{SF1} neurons reciprocally connect with non-SF1 neurons in the VMH, and could potentially receive and process social-related sensory cues. However, the role of VMHdm^{SF1} neurons in regulating social behaviors remains elusive. We performed cell-type-specific *in vivo* calcium imaging in the VMH of adult male mice as they interacted with various external stimuli. We found that the VMHdm^{SF1} neurons were robustly activated by social- but not predator-associated stimuli, with a male-biased conspecific sex representation. In addition, conspecifics with different sexes selectively recruited distinct subpopulation of the VMHdm^{SF1} neurons. Through the ablation of specific olfactory pathways, we found that male-biased populational responses of the VMHdm^{SF1} neurons required VNO-relayed pheromonal signals, which are majorly transmitted to hypothalamus through the bed nucleus of stria terminalis (BNST). By optogenetically silencing the BNST-VMHdm pathway, we could diminish the male-preference among the VMHdm neural population. Moreover, VMHdm^{SF1} neuronal activities were highly correlated with social investigative behaviors. Altogether, we hypothesized that apart from defensive state, a large

portion of VMHdm^{SF1} neurons are capable of driving conspecific-triggered social investigation. The conspecific sex representation of VMHdm/c^{SF1} neurons may modulate animal's intend to investigate upon encountering other mice, which facilitates proper behavioral decision making by accessing more information.

Disclosures: S. Lin: None. **H. Lee:** None. **S. Yang:** None.

Poster

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Program #/Poster #: PSTR221.16/KK12

Topic: F.01. Neuroethology

Title: Characterizing cellular computation in terms of biophysical expression

Authors: *B. G. MEULEMEESTER;
Max Planck Inst. for Neurobio. of Behavior, Bonn, Germany

Abstract: Morphology and the distribution of ion channels on the dendrite are major determinants of cellular computation. How the interplay of spatially distributed ion channels affects somatic responses remains poorly understood. In general, similar cellular dynamics can be achieved with vastly different ionic currents, while minor variations in ionic currents can yield vastly different cellular dynamics. Here, we generate millions of biophysically detailed models of layer 5 pyramidal tract (L5PT) neurons, which map out the spectrum of possibilities of how channels can be distributed to achieve the characteristic dendritic and somatic electrophysiology of this celltype. We show how to utilise Explainable Artificial Intelligence (XAI) to reveal nonlinear multidimensional relationships between the distribution of channels and somatic output that can be empirically tested. Our approach thereby is an important step towards linking electrophysiological responses to their mechanistic origin.

Disclosures: B.G. Meulemeester: None.

Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.17/KK13

Topic: F.01. Neuroethology

Title: Broad receptive fields are the basis for efficient and robust population coding in cortex

Authors: *M. ROYO CANO[1], A. BAST[1], R. FRUENGEL[1], C. P. DE KOCK[2], M. OBERLAENDER[1];
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Abstract: The ability to combine sensory signals with internal information streams is a hallmark feature of the cerebral cortex, forming the basis for perception and behavior. It is thought that the pyramidal tract neurons in cortical layer 5 (L5PTs) are key for such combination processes. Along their extensive dendrites, these major cortical output cells combine information streams arriving at all layers, and then broadcast the results of this combination to several subcortical brain regions. It is yet unclear how populations of L5PTs encode information about stimulus features in their sensory-evoked responses. Here we show that sampling the fast responses simultaneously from any population of ~150 L5PTs anywhere within a primary sensory area is sufficient to decode any stimulus. We demonstrate that this robust and redundant encoding of stimuli relies on three properties that are characteristic for L5PTs: (1) fast responses that precede those in layer 4, (2) receptive fields that are much broader than those of their thalamocortical and cortical input neurons, and (3) the shapes of the broad receptive fields vary substantially between even neighboring L5PTs. Thus, while broad receptive fields lead to a loss of information at single cell level, we found that they are the basis for a highly efficient population code in L5PTs that differs from the one provided by their input neurons in thalamus and cortex - i.e., narrow receptive fields. Our findings set the stage to dissect how cortical output encodes sensory and internal information streams.

Disclosures: M. Royo Cano: None. **A. Bast:** None. **R. Fruengel:** None. **C.P. De Kock:** None. **M. Oberlaender:** None.

Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.18/KK14

Topic: F.01. Neuroethology

Support: Deutsche Forschungsgemeinschaft (grant SPP2041)
Deutsche Forschungsgemeinschaft (grant SFB1089)

Title: Organizing principles of cortical interneurons

Authors: *F. YÁÑEZ[1], F. MESSORE[2], G. QI[3], D. FELDMEYER[3], B. SAKMANN[4], M. OBERLAENDER[1];
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Abstract: Cortical inhibitory neurons are diverse. At the single-cell level, even attributes such as morphology and intrinsic physiology exhibit complex patterns of variation, making them

difficult to characterize. Here we systematically assess the degree and character of the variability of these properties across the entire cortical depth of the rat barrel cortex. We analyzed 306 morphological reconstructions and current injection responses, which represented the distribution of inhibitory neurons per depth. Each neuron was comprehensively characterized by morphoelectric features. We assigned inhibitory neurons into 13 intrinsic physiological classes, 20 morphological classes, and 25 morphoelectric classes using unsupervised multimodal clustering. These classes are consistent with previous reports in the mouse primary visual cortex. Soma depth is the primary determinant for defining morphoelectric types. The spatial extent of both axons and dendrites increases as a function of cortical depth, regardless of morphoelectric type. The spike-frequency also increases with cortical depth, whereas the spike-frequency adaptation remains unaffected by it. A simple depth-independent relationship, where the spike-frequency exceeds the spike-frequency adaptation, delineates a class of inhibitory neurons resembling the distribution of parvalbumin interneurons, including small to large basket, chandelier, and translaminar cells. The assignment based on depth-independent relationships shows a strong correspondence with the distributions of parvalbumin-, somatostatin-, and VIP-expressing interneurons in both rat barrel and mouse visual cortex. Thus, simple organizing principles may largely account for the diversity of inhibitory neurons through the adjustment of their morphoelectric properties in cortex.

Disclosures: F. Yáñez: None. **F. Messore:** None. **G. Qi:** None. **D. Feldmeyer:** None. **B. Sakmann:** None. **M. Oberlaender:** None.

Poster

PSTR221. Sensory Control of Behavior

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR221.19/KK15

Topic: F.01. Neuroethology

Support: NSF grant 1942960
NSF grant 1726534

Title: Sensory coding for detection and localization isinfluenced by the relationship between the spatial organization of receptors and signals.

Authors: *G. MARSAT[1], K. RAMACHANDRA[2];
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Abstract: Detection and localization of signals relies on arrays of receptors and their spatial organization plays a key role in setting the accuracy of the system. Electrosensory signals in weakly electric ghost knifefish are captured by an array of receptors covering their body. While we know that spatial resolution for small objects, such as prey, is enhanced near the head due to a high receptor density, it is not clear how receptor organization influences the processing of global and diffuse signals from conspecifics. We investigated the detection and localization

accuracy for conspecific signals and determined how they are influenced by the organization of receptors. To do so we modeled the signal, its spatial pattern as it reaches the sensory array, and the responses of the heterogeneous population of receptors. Our analysis provides a conservative estimate of the accuracy of detection and localization (specifically azimuth discrimination) of a conspecific signal. We show that beyond 20 cm the conspecific signal is less than a few percent the strength of the baseline self-generated signal. As a result, detection and localization accuracy decreases quickly for more distant sources. Detection accuracy at distances above 40 cm decreases rapidly and detection at the edge of behaviorally observed ranges might require attending to the signal for several seconds. Angular resolution starts to decrease at even shorter distances (30 cm) and distant signals might require behavioral or neural coding mechanisms that have not been considered here. Most importantly, we show that the higher density of receptors rostrally enhances detection accuracy for signal sources in front of the fish, but contributes little to the localization accuracy of these conspecific signals. We discuss parallels with other sensory systems and suggest that our results highlight a general principle. High receptor convergence in systems with spatially diffuse signals contributes to detection capacities, whereas in systems with spatially delineated signals, receptor density is associated with better spatial resolution.

Disclosures: **G. Marsat:** None. **K. Ramachandra:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.01/KK16

Topic: F.01. Neuroethology

Title: Gut microbes shape nesting behavior in passerine birds

Authors: *C.-Y. CHEN[1,2], S.-K. CHEN[1], C.-M. HUNG[2];
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Abstract: The bidirectional gut-brain communication, known as the gut-brain axis, plays a crucial role in integrating the gut and central nervous activities, with the gut microbiota acting as a key mediator. This concept of the microbiota-gut-brain axis has gained significant attention in recent years. Studies have revealed how gut microbiota can influence animal behavior by modulating various neurological processes, including neurotransmitter turnover, neurogenesis, and neuronal morphology. While the role of the oxytocin system and parenting behavior has been extensively studied in rodents, our understanding of how the microbiota-gut-brain axis influences parenting behavior, particularly before and in the absence of stimuli associated with parturition, egg laying, or offspring, remains limited. Nesting behavior in birds provides a robust model to investigate the modulatory effects of gut microbiota on early parenting behaviors. In this study, we aimed to explore the relationship among gut microbiota, neuronal activity, and nesting behaviors using two avian models: zebra finch (*Taeniopygia guttata*) and society finch (*Lonchura striata domestica*). Through 16S rRNA amplicon sequencing, we observed variations

in the composition of gut microbiota between the nesting group and two non-nesting control groups in both finch species. Furthermore, sexual dimorphic nesting behaviors, such as male finches engaging in fetching nesting materials and female finches spending more time in the nesting box, appeared to correlate with the changes in gut microbial communities. Specifically, a bacterial family belonging to the phylum Campylobacterota (former class Epsilonproteobacteria) emerged as the predominant group in nesting finches. Interestingly, short-term antibiotic treatment targeting Gram-negative bacteria led to an increase in the daily usage of nesting material compared to the finches drinking water. Collectively, these findings suggest that gut microbial signals are closed related to the programming of nesting behaviors in the passerines.

Disclosures: **C. Chen:** None. **S. Chen:** None. **C. Hung:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.02/KK17

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF grant #1754513
NSF grant #1845845

Title: The effects of social experience on host gut microbiome in male zebrafish (Danio rerio)

Authors: E. SCOTT, M. S. BREWER, A. L. PERALTA, *F. A. ISSA;
Biol., East Carolina Univ., Greenville, NC

Abstract: Although the gut and the brain vastly differ in physiological function, they have been interlinked in a variety of different neurological and behavioral disorders. The bacteria that comprise the gut microbiome communicate and influence the function of various physiological processes within the body including nervous system function. However, the effects of social behavior in the context of aggression and chronic social stress on gut microbiome remain poorly understood. Here, we examined whether social behavior impacts the host zebrafish (*Danio rerio*) gut microbiome. Zebrafish are social and engage in agonistic interactions that culminate in the formation of stable dominance relationships. Although the neural bases of social aggression and stress in zebrafish is well documented, their effects on gut microbiota remain poorly examined. We studied how social dominance during the first two weeks of social interactions changed the composition of zebrafish gut microbiome by comparing gut bacterial composition, diversity and relative abundance among socially dominant, submissive, social isolates, and control group-housed communal fish. Using amplicon sequencing of the 16S rRNA gene, we report that social dominance significantly affects host gut bacterial community composition but not bacterial diversity. At the genus-level, *Aeromonas* and unclassified Enterobacteriaceae relative abundance decreased in dominant individuals while commensal bacteria (e.g., *Exiguobacterium* and *Cetobacterium)* increased in relative abundance. Conversely, the relative abundance of

Psychrobacter and *Acinetobacter* was increased in subordinates, isolates, and communal fish compared to dominant fish. The social status-dependent shift in commensal and pathogenic bacteria highlights the impact of social aggression and the accompanying stress on gut microbiome with potentially similar effects in other social organisms.

Disclosures: E. Scott: None. **M.S. Brewer:** None. **A.L. Peralta:** None. **F.A. Issa:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.03/KK18

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: UNAM-DGAPA IN202818
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CONAHCYT CF-2023.G-206

Title: Sexual and social exposure increase in vitro proliferative capacity and neuronal fate in neural progenitor cells derived from the subventricular zone of the adult prairie vole brain

Authors: *D. AVILA-GONZÁLEZ[1,2], I. ROMERO-MORALES[1], O. MARTÍNEZ-ALARCÓN[1], L. J. YOUNG[3], F. CAMACHO-BARRIOS[2], A. E. CASTRO[2], R. G. PAREDES[4], N. F. DÍAZ[1], W. PORTILLO[2];
[1]Inst. Nacional de Perinatologia, Mexico City, Mexico; [2]Inst. de Neurobiología, Querétaro, Mexico; [3]Emory Univ., Emory Univ., Decatur, GA; [4]Escuela Nacional de Estudios Superiores Unidad Juriquilla, Querétaro, Mexico

Abstract: Background: The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent that establishes an enduring pair bond after cohabitation with (6 h) or without (24 h) mating. Previously, we reported that social interactions and mating modulated cell proliferation and new neuron survival in adult prairie voles. Here, we examined the proliferation and differentiation potential of neural progenitor cells (NPC) isolated from the subventricular zone (SVZ) of both female and male adult voles, based on their sociosexual experiences. The animals were divided into three groups:1) control, sexually naïve female and male voles with no interaction with the opposite sex; 2) social exposure, in which males and females were exposed to sensory cues from voles of the opposite sex and without physical contact; and 3) social cohabitation with mating, in which male and female voles mated to initiate pair-bond formation. NPC were then extracted from the SVZ and cultured to form neurospheres. Results: We

observed an increase in the number and size of neurospheres as well as more significant immunoreactivity to Nestin and a greater number of Nestin+/Edu+ cells, indicating an increase in the proliferation potential of SVZ-isolated cells from prairie voles with sociosexual experience. Additionally, there was an increase in mature neurons (MAP2+) and a decrease in glial cells (GFAP+) differentiating from NPC, with sex-dependent differences. Furthermore, during the neurosphere culture, we conducted additional experiments using various hormones and factors (brain-derived neurotrophic factor, estradiol, prolactin, oxytocin, and progesterone). Interestingly, we found that the ability of SVZ-isolated cells to generate neurospheres and differentiate into neuronal or glial lineages in response to hormones or factors is also influenced by the sex and sociosexual context. Conclusions: Sociosexual experiences that promote pair-bonds induce changes in the properties of NPC isolated from the subventricular zone (SVZ) in adult prairie voles, leading to increased proliferation and inducing a bias in their differentiation potential. Interestingly, the differentiation potential of SVZ-isolated cells was conserved under *in vitro* conditions, suggesting a commitment to a neuronal lineage in a sociosexual context. Moreover, hormonal and growth factors treatments can affect the generation of neurospheres and neural differentiation, depending on the sex and sociosexual context. The next question is whether the enhanced proliferation and neuronal fate in SVZ-cells are essential for establishing, strengthening, and maintaining pair-bond formation.

Disclosures: D. Avila-González: None. **I. Romero-Morales:** None. **O. Martínez-Alarcón:** None. **L.J. Young:** None. **F. Camacho-Barrios:** None. **A.E. Castro:** None. **R.G. Paredes:** None. **N.F. Díaz:** None. **W. Portillo:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.04/KK19

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: MSCA Postdoctoral Fellowships: 101108434 — PHYSPROSOC
JSPS KAKENHI 23H02593

Title: Autonomic biomarkers correlate brain activity during social behaviors in zebra finches

Authors: *J. KATIC[1,2], R. ISOGAI[3], M. NIE[4], Y. IMANO[3], J. NONAKA[3], S. TAKEUCHI[4,5], Y. YOSHIDA[3], Y. YAZAKI-SUGIYAMA[2,5];
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Abstract: Social environments have an impact on rapid physiological dynamics as well as on brain activities, and both might cause behavioral changes in the short and long term (Thayer & Friedman (2002) Scand J Psychol; Alba et al. (2019) Front Hum Neurosci; Lischke et al. (2018) Sci Rep; Boeckle & Bugnyar (2012) Curr Biol). The central autonomic network, circuits between the central and the autonomic nervous system, works through series of feedback loops and subsequently modulates behavioral and physiological processes. Peripheral outputs, such as heart rate and heart rate variability, have been suggested to be used as indicators of the feedback loop in the central autonomic network. However, detailed correlations between specific biomarkers and brain activity during complex behaviors such as social interactions have yet to be elucidated.Here we developed a method to measure heart rate (HR) and body temperature continuously (BT) using a small wireless biosensing device (19mm x 10mm x 6.0mm). We recorded single-unit neuronal activity extracellularly from the Locus coeruleus (LC), the brain locus for attention/arousal control, while simultaneously measuring HR and BT with a device subcutaneously implanted on the back, in anesthetized and in free-moving male zebra finches. We previously reported that LC neurons increase their activities during vocal social communications in juvenile male zebra finches (Katic et al. (2022) Nat Commun). Here we found a significantly positive correlation between HR and activities of the LC neurons (Firing rate (spike/sec)) both in anesthetized (Spearman correlation coefficient (cc): 0.5, $p>0.001$) and in awake socially interactive conditions (cc: 0.7, $p>0.001$) (N=8). Moreover, correlation in HR, as well as in BT between two male zebra finches interacting across adjacent cages, increased when they approached one another or vocalized to each other (no interaction: cc (BT) = 0.497, $p<0.001$ and cc (HR) = 0.469, $p<0.001$; with interaction: cc (BT) = 0.66, $p<0.001$ and cc(HR) = 0.78, $p<0.001$) (N=6 bird pairs). We further found BT of a tutor and tutee pair in their home cage fluctuated synchronously over a 12h period (cc = 0.57, $p<0.001$; N=3 bird pairs). Those findings suggest HR and BT be used as biomarkers of the feedback loop in the autonomic nervous system during social behaviors. Measuring HR and BT with less invasive techniques might contribute to understanding the long-term impact of brain activities on complex social behaviors.

Disclosures: J. Katic: None. **R. Isogai:** None. **M. Nie:** None. **Y. Imano:** None. **J. Nonaka:** None. **S. Takeuchi:** None. **Y. Yoshida:** None. **Y. Yazaki-Sugiyama:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.05/KK20

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Chronic stress increases the motivation for social interaction

Authors: *A. PACHECO-SPIEWAK[1], A. FALL[2], M. VENNIRO[3];
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Abstract: Exposure to stress is a significant risk factor for the development of neuropsychiatric disorders, including post-traumatic stress disorder and anxiety. In humans, social interaction can mitigate the severity of these disorders, whereas social isolation tends to worsen the negative symptoms associated with them. Here, we investigated the impact of chronic stress on operant social interaction. We first exposed both male and female rats to chronic restraint stress (30m/d, 10d). We handled control groups without any stress-inducing procedures. We then trained rats for social self-administration (60s, 2h/d) under a Fixed Ratio 1 (FR1) schedule for 10 sessions, FR2for two sessions, FR4 for two sessions, and FR8 for two sessions. Next, we trained the rats on a progressive ratio for 3 sessions. Lastly, we tested the rats for social seeking under extinction conditions after 1, 15, or 30 days of social isolation. We found that rats exposed to chronic stress earned more social interactions compared to the no-stress group during social self-administration independently of sex or effort requirement. Using a progressive ratio procedure we confirmed that chronic stress induced higher motivation for social interaction. Finally, we demonstrated a significant increase in social seeking behavior over a period of social isolation for both the no-stress and stress groups. Although both groups showed a similar increase, the stress group exhibited a more pronounced incubation compared to the no-stress group. Our findings demonstrate that chronic stress amplifies the drive for social interaction even in the face of isolation, highlighting its impact on social behavior.

Disclosures: **A. Pacheco-Spiewak:** None. **A. Fall:** None. **M. Venniro:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.06/LL1

Topic: G.03. Motivation

Support: Korea Brain Research Institute basic research program 21-BR-01-03 & 22-BR-01-01
Grants-in-Aid for JSPS Fellows 23K06796

Title: Investigating neural mechanisms of intrinsic motivation for undirected singing in zebra finches

Authors: *S. KOJIMA[1], C. MORI[2], Y. KIM[1], N. AOKI[2], K. J. HOMMA[2];
[1]Korea Brain Res. Inst., Daegu, Korea, Republic of; [2]Teikyo Univ., Tokyo, Japan

Abstract: Behaviors driven by intrinsic motivation are critical for developing and optimizing cognitive, social, and physical functions throughout life. Intrinsic motivation arises within individuals for internal satisfaction, sharply contrasting with extrinsic motivation, which involves engaging in a behavior to gain external rewards or avoid punishment. Songbirds, such as zebra finches, offer a unique opportunity to study neural substrates of intrinsic motivation, because they spontaneously produce many renditions of songs with highly quantifiable structure for vocal

practice, even in the absence of apparent recipients (undirected singing). We recently established a simple behavioral procedure to easily manipulate and quantify intrinsic motivation for undirected singing in adult male zebra finches: Temporary suppression of undirected singing by turning off the ambient light dramatically enhanced intrinsic motivation for singing, and the degree of enhancement depended on the duration of suppression. Also, by combining this procedure with systemic administration of neuromodulator antagonists and agonists, we demonstrate that intrinsic singing motivation is critically regulated by both dopamine signaling through D2 receptors and endocannabinoid signaling through CB1 receptors. Moreover, to identify brain areas associated with the enhancement of singing motivation, we explored brain areas showing activated expression of the immediate early genes (Arc, c-fos, or Egr-1) in birds with relatively high singing motivation but not in birds with low singing motivation. We found that Arc mRNA expression in the nucleus of the hippocampal commissure (NHpC) was activated depending on the degrees of singing motivation, suggesting that neural activity in NHpC is associated with intrinsic singing motivation. We are currently examining effects of lesions in NHpC on undirected singing as well as potential neural connections between NHpC and other brain areas including the nuclei of the song system.

Disclosures: **S. Kojima:** None. **C. Mori:** None. **Y. Kim:** None. **N. Aoki:** None. **K.J. Homma:** None.

Poster

PSTR222. Social Behaviors

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Program #/Poster #: PSTR222.07/LL2

Topic: F.01. Neuroethology

Support: National Natural Science Foundation of China (NSFC); grant number 32150410370

Title: The effects of social context on monkey’s decision making

Authors: *S. ZAREI, I. M. ANDOLINA;
Ctr. for Excellence in Brain Sci. and Intelligence Technol. (Institute of Neuroscience), Shanghai, China

Abstract: Making choices in the presence of others is a fundamental aspect of animal and human behavior, and demands a high level of cognitive sophistication. It can be affected by current needs, relationship bonds, authority rules, and reciprocity behaviors. However, few studies have examined the neural and cognitive mechanisms underlying social decision-making. We developed a cognitive testing system with a transparent touchscreen display, where pairs of monkeys (Macaca fascicularis; n=6) can sit face-to-face, alternately or simultaneously performing an object-based foraging task. The same core task was adapted using four conditional rules: 1) AUDIENCE—when one subject performs the task while another is observing; 2) CO-

ACTION—when both subjects work for reward independently; 3) ENVY: when one subject performs the task but both receive a reward; and 4) ALTRUISM: when one subjects task choice can deliver a reward to both participants. We used two iRecHS2 infrared eye trackers to monitor gaze patterns, pupil size, eye blinks, and performed facial video analysis along with psychometric performance measurements. Our results showed that monkeys performed better when being watched, when performing a task as an active co-performer, and when the recipient was a cage mate. The eye-tracking results confirmed that monkeys' looking patterns were influenced by social relationships and task conditions. This suggests that social interaction plays a role in the way that monkeys process information and make decisions. We believe that this work will serve as the basis for future studies on the cognitive and neural mechanisms underlying social decision-making in both monkeys and humans.

Disclosures: S. Zarei: None. **I.M. Andolina:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.08/LL3

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01MH123513
OneMind Rising Start Award
SFARI

Title: Loss of oxytocin receptor signaling disrupts social dynamics and nucleus accumbens activity in male and female prairie voles

Authors: *K. LONG[1], N. HOGLEN[4], A. KEIP[2], D. MANOLI[3];
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Abstract: Oxytocin modulates the neural circuits that underly fundamental social behaviors. In the socially monogamous prairie vole, oxytocin signaling in the ventral striatum is hypothesized to enhance the expression of partner preference; however, it remains unclear how the ventral striatum responds to social behavior in the context of pair bonding and, moreover, how oxytocin receptor (OxtR) signaling influences short- and long-term activity of this region to regulate social attachment. Here, we examine the behavioral and neural dynamics of pair bond formation in male and female, wild-type (WT) and OxtR knockout prairie voles. Using *in vivo* longitudinal fiber photometry, we measure calcium activity of the nucleus accumbens (NAc) across different stages of pair bond formation as animals interact with their bonded partners or novel strangers. We demonstrate that social dynamics are sex-specifically disrupted in $OxtR^{-/-}$ voles during initial interactions with a partner and subsequent interactions with novel conspecifics. Furthermore, by

analyzing NAc neural traces relative to individual social behaviors and behavioral modules, we show that loss of OxtR sex-specifically disrupts calcium activity in the NAc surrounding social interactions and that neural signatures during initial interactions predict later behavior in male $OxtR^{-/-}$ voles. Together these data suggest that loss of OxtR signaling alters ventral striatal signatures of social interaction over the course of pair bonding, providing novel insight into the role of oxytocin signaling for the development of social attachment.

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Poster

PSTR222. Social Behaviors

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NINDS R00NS114166
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Brain & Behavior Research Foundation Young Investigator Award
GR114536
NIH/NIGMS T328857285
Connecticut Mental Health Center, CMHC

Title: Oxytocin supports the emergence of sensory and social information processing in the developing somatosensory cortex

Authors: *L. LIN[1], I. NWABUDIKE[1], R.-J. LIU[1], A.-N. SLIBY[1], S. MOHAMMED[2], A. DONATELLE[3], N. DE MARCO GARCIA[3], A. CHE[1];
[1]Dept. of Psychiatry, Yale Sch. of Med., New Haven, CT; [2]Notre Dame of Maryland Univ., Baltimore, MD; [3]BMRI, Weill Cornell Med. Col., New York, NY

Abstract: Abnormalities in sensory perception and social abilities are prevalent and often comorbid features in a wide range of mental illnesses, in particular autism spectrum disorders (ASD). A central challenge has been to identify alterations in neurobiological mechanisms that underlay processes as distinct as sensory perception and social cognition. Considerable evidence indicates that the neuropeptide oxytocin significantly contributes to a wide range of social behaviors, and more recently, it has been linked to synaptic plasticity in sensory cortices. In this study, we tested the hypothesis that oxytocin is required for establishing proper synaptic connectivity in the developing primary somatosensory cortex (S1) for both tactile sensory perception and social information processing. We found that oxytocin receptors (OXTRs) are preferentially expressed in somatostatin (SST) interneurons in the developing S1, and the OXTR agonist TGOT induces large depolarizing currents as well as robust spontaneous firing in SST interneurons as early as the second postnatal week. Removing OXTRs specifically from SST interneurons results in persistent, over-synchronized spontaneous network activity *in vivo*,

suggesting that oxytocin signaling is involved in circuit desynchronization required for information processing. In support of this role of oxytocin in the developing S1, our preliminary data indicate that the presence of a social context (dam/dam odor), which activates oxytocinergic neurons, reduces synchronization and enhances sensory responses of L2/3 neurons in preweaning mice. In addition, mice with targeted OXTR deletion in SST interneurons show impaired texture discrimination as adults. Our results suggest that oxytocin signaling may be required for establishing circuits involved in sensory and social information processing during development.

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Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.10/LL5

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: ERC
ANR

Title: Manipulation and recording of oxytocin neurons activity in macaques to uncover the social functions of oxytocin

Authors: *A. AMELOOT, E. DISARBOIS, A. SIRIGU, J.-R. DUHAMEL;
Inst. des Sci. Cognitives - Marc Jeannerod UMR5229 CNRS, Bron, France

Abstract: Oxytocin (OT), a neuropeptide mainly produced in the hypothalamus, has been shown to act as a key modulator of social behavior. In most human and non-human primate studies, the demonstration of OT effects relies on its external administration by inhalation and the reactivity of the OT system is assessed by peripherals measurements. Due to the limited spatio-temporal resolution and several pitfalls of both approaches, there is an urgent need for more precise tools that allow direct manipulation and recording of oxytocin neurons activity in primates. Indeed, to understand the function of the OT system, we must consider not only the cellular and behavioral effects of OT signaling, but also when and why these effects occur. In other words, we must identify the factors that cause OT release and the signal it carries. To reach this aim, we (1) performed electrophysiological recording in macaque hypothalamic nuclei enriched in oxytocin neurons to identify the stimuli that trigger oxytocin release; (2) chemogenetically activated macaque oxytocin neurons to uncover the behavioral consequences of endogenous OT release. Firstly, our preliminary results highlight paraventricular and supraoptic neurons sensitive to audio, visual and tactile stimuli, with some demonstrating a preference for social stimuli over

non-social ones. Secondly, our results show that DREADD-mediated OT neurons activation strongly increased the time spent exploring the eye and nose areas during a free viewing task of neutral faces. Collectively, these findings provide new insight into the functioning of the OT system, and will lead to a more comprehensive understanding of how OT dynamically shapes adaptive and flexible social behaviors in primates.

Disclosures: A. Ameloot: None. **E. Disarbois:** None. **A. Sirigu:** None. **J. Duhamel:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.11/LL6

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: JSPS grant JP19K24681

Title: Oxytocin circuit dysfunction in BTBR mouse model of autism

Authors: *H. ARAKAWA;
Univ. of the Ryukyus, Okinawa, Japan

Abstract: Neuropeptide oxytocin (OXT) dysfunction is implicated in circuit abnormalities underlying autism spectrum disorders (ASD). The PVN (paraventricular nucleus) OXT neurons sending projections to the MeA (medial amygdala) and the BnST (bed nucleus of the stria terminalis), play important roles in social processes. We investigated the functional contributions of these OXT circuits on ASD-like socio-emotional behavior in BTBR mice, a behavior-based ASD model. OXT promoter viral tracing revealed that the $OXT^{PVN->BnST}$ projections are defect in BTBR mice. Chemogenetic activation of $OXT^{PVN->MeA}$ circuits enhances anxiety-like behavior and facilitates social approach behavior, while activation of $OXT^{PVN->BnST}$ circuits suppresses anxiety-like behavior along with inhibiting social approach. $OXT^{PVN->BnST}$ circuit activation in BTBR mice proves ineffective on socio-emotional behavior and OXT receptor mRNA expression. Altogether, OXT circuits serve as a key regulator for socio-emotional behavior; hence a defect of the $OXT^{PVN->BnST}$ circuits contributes to the development of ASD-like social phenotypes in BTBR mice.

Disclosures: H. Arakawa: None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.12/LL7

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: The effects of disrupted oxytocin receptor signaling in the medial amygdala on mouse social behavior

Authors: *C. SAPP, E. EGLESTON, H. K. CALDWELL;
Biol. Sci., Kent State Univ., Kent, OH

Abstract: The oxytocin (Oxt) system, and specifically Oxt signaling through the Oxt receptor (Oxtr), is a well-established modulator of social behavior across species. In fact, expression of the Oxtr in nodes within the social behavior neural network (SBNN) is a hallmark of the network. One node within the SBNN, the medial amygdala, is critical for the processing of a variety of social signals and for shaping behavioral output. Here, we evaluated how the absence of the Oxtr in the medial amygdala affected measures of social cognition in mice. We hypothesized that mice with site-specific deletion of the Oxtr in the medial amygdala would have impairments in social approach and social memory behaviors as compared to control mice. To test this hypothesis, adult male and female mice in which the Oxtr is flanked by loxP sites, $Oxtr^{Floxed/Floxed}$, were stereotaxically injected an AAV virus that expresses CRE recombinase in order to site-specifically genetically delete the Oxtr. Two weeks post-surgery, mice were tested for anxiety-like behavior using open field and elevated plus maze tests and social approach and social memory using a 3-chamber social cognition test. Male mice were also tested for aggressive behaviors using a resident-intruder test. While data analysis is not yet complete, the evaluation of these behaviors will allow for an assessment of the role of the Oxtr in the medial amygdala of mice.

Disclosures: C. Sapp: None. **E. Egleston:** None. **H.K. Caldwell:** None.

Poster

PSTR222. Social Behaviors

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Program #/Poster #: PSTR222.13/LL8

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Supported by NIH grants R01 MH121603 and R03 MH120549 to AP and GJD; GSU Brains and Behavior Seed Grant Program

Title: Deleting cells expressing the oxytocin receptor in the lateral septum alters male social communication and female copulatory behavior

Authors: *A. R. SELKE[1], B. DADKHAH[2], L. IDDRISU[3], G. J. DEVRIES[4], A. PETRULIS[5];
[1]Neurosci., Georgia State Univ., Decatur, GA; [2]Neurosci., Georgia State Univ., Duluth, GA;

[3]Neurosci., [4]Biol., Georgia State Univ., Atlanta, GA; [5]Neurosci. Inst., Georgia State Univ. Neurosci. Inst., Atlanta, GA

Abstract: The neuropeptide oxytocin (OXT) and the oxytocin receptor (OXTR) regulate social behaviors and communication in mammals, including humans. One brain region that contains relatively high levels of OXTR is the lateral septum (LS), an area known to facilitate sex- and experience-dependent social behaviors. To investigate the behavioral role of the OXTR system in the LS, we deleted OXTR-expressing neurons in the LS of socially-experienced male and female mice via viral delivery of a Cre-dependent activated caspase cell-death construct in OXTR-Cre male and female mice using wildtype (Cre-) littermates as controls and studied the effects of these lesions on social investigatory, communicative, sexual, and aggressive behaviors toward novel male and female conspecifics. Deletion of OXTR cells reduced OXTR binding in the LS of both male and female subjects relative to controls. In males, these lesions decreased the total amount of marks to both caged male and female conspecifics but increased the total area marked in the presence of a caged female. In females, these lesions facilitated copulatory behavior by increasing the number of mounts received and shortening male intromission latencies. Other behaviors, such as social investigation, ultrasonic vocalizations, and resident-intruder aggression were not altered. Our results suggest that OXTR neurons in the LS may normally suppress female copulatory behavior and regulate male urine marking patterns in a context-dependent manner. Therefore, OXT or vasopressin acting on OXTR in the lateral septum may modulate specific neuronal circuits to support or repress prosocial behaviors in males and females, respectively.

Disclosures: **A.R. Selke:** None. **B. Dadkhah:** None. **L. Iddrisu:** None. **G.J. Devries:** None. **A. Petrulis:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

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Program #/Poster #: PSTR222.14/LL9

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH grants R01 MH121603
NIH grants R03 MH120549
GSU Brains and Behavior Seed Grant Program

Title: Sex and social experience mediate vasopressin 1a receptor distribution in the lateral septum of mice

Authors: *C. N. FRIESEN, N. SCHAPPAUGH, A. SELKE, G. N. DE VRIES, A. PETRULIS; Neurosci. Inst., Georgia State Univ., ATLANTA, GA

Abstract: Social animals must successfully navigate aggressive and affiliative interactions to find a mate and reproduce. Across taxa, the vasopressin system mediates the effects of social

experiences on social behavior, including defensive, pair bonding, mating, and parental behavior. Vasopressin receptors, such as vasopressin 1a receptor (V1aR), are distributed across several brain regions in the so-called Social Behavior Network (SBN). In mammals, the distribution of V1aR throughout the brain plays a key role in social behavior patterns and may be sexually differentiated. To date, the effects of sex and social experience (both reproductive and aggressive opportunities) on V1aR distribution and expression across the whole brain of male and female mice is unknown. We hypothesize that the distribution of V1aR across brain regions that regulate social behavior (e.g., lateral septum, ventral pallidum, vertical and horizontal diagonal band) will differ between males and females. We also expect that socially experienced individuals will be characterized by distinct changes in V1aR expression in a region-dependent, and possibly sex-dependent, manner. To test this, we examined the combined effects of reproductive and aggressive social experience on V1aR distribution throughout multiple brain regions involved in social behavior in both male and female mice using receptor autoradiography. We compared V1aR receptor binding across males and females that were either socially naïve (housed with two littermates) or socially experienced (three reproductive aggressive and opportunities). We found no differences in the distribution of V1aR based on either sex or social experience in the ventral pallidum and the vertical and horizontal diagonal band. In contrast, we observed a significant effect of sex and an interactive effect of sex and experience on V1aR binding in the lateral septum: socially naive females had greater V1aR binding than naive males, whereas socially-experienced males and females did not differ in binding levels. While preliminary in situ hybridization (ISH) suggests there are no effects of sex or social experience on V1aR mRNA levels in the lateral septum, ongoing work using receptor autoradiography and ISH is examining the activity of V1aR in other areas of the brain that regulate social behavior. This research will further our understanding of sex differences and the potential plasticity of the V1aR system across the social brain.

Disclosures: C.N. Friesen: None. **N. Schappaugh:** None. **A. Selke:** None. **G.N. de Vries:** None. **A. Petrulis:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.15/LL10

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Vasopressin-responsive neurons in the dorsal raphe modulate prosocial behavior in a sex-specific manner

Authors: *D. J. KAHN[1], T. N. PATEL[2], H. LANGKAMER-SMITH[2], B. D. ROOD[1]; [1]Mol. Cell Biol. & Neurosci., [2]Rowan Univ. Grad. Sch. of Biomed. Sci., Stratford, NJ

Abstract: Social interaction is vital to the human experience, where social deficits can negatively impact quality of life in various social disorders. Currently, treatment for social

deficits is limited to cognitive behavioral therapy and therapeutics such as selective serotonin reuptake inhibitors, which only targets a portion of a complex system underlying social behavior. The neuropeptide, arginine-vasopressin (AVP), and the neuromodulator, serotonin, both play roles in social behaviors ranging from mating to aggression. AVP in the brain is largely produced in a few different nuclei, each of which has distinct functions and AVP projection patterns. In particular, the bed nucleus of the stria terminalis plays a role in affiliative (prosocial) behavior and sends AVP afferents to various nuclei such as the dorsal raphe (DR). The DR is the largest hub for serotonin production in the brain, implicating the DR as an interesting target for investigating social behavior. Previous data suggests that cells expressing vasopressin 1a receptors (Avpr1a) are present in the DR, these cells depolarize in response to AVP, and AVP indirectly excites serotonin neurons. Further, exposure to a female stimulus animal activates (increases Fos expression) DR Avpr1a neurons of both male and female subjects. Based on these data, we hypothesized that DR Avpr1a-expressing cells may influence prosocial behavior. Using a novel mouse model expressing Cre-recombinase under the control of Avpr1a regulatory elements in combination with virally delivered inhibitory DREADDs, we tested the hypothesis that chemogenetic inhibition of Avpr1a-expressing cells in the DR would decrease prosocial behavior. Our data show decreased social behavior in response to a female stimulus in both male and female mice treated with DREADD ligand CNO as compared to vehicle treatment. Specifically, DREADD inhibition reduced anogenital investigation and time spent following or chasing the conspecific. Interestingly, CNO treatment increased aspects of social behavior between male subject and male stimulus, suggesting that the role of Avpr1a neurons in the DR depends both on sex of the subject and sex of the stimulus. Overall, this study helps elucidate the role of Avpr1a-expressing neurons in the DR during social behavior, and our mouse model will accelerate research surrounding Avpr1a-expressing neurons in the brain.

Disclosures: D.J. Kahn: None. **T.N. Patel:** None. **H. Langkamer-Smith:** None. **B.D. Rood:** None.

Poster

PSTR222. Social Behaviors

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH/DA047976

Title: Volitional social interaction increases intrinsic excitability in insula neurons

Authors: *R. MARINO[1], K. PAPASTRAT[2], C. LIS[3], L. A. RAMSEY[5], M. VENNIRO[4]; [1]Univ. of Maryalnd, Baltimore, MD; [2]UMB Sch. of Med., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [3]Venniro Lab., [4]Univ. of Maryland Sch. of Med., Univ. of Maryland Sch. of Med., Baltimore, MD; [5]NIDA IRP, NIDA IRP, Baltimore, MD

Abstract: Background: We recently developed a volitional social self-administration model showing the strong motivation of rodents to engage in social interactions. The insular cortex (IC) plays a critical role in the expression of social and motivated behaviors in both humans and laboratory animals. Here, we recorded from pyramidal neurons in the insular cortex to determine the effect of social self-administration on intrinsic excitability. **Methods:** We trained male and female rats for social self-administration (60 s, FR1, 2 h/d, 12 d). Six days after the last social self-administration session, we performed current-clamp recordings in IC pyramidal neurons using *ex vivo* brain slice electrophysiology. We injected increasing current pulses (40-600 pA, 1s) to elicit action potentials in recorded neurons. As a control group, we used naïve rats to determine baseline excitability in IC neurons. **Results:** Our results showed that volitional social interaction increased the number of spikes elicited per unit of current injected in IC neurons compared to the naïve group. Furthermore, analysis of spike features demonstrated a significant reduction of spike half-width after social-self-administration. **Conclusion:** Our findings identified a new target area mediating social motivation that may inspire clinical studies to determine whether social-based treatments will restore normal insula function. Future studies will assess the role of large conductance, voltage and calcium-gated potassium channels (BK channels) in social self-administration-induced changes in IC excitability.

Disclosures: R. Marino: None. **K. Papastrat:** None. **C. Lis:** None. **L.A. Ramsey:** None. **M. Venniro:** None.

Poster

PSTR222. Social Behaviors

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Max and Minnie Tomerlin Voelcker Foundation
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Title: Berberine enhances BTBR mouse social interaction, reduces serum steroids and elevates brain serotonin levels

Authors: P. B. STEWART[1,2], R. J. HUNT[1,3], A. C. SHAKOCIUS[1], J. E. SCHULTZ[1], C. A. MILLER[1], L. ARNOLD[1], L. C. DAWS[1], *G. G. GOULD[1];
[1]Physiol., U Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [2]Biol., Univ. of the Incarnate Word, San Antonio, TX; [3]Biol., Ft. Lewis Col., Durango, CO

Abstract: Social withdrawal is a treatment-resistant core symptom of autism, along with restrictive-repetitive behaviors. Deficient serotonin (5-HT) signaling is strongly implicated as a contributing factor to autism and other psychiatric disorders. The limited clinical efficacy of

selective 5-HT reuptake inhibitors (SSRIs) to improve social behaviors in these conditions has brought into question their therapeutic utility for this debilitating symptom. Given this, our goal was to characterize the acute and chronic effects of blocking auxiliary 'uptake 2' transporters of 5-HT on social and repetitive behaviors in mice. Organic cation transporters (OCTs) are ancillary 5-HT transporters found in brain that are blocked by steroid hormones such as corticosterone. Male BTBR T+Itpr3tf/J (BTBR) mice have elevated serum corticosterone in adolescence, and they exhibit inherent deficits in social interaction and restrictive-repetitive behaviors. In 3-chamber sociability tests the OCT blocker decynium-22 (D-22) enhanced interactions in otherwise socially deficient BTBR mice and was without adverse effects on the social behaviors of C57BL/6J (B6) mice ($p < 0.05$, N= 8-9). The OCT substrate berberine similarly enhanced social interactions in these strains. However, we found berberine to be a poor blocker of 5-HT uptake compared to D-22. For example, a lower concentration of D-22 is required to inhibit cortical [^{3}H] 5-HT uptake, with a Michaelis constant (Km) ≈ 0.5 μM, while about 500x more berberine is required to equivalently inhibit it (Km ≈ 240 μM). Neither D-22 nor berberine reduced marble burying. However, we and others found that D-22 and berberine increase brain serotonin and reduces serum corticosterone. Our findings indicate berberine treatment may be a promising strategy for improving social behavior.

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Poster

PSTR222. Social Behaviors

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIDA Grant DA047976
NIMH Grant MH129310

Title: Norepinephrine α1 receptor mediates reciprocal social interactions

Authors: *C. A. LIS, K. M. PAPASTRAT, Z. HUANG, J. GARCIA, V. BRECHER, M. VENNIRO;
Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: We recently developed a model of volitional social interaction in which a rat (resident) gains access to a social peer (partner) by pressing a lever. In this model, the partner's exposure to interactions is resident controlled. However, in humans, social interactions are coordinated and reciprocal. Here, we developed a new social coordination model and identified its pharmacological mechanisms. In Experiment 1, we trained both male and female rats to achieve social interaction by coordinating a series of actions between the resident and partner rats. The resident rat would press a lever to activate a lever for the partner rat, who would then

complete the chain of actions to open a door that separated the two sides of the social chamber. After establishing stable social coordination, we manipulated the effort to achieve social interaction for both residents and partners concurrently (FR2-4) or separately (FR4-16 and PR). In Experiment 2, we determined the effect of systemic injections of α1 (1 and 2 mg/kg), α2 (0.1 and 0.5 mg/kg), D1 (10 and 20 µg/kg) and D2 (50 and 100 µg/kg) antagonists on social coordination to determine its pharmacological mechanisms. Independent of sex or effort conditions rats coordinated their actions to achieve social interaction. Moreover, α1, but not D1, α2 or D2 receptors, selectively mediated social coordination. We introduced a novel animal model of social coordination to study the mechanisms mediating reciprocal social interactions. Furthermore, we demonstrated that an α1 antagonist in either resident or partner selectively decreased social coordination.

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Poster

PSTR222. Social Behaviors

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Program #/Poster #: PSTR222.19/LL14

Topic: F.01. Neuroethology

Support: NIH R01 MH119041

Title: Neural mechanisms underlying the reinforcement of gregariousness in flocking songbirds

Authors: *A. MAKSIMOSKI, C. ZHAO, T. LEVENSON, L. V. RITERS;
Univ. of Wisconsin, Madison, WI

Abstract: Group living is vital for the survival of many species, yet relatively little is known about how the brain reinforces sociality. It has been proposed that sociality is reinforced by both stimulating a positive affective state from interactions with conspecifics and reducing a negative affect otherwise caused by social separation. Social reward and the underlying neural circuitry have been relatively well studied, but much less is known about the role played by the reduction of a negative state in the reinforcement of gregariousness. Separation in social species often induces distress behaviors akin to those displayed during pain and brain regions implicated in social pain are the same as those that underlie physical pain. As such, social contact buffers against the painful stimuli and analgesics can quell distress during social separation. It is thus possible that for social species, interactions with conspecifics both activate neural mechanisms involved in reward and suppress those that underlie pain and distress. The goal of the present study is to begin to test this hypothesis. We observed singing and other social behaviors in male European starlings (*Sturnus vulgaris*) in fall when they form large non-breeding flocks (n=12) and then conducted conditioned place preference (CPP) testing to assess the reward value of the presence of flock mates. Furthermore, the degree to which separation from a flock suppressed

pain mechanisms was assessed indirectly by measuring analgesia in birds when in flocks and when separated for 24 hours from flock mates. On the last period of 24h separation, we collected neural tissue either immediately after separation or after being reunited with the home flock for 90 mins. We ran immunolabeling for immediate early genes in key brain regions implicated in reward and analgesia (i.e., the nucleus accumbens, medial preoptic nucleus, ventral tegmental area, and periaqueductal gray). Results demonstrate that birds that sing in flocks (i.e., produce song that functions to maintain flock cohesion) develop strong CPPs for places that had been paired previously with flock mates. Conversely, birds that did not sing in flocks developed preferences for places in which they had been housed alone. This demonstrates individual differences in the degree to which social contact is rewarding. In addition, relative to flocking, social separation was found to reduce analgesia, consistent with the hypothesis that in gregarious species social contact reduces a painful state. Individual differences in immediate early gene expression in association with individual differences in social reward and analgesia will be discussed.

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Poster

PSTR222. Social Behaviors

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: National Institute on Deafness and Other Communication Disorders/DC010915
Brain and Behavior Research Foundation/28897
National Institute of Development Administration/DA047976

Title: A socio-sensory mechanism buffering drug craving

Authors: *K. PAPASTRAT[1], C. LIS[1], H. PICHARD[2], A. C. PUCHE[1], L. A. RAMSEY[3], M. VENNIRO[1];
[1]Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Philosophy and Bioethics, John Hopkins Univ., Baltimore, MD; [3]NIDA IRP, Baltimore, MD

Abstract: Social interactions are rewarding and protective against substance use disorders, but it is unclear which specific aspect of the complex sensory social experience drives these effects. Here, we investigated the role of olfactory sensory experience in social interaction and its protective effect against drug craving in rats. First, we tested the necessity of the olfactory system experience on acquisition of volitional social interaction on male and female rats by removing the olfactory bulbs (bulbectomy). Then, we examined the effect of the olfactory system on the maintenance of previously learned volitional social interaction. Next, we examine the effect of bulbectomy on rats given a choice between social interaction and cocaine. Finally,

we tested the impact of the olfactory sensory experience by training rats on operant partner-associated odors and the effect of odor-based voluntary abstinence on incubation of cocaine craving. Removing the olfactory bulbs prevented acquisition and maintenance of operant social interaction, while maintaining food or cocaine self-administration, regardless of sex or training conditions. Rats with an intact olfactory system preferred social interaction over cocaine, whereas rats with impaired sensory communication resumed cocaine self-administration. Rats also abstained from cocaine when given access to a partner odor, decreasing the incubation of cocaine craving. Our data suggests the olfactory sensory experience is necessary and sufficient for volitional social reward. Furthermore, the olfactory system provides a mechanism that buffers drug craving. Based on these findings, translational research should explore the use of social sensory-based treatments utilizing odor-focused foundations for individuals with substance use disorders.

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Poster

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R00/DA047976

Title: Cerebellum activity mediates social craving

Authors: *Z. HUANG[1], C. DOTY[2], R. MARINO[3], M. VENNIRO[3];
[1]Neurobio., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Univ. of Maryland Sch. of Med. Program in Neurosci., Baltimore, MD; [3]Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: In humans, the desire to interact socially increases during periods of social deprivation. In rodents, social interactions are highly rewarding, and social deprivation increases the motivation for play behaviors similar to craving for other non-social rewards. Here, using rats, we introduce an operant model of volitional social craving and investigate its underlying neural mechanisms. We trained male and female rats first for food (2h/d, 6d) and then for social (2h/d, 12d) self-administration. Next, we assessed social seeking after 1 and 15 days of social deprivation. On the day 15 test, we identified the social seeking-related activated brain regions (using the activity marker Fos). Next, using chemogenetics, we investigated the role of the cerebellum in social craving because of its role in reward expectation and modulation of social behaviors. We found no sex differences in social self-administration. Social seeking in the seeking tests was higher after 15 days of social deprivation than after 1 day. Social seeking was associated with selective activation of the medial prefrontal cortex, anterior insular cortex,

dorsomedial striatum, claustrum, dorsal bed nucleus of the stria terminalis, central amygdala, medial thalamus, periaqueductal gray, dorsal raphe and cerebellum. After 15 days of social deprivation, inhibition of cerebellum activity by chemogenetic manipulation decreased social seeking in both male and female rats. We introduced a novel social craving rat model and identified a novel role of cerebellum in the expression of increased motivation to seek social interaction during periods of social deprivation.

Disclosures: Z. Huang: None. **C. Doty:** None. **R. Marino:** None. **M. Venniro:** None.

Poster

PSTR222. Social Behaviors

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Title: Prefrontal encoding of group social behavior during environmental stress

Authors: *T. RAAM[1], L. GU[2], Q. LI[3], N. RAMESH[4], S. M. CORREA[5], W. HONG[6]; [1]Neurobio., [2]Biol. Chem., UCLA, Los Angeles, CA; [3]Bioengineering, UCLA, LOS ANGELES, CA; [5]Integrative Biol. & Physiol., [6]Neurobio. and Biol. Chem., [4]UCLA, Los Angeles, CA

Abstract: Social interactions are critical to the well-being of a wide variety of species. While a growing body of literature has identified neural circuits for dyadic social interactions between two animals, our understanding of higher order interactions at the level of larger groups is weak. Many species organize into social groups, in which the individual contributes to and benefits from the well-being of the whole. However, little is known about the neural basis of group behaviors in response to environmental stressors. To address this gap, we are using a novel approach to study how groups of mice self-organize into huddles in response to thermal cold stress. Here, we used computer-vision based multi-animal pose estimation tools to identify five unique huddling states in groups of four mice. We found that huddling behavior is modulated by group size--individual mice huddle more in groups than in pairs, suggesting that social groups have emergent properties that dyads do not have. Moreover, we found that groups adapt their huddle states according to the degree of the ambient temperature, and that huddle states

dynamically evolve throughout a session. When assessing behavior at the level of the individual, we found that individual mice demonstrate active (self-initiated) and passive (partner-initiated) behavioral strategies to engage or disengage in huddles with other animals. Interestingly, these active and passive strategies are heavily dependent on the size of the huddle being entered or exited. We then asked which neural circuits coordinate the decisions to huddle in response to cold stress. Previous work suggests that medial prefrontal cortex (mPFC) is a critical node for regulating dyadic and group level social behaviors, as well as decision making more broadly. Using Miniscope calcium imaging, we found a unique population of cells in mPFC that encode active and passive decisions to engage or disengage from a huddle, but do not encode other social behaviors. Using mPFC population activity, we found that huddling behaviors are separable from other behaviors in population space, and can be accurately decoded from other behaviors using SVM decoders. We also found that mPFC neurons encode the social identity of huddling partners. Together, these data suggest a critical role for mPFC in encoding group-level responses to stress and present a novel avenue towards studying social interactions in larger groups.

Disclosures: **T. Raam:** None. **L. Gu:** None. **Q. Li:** None. **N. Ramesh:** None. **S.M. Correa:** None. **W. Hong:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.23/LL18

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: VA Academy of Science undergraduate research award
Thomas F. and Kate Miller Jeffress Memorial Trust Award

Title: Novel and established methods to assess social dominance in male laboratory mice.

Authors: *R. P. WATERS, **T. PHILBECK**;
Univ. of Mary Washington, Fredericksburg, VA

Abstract: The social interactions of mice can provide valuable information on their behavioral and physiological state. The Tube Test is a practical and inexpensive method that is commonly used to estimate the social rank of mice living in social cages, but this test is not universally accepted as a reliable and objective measure of social dominance. We designed a study to assess the validity of this test, which will enhance our ability to determine social relationships among mice in our lab. We kept two strains of mice (outbred CD-1 and inbred C-57) in strain specific dyads and performed multiple iterations of the Tube Test over 29 days. We compared these results to home cage aggression, hormone levels, and anatomical features that are associated with social dominance in mice (albeit using more expensive and labor-intensive methods than the Tube Test). Performance in the Tube Test did not predict plasma hormone levels or preputial

gland size. We observed a relationship between Tube Test performance and home cage aggression, in which mice that consistently lost the Tube Test exhibited higher levels of aggressive attacks toward their cagemates ($p \leq 0.05$). Our results suggest that care should be taken when assigning social ranks to mice based on results from the Tube Test alone.

Disclosures: R.P. Waters: None. **T. Philbeck:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.24/LL19

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Social relationships have varied impacts on neuroendocrine state

Authors: *N. H. PRIOR[1], C. HAAKENSON[3], S. CLOUGH[4], G. F. BALL[4], B. A. SANDKAM[2];
[2]Neurobio. and Behavior, [1]Cornell Univ., Ithaca, NY; [3]NIH, Bethesda, MD; [4]Psychology, Univ. of Maryalnd, College Park, MD

Abstract: Social relationships are important for many species. Focused research on a few systems has contributed neurobiological models of pair bonding; however, it remains unclear how generalizable these models are across species and across contexts within a species. Zebra finches are a fascinating system to explore the neurobiology of social bonding because they form and maintain multiple types of relationships. To test whether different brain networks support these different relationships, we quantified the effect of same vs opposite sex relationships on neuroendocrine state after 24 hours or 2 weeks of co-housing. We characterized neuroendocrine state based on the expression of 22 genes related to steroid hormones, dopamine, opioids, and nonapeptides in 6 brain regions associated with social communication and pair bonding (nucleus accumbens (NAc), nucleus taenia of the amygdala (TnA), medial preoptic area (POM), ventral tegmental area (VTA), periaqueductal grey (PAG), and the secondary auditory cortex (NCM/CMM). Overall, we found evidence that both independent and overlapping networks regulate social bonding across contexts. Social relationships impacted neuroendocrine state in 4 regions for males (NAc, TnA, POM, and PAG) and 3 regions for females (NAc, TnA, and POM). Neuroendocrine state in the NAc reflected the formation of opposite sex partnerships; however, we found the effect of pair bonding to produce different patterns of gene expression than what has been previously reported in voles. More specifically, we found that both DRD1 and DRD2 expression were up-regulated following opposite sex pair bonding in males, and that OPRM1:OPRK1 expression was up-regulated following social bonding (for both same- and opposite- sex pairs). Finally, our dataset is ideal to test additional gene-behavior relationships. Here we also report additional analyses showing the relationships between steroid and nonapetide related genes in VTA and NCM and individual variation in patterns of social interactions. Combined, our results support the view that there are numerous mechanisms

supporting a diversity of social relationships. Furthermore, this work emphasizes the importance of having nuanced understandings of how individual variation in social interactions shape social bonding phenotypes.

Disclosures: **N.H. Prior:** None. **C. Haakenson:** None. **S. Clough:** None. **G.F. Ball:** None. **B.A. Sandkam:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.25/LL20

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF

Title: The neuromolecular integration of a dynamic social world

Authors: *R. DEANGELIS[1], J. HAN[2], I. MILLER-CREWS[3], H. A. HOFMANN[4];
[1]Inst. for Neuroscience, Integrative Biol., [2]Inst. for Cell and Mol. Biol., [3]Integrative Biol., [4]Inst. for Neuroscience, Inst. for Cell and Mol. Biology, Integrative Biol., Univ. of Texas, Austin, TX

Abstract: Parental care and pair bonding are examples of social states in which behavioral responses to environmental stimuli often follow predictable patterns. For example, in pair bonding species, unbonded animals often approach opposite sex individuals, yet once a pairbond is established, they may avoid them and even respond aggressively. The behavior patterns which emerge following the formation of these enduring and rewarding bonds are a consequence of modifications to the dopaminergic reward system. However, investigations into the underlying neural mechanisms mediating behavior rarely account for differences in social (or motivational) states. Here, we use the monogamous bi-parental Convict Cichlid as a model system to investigate how social bonds shape the brain and behavior by determining the extent to which the same brain regions, cell types, and gene expression patterns are shared versus unique across distinct behavioral displays with shared motivational outcomes. We find that pair bonded males and females synchronize their responses as they navigate different environmental challenges, with each pair presenting a unique behavioral profile. Through immunofluorescent labeling of a neural activity marker and tyrosine hydroxylase, we demonstrate that differentially motivated aggressive and affiliative responses are mediated through brain region specific activity patterns of the dopaminergic system. Then, by integrating single-nuclei RNA sequencing and spatial transcriptomics we identify the spatiotemporal patterns of behaviorally responsive cells and brain areas regulating two forms parental behavior (offspring retrieval vs. defense). Specifically, we show that oxytocin and vasopressin are more highly expressed in nonapeptide neurons during offspring defense, a pattern that corresponds to our spatial dataset. Finally, by characterizing cellular activity through immediate-early gene expression, we find that offspring retrieval and defense are associated with brain region and cell type specific gene expression differences.

Together, our results show how integration across hierarchically organized biological levels reveals how socially regulated neural circuits promote context-appropriate behavior.

Disclosures: R. Deangelis: None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.26/LL21

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant IOS/DEB 1638861
Stengl-Wyer Research Grant Program

Title: Ecology and Life History Explain Variation in Socially Critical Brain Regions across Vertebrates

Authors: J. HAN[1], R. L. YOUNG[1], ***H. A. HOFMANN**[1,2];
[1]Integrative Biol., [2]Inst. for Neurosci., The Univ. of Texas at Austin, Austin, TX

Abstract: Social behavior can vary tremendously within and across species. We now have a basic understanding of how the brain generates context-appropriate behavior in an ever-changing world. Considerable progress has also been made towards reconstructing the evolution of the neuromolecular mechanisms that regulate and generate complex behavior (such as the vertebrate Social Decision-Making Network, SDMN), underscoring the conserved roles of evolutionarily ancient fore- and midbrain nuclei as well as hormonal and neuromodulatory systems in the regulation of social behavior. In addition, similar gene expression networks can underlie the convergent evolution of similar social phenotypes even across distantly related taxa, suggesting the repeated and parallel deployment of conserved molecular and neural pathways. Together, these results suggest that the last common vertebrate ancestor alive ~500 MYA already had the neuromolecular apparatus in place to meet the challenges and opportunities imposed by fluctuating internal states and external environments (e.g., finding mates, defending resources, avoiding predators). Here, we introduce a phylogenetic comparative transcriptomics approach to uncover how transcriptome variation reflects variation in ecology, demography, and life history across 32 vertebrate species. We first reconstructed the evolutionarily conserved "core transcriptomes" of subpallial amygdala and hippocampus, two key nodes of the SDMN. We then estimated rates expression evolution to identify gene sets associated with convergently evolved social phenotypes. Finally, we discovered gene co-expression modules that correlate with variation in species attributes across vertebrates. Our novel approach begins to identify the causes and consequences of variation of an ancient brain system underlying social behavior.

Disclosures: J. Han: None. **R.L. Young:** None. **H.A. Hofmann:** None.

Poster

PSTR222. Social Behaviors

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR222.27/LL22

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Klingenstein-Simons Foundation 00083041

Title: Neural correlates of behavior during novel large group interactions in the highly social spiny mouse (Acomys cahirinus)

Authors: *D. HO[1], J. P. CURLEY[2], A. M. KELLY[1];
[1]Dept. of Psychology, Emory Univ., Atlanta, GA; [2]Dept. of Psychology, Univ. of Texas At Austin, Austin, TX

Abstract: The overwhelming majority of laboratory studies on complex social behaviors in rodents examine only dyadic interactions. This limits our understanding about complex social behaviors as dyadic interactions alone do not capture the full suite of behaviors an animal may exhibit when interacting in a more dynamic social environment (i.e., a large group). The African spiny mouse (*Acomys cahirinus*), an emerging model for studying sociality, is a promising candidate for addressing this longstanding knowledge gap. In both wild and lab environments, spiny mice are highly prosocial and gregarious, readily affiliating with unrelated stranger conspecifics. Here, we tested the suitability of spiny mice as a model for examining how the brain enables animals to get along in novel large groups. Male and female spiny mice were tested in same-sex groups of 14 animals, where all but one animal was novel to each focal animal. In all groups, we observed little-to-no aggression between animals and found that both sexes readily affiliate with novel conspecifics, confirming their feasibility for use in large-group interaction experiments. We also examined brain-behavior relationships, with a specific focus on nonapeptide neuronal populations. Vasopressin (VP) and oxytocin (OT) modulate a variety of social behaviors ranging from affiliation to aggression and anxiety. How the nonapeptides relate to grouping behaviors in mammals is not well known. Thus, we correlated behavior from the novel group interactions with OT and VP densities from several distinct neuronal populations throughout the midbrain. We found that the number of OT, as well as VP, neurons in the anterior hypothalamus (AH) negatively correlated with the amount of time spent investigating conspecifics at the beginning of the novel group interaction. This suggests that the AH OT and VP cell groups may inhibit social boldness. Indeed, AH VP facilitates aggression in territorial species. However, in a non-territorial, highly social species, this cell group may have evolved to inhibit prosocial behaviors and/or social boldness as opposed to directly modulating overt aggression. Other analyses include relating status in social networks to nonapeptide neuroanatomy. Together this study demonstrates that spiny mice are ideal for examining nonreproductive social behavior in same-sex large groups, and provides a promising foundation for future studies investigating complex social interactions in a group setting, such as long-term group dynamics and group acceptance of newcomers, as well as the neural mechanisms underlying these social behaviors.

Disclosures: **D. Ho:** None. **J.P. Curley:** None. **A.M. Kelly:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.01/LL23

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Burrough's Wellcome Fund CASI award
Baszucki Brain Research Fund

Title: Conserved mechanisms coordinating body-wide metabolism and exploratory behavior

Authors: *D. HOCHBAUM, B. SABATINI;
Harvard Med. Sch., Boston, MA

Abstract: Changes in the metabolic state of an animal are often coupled to adaptations in behavior. For example, in seasonal environments, when food is abundant, metabolism ramps up, and animals tend towards more exploratory behaviors such as foraging, mating, and expansion of territory, which innately involve risk taking. When food is scarce, metabolism slows, and animals tend to become more risk averse. Territories shrink, animals exploit nearby resources, and in some cases, they barely move (hibernate). In humans the innate coupling of behavior to metabolism is clear in the context of metabolic disorders, such as hyper- or hypo-thyroidism, which present with psychiatric symptoms of mania (hyperthyroidism, increased metabolism), or depression (hypothyroidism, slowed metabolism). While the coordination between bioenergetics and behavior is readily apparent, how it is achieved remains unclear. Here, we show how one potent regulator of whole-body metabolism, thyroid hormone, acts locally in the brain by turning on transcriptional programs in neurons that result in the rewiring of cortical microcircuitry. By directly manipulating these programs, we find that they are required to alter exploratory behaviors. Thus, although thyroid receptors are expressed in many organs and in many brain areas, notably the hypothalamus, T3-dependent transcriptional cascades in frontal cortex rewire neural circuits to coordinate two seemingly disparate biological phenomena: exploratory drive and whole-body metabolic state.

Disclosures: **D. Hochbaum:** None. **B. Sabatini:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.02/LL24

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01MH117111
NIH Grant T32MH067564

Title: Oxytocin-dopamine convergence in the mouse medial prefrontal cortex

Authors: *S. FREDA, M. F. PRIEST, Y. F. LOWE, I. J. RIETH, L. H. YOON, Y. KOZOROVITSKIY;
Northwestern Univ., Evanston, IL

Abstract: Oxytocin (Oxt) and dopamine (DA) are highly conserved neuromodulators implicated in prosocial behaviors. Central Oxt and DA projections extend widely throughout the mammalian brain, with enrichment in distal limbic networks linked to reward and salience processing. Interactions of these two systems are integral for higher-order social behaviors and recent studies in our laboratory reveal *direct* connectivity between paraventricular Oxt neurons and dopamine-synthesizing neurons of the ventral tegmental area and substantia nigra pars compacta. To further dissect convergent sites of Oxt-DA signaling, we performed brain-wide quantitative anterograde and retrograde tracing of paraventricular oxytocin neurons in male and female mice. We found Oxt projections in numerous nuclei of the mesocorticolimbic dopamine system, supporting a multisite Oxt-DA interaction framework in regions linked to social cognition. Our cortical projection maps also identify the medial prefrontal cortex (mPFC) as a nucleus with sex-conserved and strong Oxt connectivity, compared to other sensory processing cortices. At the behavioral level, mPFC Oxt release underlies maternal care, sociosexual approach, and social recognition. Yet, our understanding of how Oxt generates prosocial behavioral output by reconfiguring neocortical activity is limited, outside of the auditory cortex. Our project describes how Oxt release may alter mPFC activity in a layer- and projection-specific manner and dissects how Oxt and DA may concurrently modulate mPFC neuronal output. Our quantitative mapping of endogenous cortical oxytocin receptors uncovers how local Oxt release may simultaneously engage superficial layer inhibitory neurons and deep layer pyramidal neurons to modulate circuit-wide activity. In addition, we provide evidence that Oxt and DA target the same deep layer mPFC pyramidal neurons to potentially form a distinct microcircuit in the mPFC. *Ex vivo* slice recordings of mPFC neurons reveal layer-dependent modulatory effects of oxytocin, supporting our receptor mapping data. We are currently using custom designed, multichannel optrodes to further dissect our circuit-level predictions of mPFC oxytocin release and potentially describe novel mechanisms of Oxt-DA modulation relevant to social processing.

Disclosures: S. Freda: None. **M.F. Priest:** None. **Y.F. Lowe:** None. **I.J. Rieth:** None. **L.H. Yoon:** None. **Y. Kozorovitskiy:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.03/LL25

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant RO1DA026437

Title: Intranasal oxytocin results in increased head motion during functional MRI scanning

Authors: *S. HOULTON, J. VAIDYA, L. STRATHEARN;
Univ. of Iowa, Iowa City, IA

Abstract: Oxytocin (OT) is a neuropeptide synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. OT is associated with prosocial behaviors, such as parent-child bonding, eye contact, and sexual activity. Intranasally-administered OT has been widely used to study its effects on the human brain using functional magnetic resonance imaging (fMRI). Motion artifact is one of the most critical factors in interpreting fMRI results, as it can distort the images and functional results. Here, we show differences in head motion due to OT compared to placebo in mothers with addiction problems. We used fMRI to investigate the differences in neural response between mothers with drug addiction problems and healthy control mothers, while viewing images of their own vs. unknown infant faces. We used a double-blind, placebo-controlled crossover design to investigate the contrasts in neural activation following OT or placebo. 24 control and 25 addiction participants inhaled a nasal spray either containing 24 international units (IU) of OT or a water-based placebo, 50 minutes before the fMRI scan. There were four task runs and one resting state run. Motion threshold was defined as framewise displacement (FD), an index of head movement in 6 translational and rotational axes from volume to volume, $\geq$ 3mm. Using a Poisson regression, we investigated the number of times participants exceeded the 3mm threshold in FD. To ensure that the motion was not specifically related to the baby-face task paradigm, we analyzed the resting state data separately and later, combined them. We found that mothers with addiction problems more frequently exceeded the motion threshold compared to control mothers ($z = 2.372$, $p = 0.018$). Interestingly, the OT condition was independently and strongly associated with increased motion ($z = 6.005$, $p = 1.91e-09$). There was no significant interaction between group and condition. OT results in an increase in motion, regardless of group. Past fMRI studies using OT have reported differences in neural activation, but without fully considering the impact of motion artifact on these results. We are exploring different methods of analyzing motion artifact after intranasal OT administration, and how to best report this information.

Disclosures: S. Houlton: None. **J. Vaidya:** None. **L. Strathearn:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.04/LL26

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: RGPIN-2018-04699

Title: Interplay between estrogen receptors and oxytocin receptors in social recognition processing in the medial amygdala of female mice

Authors: *D. CANTINI[1], M. CHA[1], E. CHOLERIS[2];
[2]Elena Choleris, [1]Univ. of Guelph, Guelph, ON, Canada

Abstract: Estrogens act via gene regulation and rapid cellular signalling. The potent and most abundant estrogen 17β-estradiol has been shown to facilitate social recognition of conspecifics in mice on a rapid time scale in various brain regions. The medial amygdala is heavily involved in olfactory processing of social odours in mice and expresses the three known estrogen receptors (ER): ERα, ERβ, and G Protein-Coupled ER (GPER). Selective agonists for each of the three ERs rapidly facilitate social recognition in the medial amygdala of female CD1 mice. Fully functional oxytocin receptors (OTR) within this region are necessary for the rapid effects of 17β-estradiol on social recognition, suggesting an E2/OTR interplay. The objective of this study is to elucidate which of the three ERs interplay with OTRs in the medial amygdala of female mice to elicit rapid facilitation of social recognition. Female mice were ovariectomized and had bilateral cannulae implanted into the medial amygdala. A subeffective dose of OTR antagonist (OTRA) was infused into the medial amygdala before the infusion of one of the ER agonists (ERα agonist PPT; ERβ agonist DPN; GPER agonist G1). A difficult social recognition paradigm designed to measure the rapid facilitating effects of treatment was administered. If the facilitating effect of the ER agonist is impaired by the administration of the OTRA, we can infer that the specific ER is implicated in the interplay with OTR. Administration of subeffective OTRA before DPN resulted in no impairment in social recognition. Preliminary results show that administration of subeffective OTRA before G1 resulted in impairment in social recognition, suggesting that GPER, but not ERβ, interplays with OTRs within the medial amygdala in the rapid facilitation of social recognition. Elucidating the interplay between steroid hormones and oxytocin in social brain regions will provide a better understanding of the biology of healthy social behaviour.
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Disclosures: D. Cantini: None. **M. Cha:** None. **E. Choleris:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.05/Web Only

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Japanese KAKENHI grant

Title: Hippocampus-synthesized estrogen and androgen modulate dendritic spines and LTP in non-genomic manner

Authors: *S. KAWATO, M. SOMA, M. OGIUE-IKEDA;
Univ. of Tokyo, Tokyo, Japan

Abstract: We demonstrated (1) hippocampal synthesis of estrogen and androgen, and (2) non-genomic synaptic modulation by these sex-steroids. [Synthesis] We showed expression as well as neuronal/synaptic localization of essential enzymes in the adult male rat hippocampus. Mass-spectrometric analysis demonstrated that hippocampal levels of estradiol (E2), testosterone (T) and dihydrotestosterone (DHT) were about 8 nM, 17 nM and 7 nM, higher than those in plasma. Rapid E2 synthesis with in the hippocampus can be measured via blocking of LTP (long-term potentiation) by 20 min perfusion of Aromatase inhibitor in hippocampal slices. Castration did not decrease male hippocampal E2, because E2 is synthesized from hippocampal T. Castration, however, significantly decreased T and DHT in the hippocampus, indicating an import of T via the blood circulation. Female hippocampal levels of E2 (0.5-4 nM), and T (1 nM) were less than male, but much higher than those in plasma. [Synaptic Modulation] E2-induced rapid non-genomic modulation (1 h) was demonstrated by analysis of dendritic spines and LTP of adult male rat hippocampal 'acute' slices (steroid-depleted slices). Dendritic spine analysis was performed for CA1 pyramidal neurons in hippocampal slices. Spine density and spine head diameters were obtained by mathematical software Spiso-3D which identifies spines by calculating geometrical parameters. E2 at 1 nM rapidly increased the density of small-head spines. T and DHT at 10 nM increased the density of small-head and large-head spines. Signaling pathways are: synaptic ERalpha or AR → LIMK, MAPK, PKA, PKC, Src → cofilin or cortactin → actin polymerization→ new spines. LTP analysis showed that 1 nM E2 induced full-LTP (E2-LTP) upon sub-threshold stimulation, although without E2 the sub-threshold stimulation did not induce full-LTP. Kinase inhibitors against MAPK, PKA, PKC blocked E2-LTP. References: Kimoto et al., 2001 Endocrinol, Hojo et al., 2004 PNAS, Mukai et al., 2007 J. Neurochem, Hojo et al., 2009 Endocrinol, Mukai et al. 2011 Cereb Cortex, Ooishi et al. 2011 Cereb Cortex, Okamoto et al., 2012, PNAS, Kato et al., 2013, Frontier Neurosci. Hasegawa et al., 2015 Brain Res., Hatanaka et al., 2015 Brain Res., Murakami et al., 2015 Brain Res., Soma et al., 2018 Frontier Neurosci., Hojo and Kawato 2018 Frontier Neurosci.

Disclosures: S. Kawato: None. **M. Soma:** None. **M. Ogiue-Ikeda:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.06/LL27

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01MH123523

Title: Single-cell multiomic characterization of the impact of sex and estrous cycle on cellular phenotype in the ventral hippocampus

Authors: *M. C. TICKERHOOF[1], M. SUZUKI[2], M. KUNDAKOVIC[1];
[1]Dept. of Biol. Sci., Fordham Univ., Bronx, NY; [2]Dept. of Nutr., Texas A&M Univ., College Station, TX

Abstract: Depression and anxiety disorders are up to twice as prevalent in women as in men. The natural hormonal shifts occurring over the course of the menstrual cycle are an important contributing factor, as demonstrated by the prevalence of these disorders nearly doubling during the reproductive period, as well as exacerbated symptoms during the premenstrual period in more than a half of individuals with such disorders. In rodents, anxiety- and depression-related behaviors also vary across the estrous cycle, wherein female mice demonstrate higher avoidance during diestrus (low estrogen, high progesterone) and decreased avoidance during proestrus (high estrogen, low progesterone). We previously linked these hormone-driven behavioral changes to alterations in neuronal gene expression and chromatin organization within the ventral hippocampus (vHip), a region important in stress susceptibility and emotion regulation. However, these molecular findings were characterized at the level of bulk analysis of sorted neuronal (NeuN+) nuclei, and thus shifts in regulation of gene expression within select cell populations, including glial cells, can be obscured. In this study, we aim to characterize both gene expression and chromatin accessibility within the vHip across the estrous cycle and sex at single-nucleus resolution. Our study includes two female groups in proestrus (n=6) and diestrus (n=6), and males (n=6). After vHip dissection, nuclei are isolated and processed using the 10X Chromium Single Cell Multiome kit, targeting 10,000 nuclei per replicate. Our analysis so far has revealed 33 different cell clusters including 19 distinct subclusters of excitatory neurons (NeuN+/Slc17a7+), 5 distinct subclusters of inhibitory neurons (NeuN+/Gad1+), and the remainder clusters of non-neuronal cells including rare populations like ependymal cells and pericytes. Each subcluster has unique patterns of genes known to have a role in estrogen responsivity (e.g. Esr1), chromatin remodeling (e.g. Egr1), and anxiety-related behavior (e.g. Dlk1). These results demonstrate heterogeneity within broad cell types, with unique neuronal and glial subclusters that were missed in bulk neuron-only analysis. This project is ongoing, and we predict that gene expression and regulation will differ within distinct cell types depending on sex and ovarian hormone status, particularly for genes relevant for neuronal plasticity and anxiety- and depression-related behaviors. This work will provide essential knowledge into the neurobiological mechanisms underlying sex bias in depression and anxiety disorders, paving the way for treatments that take hormonal state into account.

Disclosures: M.C. Tickerhoof: None. **M. Suzuki:** None. **M. Kundakovic:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.07/LL28

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC grant RGPIN-2018-04699

Title: Rapid effects of estrogens and androgens on short-term memory in the dorsal hippocampus of male mice

Authors: *K. E. LADOUCEUR[1], K. NIELSEN[1], E. CHOLERIS[2];
[2]Elena Choleris, [1]Univ. of Guelph, Guelph, ON, Canada

Abstract: It is established that sex-steroid hormones can influence learning and memory within a rapid timeframe that is independent of gene transcription. Previous research has found that in ovariectomized female mice, the potent estrogen, 17β-estradiol (E2), facilitates social recognition, object recognition and object placement short-term memory in the dorsal hippocampus. The rapid effects of sex steroid hormones on short-term memory in the dorsal hippocampus of male mice have yet to be elucidated. The primary circulating steroid hormone in males, Testosterone, can be converted to either estrogens (by the enzyme aromatase) or dihydrotestosterone (DHT, by the enzyme 5α-reductase). Thus both estrogens or androgens may exert rapid effects on the male dorsal hippocampus. For this reason, we examined the rapid influence of E2 and the non-aromatizable androgen, DHT on social recognition, object recognition or object placement short-term memory. Castrated male mice receive an intradorsal hippocampal infusion of either E2 (25, 50, 100, 150 or 200 nM), DHT (0.5, 0.25 or 0.0625 μg/μL) or the artificial cerebral spinal fluid vehicle (+0.02% ethanol) and their short-term memory performance is assessed in one of three tasks; object recognition, object placement and social recognition. Each task is designed such that castrated male mice cannot perform it, thus allowing the assessment of facilitating effects. This research further elucidates the rapid modulation of brain functions by the sex-steroids in males.

Disclosures: K.E. LaDouceur: None. **K. Nielsen:** None. **E. Choleris:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.08/MM1

Topic: F.02. Neuroendrocrine Processes and Behavior

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R15GM118304
UWM Discovery and Innovation Grant (101x418)
Distinguished Graduate Student Fellowship
Distinguished Dissertator Fellowship

Title: Determining an estrogenic spatial engram in the mouse dorsal hippocampus

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Abstract: Rationale: Object exploration triggers neuronal activity in the dorsal hippocampus (DH), which forms new contacts between neurons to form a memory engram. However, weak learning leads to the fading of initial contacts and failure to recall learned information. The potent estrogen, 17β-estradiol (E2), facilitates consolidation of spatial memory by producing stronger synaptic connections than learning alone, potentially by drawing more neurons into the engram. However, it is unknown which cells become members of this engram. We hypothesize that E2 activates greater numbers of pyramidal neurons in the DH than learning alone to support spatial memory-related engrams. **Objective:** Our objective was to leverage the activity-dependent Tet-Tag viral approach to compare the effects of object training alone, E2 infusion into the DH alone, and training+E2 on activation of hippocampal CA1 pyramidal neurons in male and female gonadectomized mice. **Methods:** Adult male and female C57BL/6 mice were gonadectomized, infused bilaterally with a 1:1 viral mixture of AAV9-c-fos-tTa and AAV9-TRE-EGFP into dorsal CA1, and implanted with DH-targeting bilateral guide cannulae in a single surgical session and given 1 or 2 weeks to recover before behavioral training. Mice were fed doxycycline to repress green fluorescent protein (GFP) production until 3 days prior to object training, where they explored 2 identical objects for an accumulated 30 s. Immediately after training, mice were bilaterally infused into the DH with vehicle or 5 µg/hemisphere E2 and replaced on doxycycline 30 min later. Homecage controls were handled for 30 s and either placed back into their cages or infused with vehicle or E2 into the DH and placed back on doxycycline 30 min later. All mice were perfused 10 days later. Brains were sectioned and immunohistochemically labeled for DAPI, GFP, and CaMKIIα to label virally induced expression of GFP in pyramidal neurons. Dorsal CA1 of each section (n = 4-6/brain) was quantified bilaterally for GFP, CaMKIIα, and their overlap. **Results:** Preliminary results showed that after 1 week of recovery, GFP tagging of neurons in the female CA1 was not affected by training or treatment status. However, after 2 weeks, training+E2 led to significantly more GFP tagging than training+vehicle and homecage control conditions, suggesting a minimum timepoint for sufficient viral expression to examine the estrogenic spatial engram. **Conclusions:** These initial data support our hypothesis of a combinatory recruitment of cells into the engram by learning and E2 signaling. Future work will continue to identify these cells in males and females to determine how estrogens influence the spatial engram.

Disclosures: A. Fleischer: None. **S. Ramirez:** None. **K.M. Frick:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.09/MM2

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01MH107886 awarded to KMF
R01MH122414 awarded to TJJ
1F32MH118782-01A1 awarded to KSG

SURF funding awarded to RKK from the UWM Office of Undergraduate Research

Title: Examining a role for proteasome activity in estradiol-induced facilitation of hippocampal memory consolidation and CA1 spine density in ovariectomized female mice

Authors: *S. B. BEAMISH[1], K. S. GROSS[1], R. K. KUEHN[1], T. J. JAROME[2], K. M. FRICK[1]; [1]Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Sch. of Animal Sciences, Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: 17β-estradiol (E_2) enhances hippocampal function and long-term memory formation, yet the molecular mechanisms through which E_2 exerts its effects remain unclear. One overlooked mechanism that may be integral to the memory-enhancing effects of E_2 is protein degradation mediated by the ubiquitin-proteasome system (UPS). In this system, proteins are tagged with ubiquitin and become substrates for degradation by the 26S proteasome complex. The present study examined the extent to which proteasomal protein degradation is necessary for E_2 to enhance hippocampal memory consolidation and CA1 spine density in ovariectomized (OVX) female mice. Because E_2 in the dorsal hippocampus (DH) enhances the consolidation of spatial memory and object recognition in the object placement (OP) and object recognition (OR) tasks, we first sought to establish a role for proteasome activity in memory formation in these tasks. Adult female C57BL6/J mice were bilaterally OVX and implanted with bilateral guide cannulae into the DH. After recovery, mice were allowed to accumulate 30 s exploring two identical objects in an open field. Immediately after training, mice received bilateral DH infusion of vehicle or the proteasome inhibitor β-lactone (β-lac; 4, 8, or 16 ng/hemisphere), and OP and OR were tested 24 h and 48 h later, delays at which vehicle-infused mice display intact memory consolidation. During testing, one object was moved (OP) or replaced with a novel object (OR); mice that remember the training objects spend more time than chance (15 s) with the moved or new object. DH infusion of 8 or 16 ng/hemisphere, but not 4 ng/hemisphere, of β-lac impaired OP and OR memory consolidation in OVX mice. Next, to determine whether UPS activity is necessary for the memory-enhancing effects of E_2, new OVX mice were implanted with bilateral DH cannulae to deliver vehicle or the behaviorally subeffective dose of 4 ng/μl β-lac and a third cannula targeting the dorsal third ventricle (ICV) to deliver vehicle or E_2. Mice were trained in OP or OR, and then immediately received ICV and DH infusions, respectively, of vehicle+vehicle, E_2+vehicle, vehicle+β-lac, or E_2+β-lac. Memory was tested 24 h (OP) and 48 h (OR) later, delays at which vehicle-infused mice do not display intact memory consolidation. Mice treated with E_2+vehicle spent significantly more time than chance with the moved and novel objects, whereas E_2+β-lac treated mice did not. These data provide the first evidence that proteasome activity is necessary for E_2 to enhance hippocampus-dependent memory in OVX female mice. Ongoing work is assessing the extent to which β-lac prevents E_2-induced enhancements in CA1 spine density.

Disclosures: S.B. Beamish: None. **K.S. Gross:** None. **R.K. Kuehn:** None. **T.J. Jarome:** None. **K.M. Frick:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.10/MM3

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 2R15GM118304-02
NIH Grant R01MH107886
UWM Research Foundation Catalyst Award sponsored by the Lynde and Harry Bradley Foundation

Title: Systematic oral treatment with a highly selective and potent estrogen receptor beta agonist (EGX854) alleviates hot flashes and improves memory in a mouse model of menopause

Authors: *F. H. ABDELAZIM[1], A. W. FLEISCHER[1], S. CHAUDHURY[2], E. A. WETZEL[2], K. J. MARKS[3], S. BROWN-FORD[3], A. A. GLEASON[3], W. A. DONALDSON[2], D. S. SEM[3], K. M. FRICK[1];
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Abstract: Rationale Menopause is a naturally occurring transition in women's lives marked by a decline in the production of sex steroid hormones, including estrogens. This estrogen loss often leads to hot flashes and an increased risk of memory dysfunction and Alzheimer's disease. Although estrogen therapy (ET) can effectively treat hot flashes and reduce the risk of memory loss and dementia, it carries risks of cancer and heart disease. Estrogen receptor agonists acting on estrogen receptors alpha (ERα) and beta (ERβ) can alleviate hot flashes and enhance memory function. However, ERα activation is associated with cell proliferation whereas ERβ activation reduces tumor proliferation. As such, ERβ selective agonists are a promising avenue for symptom relief without the negative effects of traditional ET. **Objective:** Our goal was to determine the extent to which long-term oral treatment with a novel highly potent and selective ERβ agonist (EGX854) could alleviate hot flashes and enhance memory in a mouse model of menopause. **Methods:** Efficacy of EGX854 (1,100-fold ERβ selectivity) was compared to another selective ERβ agonist (EGX358, 750-fold ERβ selectivity) that we previously showed reduces hot flashes and enhances memory in young ovariectomized mice. Here, 8 week-old female C57BL/6 mice (n=14/group) were ovariectomized then treated daily with vehicle (10%DMSO), EGX358 (0.5 mg/kg), or one of three doses of EGX854 (0.005, 0.05, or 0.5 mg/kg) via oral gavage for 2 weeks prior to behavioral testing. Mice continued treatment and assessment of memory, hot flashes, anxiety, and depression- like behaviors for 8 weeks. Memory was assessed using object placement (OP) and object recognition (OR) tasks. Thermal imaging of tail skin temperature (T_{skin}) assessed hot flashes after injection of vehicle or senktide, an NK-3 tachykinin receptor agonist that induces heat dissipation from the tail. Anxiety-like behavior was assessed in open field (OF) and elevated plus maze (EPM), and depression-like behavior was assessed in tail suspension (TST) and forced swim (FST). **Results:** EGX358 and EGX854 (0.005 and 0.5 mg/kg) significantly enhanced OP memory and one dose of EGX854 (0.5 mg/kg) significantly enhanced OR memory compared to vehicle. Preliminary EPM data show no effect of treatment on anxiety-like behaviors. No treatment affected body weight or uterine weight,

indicating no effects on appetite, metabolism, or cell proliferation. **Conclusion:** Long-term treatment with EGX854 significantly enhanced spatial and object recognition memory with no effects on anxiety, body weight, or uterine cell proliferation. Analyses of anxiety- and depression-like behaviors and T_{skin} are ongoing.

Disclosures: F.H. Abdelazim: None. **A.W. Fleischer:** None. **S. Chaudhury:** None. **E.A. Wetzel:** None. **K.J. Marks:** None. **S. Brown-Ford:** None. **A.A. Gleason:** None. **W.A. Donaldson:** 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.; Estrigenix Therapeutics, Inc. **D.S. Sem:** 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.; Estrigenix Therapeutics, Inc. **K.M. Frick:** 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.; Estrigenix Therapeutics, Inc..

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.11/MM4

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01MH107886
UWM Distinguished Dissertation Fellowship
UWM College of Letters and Science
UWM Office of Undergraduate Research

Title: A role for de novo mPFC estradiol synthesis in spatial and object recognition memory consolidation in ovariectomized mice

Authors: *M. R. SCHWABE, H. A. BEATY, E. M. MILKIE, K. M. FRICK;
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Abstract: Decades of research has shown that the hormone 17-β- estradiol (E2) can regulate hippocampal synaptic plasticity and enhance the consolidation of memories dependent on the dorsal hippocampus (DH) such as spatial memory and object recognition. Other brain regions, such as the medial prefrontal cortex (mPFC), also play critical roles in these types of memory, yet little is known about the involvement of E2 in these areas in mediating memory formation. In the DH, de novo E2 synthesis is essential for object recognition and object placement memory consolidation in ovariectomized (OVX) female mice, as acute post-training DH infusion of the aromatase inhibitor letrozole impairs memory consolidation in object recognition (OR) and

object placement (OP) tasks (Tuscher et al., 2016). Because mPFC activity is also critical for consolidation in these memory tasks (Tuscher et al., 2018) and exogenous E2 infusion in the mPFC enhances OR and OP memory consolidation (Tuscher et al., 2019), we hypothesized that de novo E2 synthesis in mPFC is likely necessary for OR and OP memory formation. Female C57BL/6 mice (8-9 weeks of age) were OVXed and implanted with bilateral guide cannula to target the mPFC. After recovery, mice were placed in an open field box and allowed to accumulate 30 s exploring two identical objects. Immediately afterwards, mice received an intra-mPFC infusion of vehicle (1% DMSO in sterile saline) or one of two doses of letrozole (0.025 µg or 0.05 µg/hemisphere). During testing, one training object was moved to a different quadrant (OP) or replaced with a novel object (OR). OP and OR testing was conducted 4 hours and 24 hours after training, respectively, timepoints at which vehicle-infused mice remember the identity and location of training objects. Preliminary data indicate that letrozole-infused mice exhibit impaired OR and OP memory consolidation relative to controls, supporting a key role for de novo E2 synthesis in mPFC during memory consolidation. Ongoing work aims to establish a role for aromatase-expressing cells in object learning and to better characterize specificity of aromatase expression in mPFC subregions.

Disclosures: **M.R. Schwabe:** None. **H.A. Beaty:** None. **E.M. Milkie:** None. **K.M. Frick:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.12/MM5

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH P01AG071746

Title: Relationship between estrogens, cardiometabolic health, and spatial memory in a rat model of menopause

Authors: *C. MONTANARI[1,2], E. L. DONG[2,3], S. SRINIVASAN[2,3], A. P. O. LEITE[4,2], R. MENON[2,4], A. B. WALKER[2,5], Z. DIAZ[4,2], A. F. DELARGE[1,2], M. J. MAROTEAUX[1,2], L. DESMOULINS[5,2], S. H. LINDSEY[4,2], A. ZSOMBOK[5,2], J. M. DANIEL[1,2,3];
[1]Psychology Dept., [2]Tulane Brain Inst., [3]Tulane Neurosci. Program, [4]Pharmacol. Dept., [5]Physiol. Dept., Tulane Univ., New Orleans, LA

Abstract: Research in preclinical models indicates that estrogens are neuroprotective and positively impact cognitive aging. However, clinical data have been equivocal as to benefits of menopausal estrogen therapy for the brain and cognition. Variation in response to estrogen therapy in women suggests that pre-existing disease such as hypertension and metabolic syndrome can modulate mechanisms of estrogen action. The goals of the current work are: 1) to determine if cardiometabolic health in middle-age female rats is predictive of performance on a

spatial memory task, and 2) to determine if the ability of different regimens of estradiol treatments to differentially affect memory in aging females is predicted by their impact on cardiometabolic health. Female Long-Evans rats were purchased at 70 days of age and allowed to age until 8 months of age. Rats were then trained for 24 days on a spatial memory radial-maze task. Following completion of maze training, measures of cardiometabolic health were obtained including glucose tolerance test, body composition measures using dual energy X-ray absorptiometry, blood pressure using tail-cuff plethysmography, and body weight. Regression analyses revealed no relationship between any of the cardiometabolic measures and spatial memory (number of errors averaged over last 4 days of radial-maze training). Following completion of the initial cardiometabolic testing, all rats were ovariectomized and implanted with vehicle or estradiol capsules. Capsules were replaced after 40 days and again after 5 months resulting in the following treatment groups: 1) Continuous Vehicle (receive only vehicle capsules; modeling women who never use menopausal estrogen therapy, 2) Continuous Estradiol (receive only estradiol capsules; modeling women who take and remain on estrogen therapy), 3) Previous Estradiol (receive estradiol for 40 days followed by vehicle; modeling women who take estrogen therapy for a few years and then stop), and 4) Delayed Estradiol (receive vehicle for 5 months followed by estradiol; modeling women who begin taking estrogens years after menopause). One month prior to and again 3 months following the second capsule replacement, spatial memory and cardiometabolic health status were assessed. At each of these time points, the relationships between estradiol status, spatial memory, and measures of cardiometabolic health were determined. Current results indicate that cardiometabolic health status is not related to performance on a radial-maze task in gonadally intact middle-age female rats. Assessment of the relationship between estradiol treatments, spatial memory, and cardiometabolic health is ongoing.

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Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.13/MM6

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Pilot Grant from the Nathan Shock Center for Excellence

Title: Aberrant hippocampal modulation of the dopamine system in the follicle deplete model of perimenopause

Authors: *S. PEREZ[1], A. BOLEY[1], D. J. LODGE[2];
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Abstract: Despite the known changes in mental health associated with the menopause transition (or perimenopause), there has been minimal research into the effects of perimenopause psychiatric symptoms and the brain circuits underlying them. The perimenopausal period has been associated with an elevated risk of debilitating psychiatric disorders which can negatively impact a woman's everyday life. Thus, developing a better understanding of the neurocircuitry that contributes to psychiatric alterations experienced by perimenopausal women is necessary to develop targeted therapies that can drastically improve their quality of life. Of relevance, is the dopamine system and its regulation by the hippocampus because of the role these circuits play in regulating psychiatric symptoms (such as psychosis) and cognitive function. To establish a translational model of perimenopause in rodents, we administered 4-vinylcyclohexene diepoxide (VCD) which induces a progressive loss of ovarian follicles resembling natural menopausal transition in women. Our preliminary data in this model demonstrates baseline hippocampal hyperactivity, aberrant coordinated neuronal activity, and deficits in behaviors mediated by hippocampal function. Further, we observe aberrant dopamine system function and deficits in dopamine-dependent behaviors. This is relevant because increased dopamine neurotransmission is believed to be responsible for symptoms of psychosis. Taken together, our data suggests that aberrant hippocampal activity may underlie symptoms of psychosis during perimenopause.

Disclosures: S. Perez: None. **A. Boley:** None. **D.J. Lodge:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.14/MM7

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Castration in male rats expedites habitual behavior: no effect of estrogen replacement

Authors: *Z. MOHAMMED, H. SCHOENBERG, S. VONDOEPP, R. DOUGHERTY, E. HILTON-VANOSDALL, F. CARASI-SCHWARTZ, D. TOUFEXIS;
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Abstract: Gonadal hormones are key physiological regulators of behaviors in both human and nonhuman animals. These include testosterone, estrogen, and dihydrotestosterone in males. Research in our laboratory has demonstrated that female rats exhibit habitual responding, measured as insensitivity to reinforcer devaluation, after 120 reinforcer-outcome pairings (R-O). In contrast to the rapid development of habit in female rats, responses of male rats remain goal-directed up to at least 320 R-Os. In the present study, we aimed to investigate how gonadal hormones affect the acquisition and expression of goal-directed and habitual behavior in male rats. To accomplish this, castrated male rats (n = 18) were trained to 240 R-Os, a level of training at which intact males remain goal-directed, and half of the rats then received reinforcer devaluation using lithium chloride. All rats were then tested for habitual responding in the absence of the outcome (extinction). Results showed that castration in male rats produced

habitual responding. Subsequently, we added an additional experimental group (n = 18), in which castrated males were replaced with constant physiological levels of estradiol (E2), to mimic the aromatization of testosterone to estrogen that occurs in intact male rats and thereafter, underwent the similar experimental procedure. Data showed that Castrated E2-replaced males also remained habitual at 240 R-O's. Together, these studies suggest that testicular hormones are necessary for the maintenance of goal-directed behavior in male rats following 240 R-Os. And that the estrogenic metabolite of testosterone is insufficient to restore goal-directed behavior in castrated male rats. This work provides new insights into the emerging role of sex and hormones in cognition and behavior.

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Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.15/MM8

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: CONACYT CVU1131493

Title: Characterization of the effect of a chronic hypercaloric diet or a chronic restricted diet on the metabolism and behavior of ovariectomized rats

Authors: *E. RUIZ-MASSO[1], B. B. CARRANZA-CASTILLO[1], L. QUEVEDO-CORONA[1], I. JIMENEZ-ESTRADA[2];
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Abstract: "Characterization of the effect of a chronic hypercaloric diet or a chronic restricteddiet on the metabolism and behavior of ovariectomized rats"Ruiz-Masso Eduardo[1,2], Carranza Castillo Brenda Berenice[1], Quevedo Corona Lucía[1] andJiménez Estrada Ismael[21]National School of Biological Sciences, IPN, CDMX[2] Center for Research and Advanced Studies, IPN, CDMXThe objective of this study is to characterize the alterations evoked by a bilateral ovariectomy(Ovx) made at the postnatal day 90, on the energy metabolism (food consumption, weightgain, temperature, fasting blood glucose, glucose tolerance, and adiposity), leptin hormoneexpression and several motor and cognitive behaviors (Locomotor activity evaluated withthe open field test; Anxiety behavior examined by the elevated plus maze; Depressiondetermined by the tail suspension test, and learning and memory assessed by the Barnesmaze test) of female Wistar rats subjected to: A) standard diet (CONT, CONT-Ovx), B)hypercaloric diet (DHC, DHC-Ovx), or C) food restricted diet (DR, DR-Ovx), chronicallyapplied from gestation until the postnatal week 28 (PNW 28).The results obtained show that at PNW 28, rats of the DHC and DHC-Ovx groups display anoticeable increase in

body and fat weight, with no apparent change in body size while DRand DR-Ovx rats exhibited lower body and fat weight with respect to the other animal groups(CONT, CONT-Ovx, DHC, DHC-Ovx). Blood glucose levels were significantly larger in DHCrats. Leptin concentration was higher in DHC, and DHC-Ovx groups, while the DR and DR-Ovx groups showed decreased values. In addition, animals with lower body weight (DR andDR-Ovx) showed a considerable larger locomotor activity in the open field test than the othergroups of animals. The CONT-Ovx group showed higher anxiety as compared to the CONTgroup, and the DR-Ovx group exhibited higher anxiety compared to the DR group. The mostremarkable effect of both diet and ovariectomy was observed on the depressive behavior. Itwas determined that Ovx increases depressive behaviors in DHC rats but decreased suchbehavior in DR rats compared with their counterparts. Rats on the DHC diet experiencedgreater depression compared to those on the DR diet, which showed a decrease indepressive behavior.In conclusion, the reduction of sexual hormones due to ovariectomy evokes noticeablealterations in morphological, metabolic, and behavioral characteristics in well- andmalnourished rats. One of the most remarkable effects is CONT-Ovx and DR-Ovx ratsincreased their anxiety behavior and CONT-Ovx, DHC and DHC-Ovx rats showed biggerdepressive behavior than the other groups of rats.

Disclosures: **E. Ruiz-Masso:** None. **B.B. Carranza-Castillo:** None. **L. Quevedo-Corona:** None. **I. Jimenez-Estrada:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.16/MM9

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NNS Grant LW82071603

Title: A delayed ovulation of Progestin-Primed Ovarian Stimulation (PPOS) andits neurobiologicalregulating mechanism

Authors: *Y. NIE[1], X. SHEN[2], Y. XIE[2], L. WANG[2];
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Abstract: PPOS as a new clinic ovulation stimulation protocol, its role in ovulation and regulatory mechanism is not clear. Our clinical research showed that the patients in the prolonged ovulation trigger–oocyte pickup (OPU) time interval group had significantly better clinical outcomes than the earlier group. By simulating the clinical PPOS model and using dox-modulated Fos-Tta;teto-H2B/GFP mice, we confirm the delayed ovulation, and the suppressed LH level of PPOS group which led to the reduced expression of LHCGR on the preovulatory follicles before trigger and significantly decreased the following progesterone synthesis, blood progesterone level and progesterone-receptor (PGR) expression within 4-6 hours after hCG

trigger. The PGR regulated ovulatory genes including ADAMTS1 were downregulated in the PPOS group and these sequential cascades delay its ovulation. The dox-modulated Fos-Tta;teto-H2B/GFP mice showed not only the classical reproductive brain nuclei, also retrochiasmatic area (RCh) were involved in the inhibited LH level of PPOS. In conclusion, PPOS suppresses the LH level before trigger, and decreases the synthesis of progesterone after hCG, thus delays ovulation by downregulating the LHCGR-PGR pathway, and RCh and some new brain regions were found to participate in it.

Figure 1

A

Clinical outcomes of patients using PPOS after propensity score matching

Parameters	Group 1 35.6≤Time<36.4 (hour)	Group 2 36.4≤Time<37.1 (hour)	Group 3 37.1≤Time≤37.8 (hour)	*P* value
Number of patients(n)	751	751	751	
Age(year)[#]	31.10±3.76	31.29±3.62	31.33±3.60	0.417
Oocyte retrieved(n)	12.02±6.61	12.06±6.83	11.76±6.58	0.640
MII oocyte(n)	9.80±5.80	10.06±5.87	9.86±5.68	0.655
Mature oocyte rate (%)	82.34±18.85[a]	84.54±17.26[b]	84.60±18.20[a,b]	**0.002**
Cycles with >70% mature oocytes (n)	583(77.63)[a]	621(82.69)[b]	629(83.75)[b]	**0.009**
Implantation rate (%)	29.61(551/1861)[a]	34.17(585/1712)[b]	34.37(588/1710)[b]	**0.003**
Live birth rate per transfer (%)	39.57(402/1016)	43.56(409/939)	42.06(400/951)	0.194

Shen X…, Wang L*, Kuang Y*. Front Endocrinol (Lausanne). 2019;10:694.

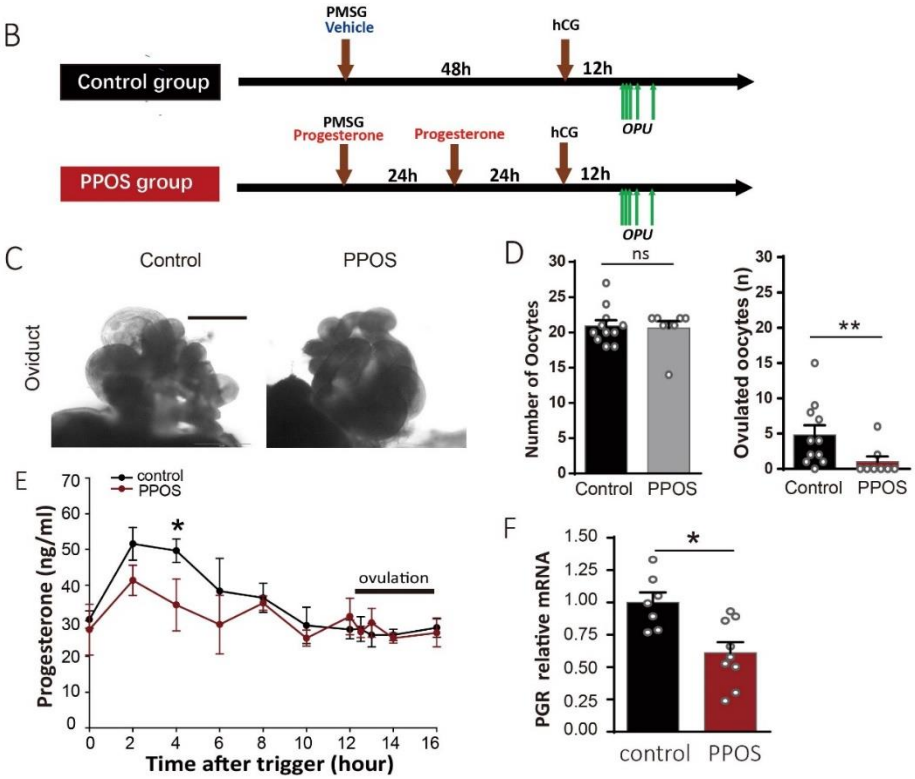

Figure 1 The decreased progesterone-PGR after trigger contributing to the delayed ovulation of PPOS. (A) The better clinical outcomes of delayed ovulation trigger–oocyte pickup interval in patients using PPOS after propensity score matching. (B) Schematic of experimental design in mice: vehicle (control group) or two doses of progesterone (PPOS group) were administered. (C) Micrographs of oviducts at 12.5 hours after hCG injection in control and PPOS groups. (n=3-5 mice in each group). Scale bar, 100μm. (D) Comparisons of the number of total oocytes and ovulated oocytes at 12.5 hours after hCG injection.(n=8-11 mice in each group). (E) The serum progesterone in the PPOS and control groups after hCG injection (n=8 mice in each group). (F) PGR relative mRNA expression levels at 4 hours after hCG injection within two groups (n=7-9 mice in each group).Statistical analysis was performed by Mann-Whitney test in D and F, and by two-way repeated ANOVA with Holm-Sidak test in E. ns, no significance (P≥0.05). * P<0.05, **P < 0.01.

Figure 2

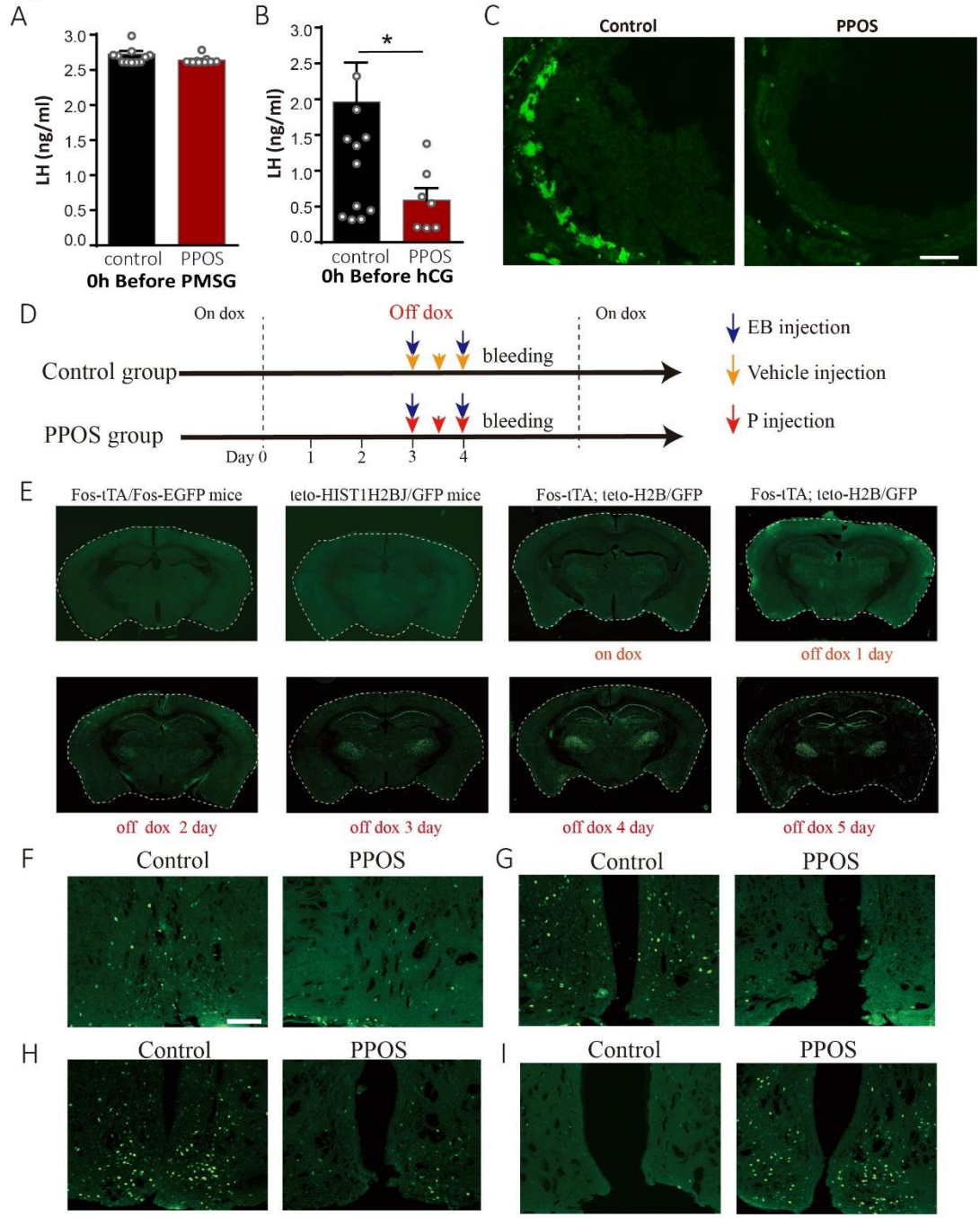

Figure 2 PPOS suppresses the LH level before trigger by modulating the reproductive related brain regions and neurons of the hypothalamus, and downregulates the LHCGR-PGR pathway after trigger. (A, B) The LH levels at 0 hour before PMSG injection (A, n=7-12 mice in each group) and 0 hour before hCG trigger (B. n=7-12 mice in each group) in the control and PPOS groups. (C) Immunofluorescence images of LHCGR expression on the ovaries in the PPOS and control groups when giving hCG injection. (n=3-5 mice in each group). (50um in the bottom panel). (D) Schematics of administration dox and blood collection protocols in the control and high PPOS groups within Fos-Tta;teto-H2B/GFP mice. (E) Fluorescence expression in Fos-Tta;teto-H2B/GFP mice before and after dox withdrawal. (F-I) Neuronal activation in AVPV (F), RP3V (G), SCh (H), RCh (I) brain regions of mice in the control and PPOS groups. (Scale bar: 100um). Statistical analysis was performed by Mann-Whitney test in A and B. ns, no significance (P≥0.05). * P<0.05.

Disclosures: **Y. Nie:** None. **X. Shen:** None. **Y. Xie:** None. **L. Wang:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.17/MM10

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Ministry of Science and Technology in Taiwan (MOST 111-2628-B-214-001-MY3)

Title: Female hyperandrogenism increases vulnerability in pain and depression in a rat model of polycystic ovary syndrome

Authors: Y.-T. SU[1], ***C.-C. WU**[2];
[1]Kaohsiung Chang Gung Mem. Hosp., Kaohsiung City, Taiwan; [2]Sch. of Med., I-Shou Univ., Yan-Chao District, Kaohsiung City, Taiwan

Abstract: Polycystic ovary syndrome (PCOS) is the most prevalent reproductive disorder affecting women, characterized by various symptoms such as hormonal imbalances, metabolic disturbances, and infertility. Studies have reported that PCOS patients have a higher prevalence of pain compared to healthy individuals, highlighting the impact of PCOS on pain perception and health-related quality of life. However, the exact underlying causes of this pain remain unclear. Additionally, pathophysiological factors contributing to pain development in PCOS include inflammation deregulation, adipokines, and insulin resistance. Although there is robust evidence supporting the association between pain perception and these risk factors, the perception and treatment of pain in PCOS patients are often overlooked in clinical practice. In this study, we aimed to evaluate the effect of PCOS on pain augmentation and disease progression by conducting a rat model of hyperandrogenism induced by letrozole, which mimics the pathogenesis of PCOS. We also generated a neuropathic pain model by spinal nerve ligation (SNL). Our data revealed that PCOS rats were more susceptible to mechanical hyperalgesia in the von Frey test, indicating heightened sensitivity to pain. Moreover, these rats exhibited increased susceptibility to depressive behavior in the forced swimming test and the tail suspension test, suggesting a potential association between PCOS and depression. Furthermore, we observed elevated levels of inflammation cytokines and calcitonin gene-related peptide (CGRP) in the serum of PCOS rats. These findings suggest that PCOS may exacerbate pain sensation and depression behavior in rats. This research highlights the importance of healthcare professionals being attentive to pain in PCOS patients and emphasizes the need for further clinical investigations and basic research to elucidate the correlation or causality between pain and PCOS.

Disclosures: **Y. Su:** None. **C. Wu:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.18/MM11

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant IOS-1754878

Title: Acute low-dose estradiol treatment decreases anxiety-like behavior of male and female juvenile Siberian hamsters

Authors: *Z. FORRESTER-FRONSTIN, A. S. MONDSCHEIN, C. N. CORDES, M. J. PAUL;
Univ. at Buffalo, Buffalo, NY

Abstract: Anxiety disorders typically arise during childhood, with a greater prevalence in girls than boys. A potential role for gonadal hormones is often overlooked because it is commonly thought that the gonads are quiescent during the juvenile period. However, the juvenile gonads secrete measurable amounts of steroid hormones, and we have recently found that prepubertal ovariectomy, but not castration, increases exploratory behavior of juvenile Siberian hamsters (*Phodopus sungorus*) in the light/dark box test — an effect typically interpreted as anxiolytic. Chronic estradiol replacement, however, did not reverse the effects of ovariectomy. Given that actions of estradiol can differ depending on the dose and duration of treatment, the present experiment tested whether two doses of acute estradiol injections would decrease exploratory behavior in the light/dark box test. Male and female Siberian hamsters were gonadectomized at postnatal day 20-21. One week later, hamsters received a single injection of estradiol benzoate (10µg/mL or 100µg/mL in 0.1mL sesame oil, SC) or vehicle (0.1mL sesame oil). Approximately 42hours after injection, hamsters were tested in the light/dark box test. The low dose of estradiol increased exploration of the light zone (females and males) and the number of light zone entries (females only); the high dose of estradiol had no effect in either sex.Ongoing experiments are testing whether the lack of effect following chronic estradiol treatment in previous studies could be due to down regulation of estradiol receptors in the brain. Although in the opposite direction than predicted, the present findings demonstrate that juvenile behavior is sensitive to the actions of estradiol on exploratory and/or anxiety-like behavior in a non-linear, dose-dependent fashion. Future experiments are needed to determine why both ovariectomy and low-dose estradiol treatments are anxiolytic in juvenile hamsters. Nevertheless, these findings suggest that gonadal steroids could play a larger role during the juvenile period than previously appreciated.

Disclosures: Z. Forrester-Fronstin: None. **A.S. Mondschein:** None. **C.N. Cordes:** None. **M.J. Paul:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.19/MM12

Topic: C.10. Brain Injury and Trauma

Support: UNAM-DGAPA-PAPIIT IN203519

Title: Finasteride administration to pregnant rats persistently affects offspring myelination

Authors: *A. G. CARDENAS-PEREZ, E. GARAY, A. CISNEROS-MEJORADO, R. ARELLANO;
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Abstract: Neurosteroids (NEs) synthesized through action of the 5α-reductase, such as allopregnanolone (AP), and tetrahidrodeoxicorticosterone (THDOC), act as allosteric modulators of membrane receptors to neurotransmitters receptors, especially on type A GABA receptors and NMDA receptors, these molecules have been involved in the myelination process. A role for NEs has been suggested in the control of myelin formation in different models. An elevated gestational concentration of AP seems to be essential for the development and neuroprotection of the fetal brain. The aim of this study was to determine the effect of NEs suppression produced by finasteride (an inhibitor of the 5α-reductase enzyme) administration applied to pregnant rats has on the myelination process of the offspring in the postnatal stage. This experimental manipulation has been used to simulate the NE environment that the prematurely born neonate might experience. Pregnant Sprague Dawley rats were treated with finasteride (25 mg/kg maternal weight) at E14 to E21 embryonic stage. The brains of offsprings from, mothers treated with FD (n=3) or of controls (n=3) that were treated with vehicle alone (same days as finasteride) were dissected at P21; then tissue sections from different brain areas were cut and stained using BGII technique, and then visualized for analysis using a microscope. Our results showed that pups from rats that were FD treated displayed a robust decrease of BGII staining, suggesting an important decay in myelin content, given that offsprings maintained this decrease up to the age of P21, this strikingly seems to signal the importance of NEs actions during the embryonic stage were the main source is the maternal synthesis of NEs and covering through the late neonatal period.

Disclosures: A.G. Cardenas-Perez: None. **E. Garay:** None. **A. Cisneros-Mejorado:** None. **R. Arellano:** None.

Poster

PSTR223. Hormones and Cognition

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR223.20/MM13

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIA Grant R01 AG015019

Title: Aging of the human cortisol rhythm and correlations with cognitive performance

Authors: *A. NEUMAN[1], C. HERTZOG[1], S. D. MOFFAT[1], *A. S. NEUMAN[2];
[2]Psychology, [1]Georgia Inst. of Technol., Atlanta, GA

Abstract: Hormone levels rarely remain stable throughout the day, fluctuating due to the body's circadian rhythm which is influenced by internal and external factors. Cortisol, a corticosteroid, is a hormone recognized for its role in acute and chronic stress responses and has been linked to Alzheimer's disease risk and cognitive decline. The circadian rhythm of cortisol has been widely studied in young people and its daily fluctuations have been well-characterized across repeated studies. However, some uncertainty still remains regarding the existence and nature of age-related changes in cortisol's daily rhythm. In this study, participants (M_{age} = 50.4 years, SD = 19.33, range = 20 – 80, N = 186, 104 females) provided saliva samples seven times a day for 10 consecutive days: immediately after waking, 30 minutes after waking, then approximately every three hours until 21:00, for a total of 70 measurements for each participant. Age and waking time predicted both mean daily cortisol output as well as variability around the daily mean. Both the standardized daily peak-trough difference and the average awakening response slope were dependent on an interaction between age, sex, and waking time. The results suggest that there are age-related changes in the cortisol circadian rhythm that may depend on sex and sleep patterns. Correlations between these circadian metrics and performance on memory and cognitive tests will also be reported.

Disclosures: A. Neuman: None. **C. Hertzog:** None. **S.D. Moffat:** None. **A.S. Neuman:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.01/MM14

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH grants R01 MH121603 and R03 MH120549 to AP and GJD
GSU Brains and Behavior Seed Grant Program

Title: Behavioral and structural phenotype of a novel vasopressin 1a receptor cre-driver mouse strain

Authors: *D. HARTSWICK[1], C. N. FRIESEN[1], A. SELKE[1], P. KINDLER[1], N. SCHAPPAUGH[1], N. RIGNEY[1], G. J. DE VRIES[2], A. PETRULIS[1];
[1]Georgia State Univ. Neurosci. Inst., Atlanta, GA; [2]Georgia State Univ. Dept. of Biol., Atlanta, GA

Abstract: The neuropeptide arginine-vasopressin (AVP) has long been known to regulate sexually differentiated social behavior, such as aggression, social communication, and sexual behavior. Much of AVP's effect on social behavior is mediated by the vasopressin 1a receptor (V1aR), the most widely expressed AVP receptor in the central nervous system. Until recently, there have been few methods to genetically manipulate V1aR. Here, we behaviorally and

anatomically characterize a novel mouse strain that expresses cre-recombinase under the control of the V1aR gene (V1aR-iCre) promoter. Cre+ and Cre- male and female adult mice did not differ in their gross anatomy, basic sensory, and locomotor functioning, or in their anxiety-like and depressive-like behaviors (elevated plus maze, open-field, forced swim test, and sucrose preference). To test whether iCre expression is linked to neural V1aR expression, we injected a viral vector that Cre-dependently expressed green fluorescent protein (GFP) into the lateral septum (LS), an area known to express high levels of V1aR. We observed a pattern of GFP expression consistent with the known distribution of V1aR cells in LS in Cre+ males and females, but not Cre- littermates. Autoradiographic measurement of V1aR binding in LS revealed no differences between Cre+ and Cre- males and females, indicating that cre-recombinase insertion in the V1aR gene did not measurably disrupt V1aR expression in the brain. The overall similarity of behavior and V1aR expression between Cre+ and Cre- animals, combined with successful Cre-dependent expression, suggests that these mice are a viable strain for genetic manipulation of V1aR cells in the brain. Future work will characterize a new V1aR conditional knockout mouse strain to further expand the methodologies available to study the AVP/V1aR system.

Disclosures: **D. Hartswick:** None. **C.N. Friesen:** None. **A. Selke:** None. **P. Kindler:** None. **N. Schappaugh:** None. **N. Rigney:** None. **G.J. de Vries:** None. **A. Petrulis:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.02/MM15

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01 MH121603
NIH Grant R03 MH120549
GSU Brains and Behavior Seed Grant Program

Title: Vasopressin 1a receptor expression profiles in the social behavioral network

Authors: *N. A. SCHAPPAUGH[1], G. J. DE VRIES[2], A. PETRULIS[1];
[1]Georgia State Univ. Neurosci. Inst., Atlanta, GA; [2]Biol., Georgia State Univ., Atlanta, GA

Abstract: The neuropeptide arginine-vasopressin (AVP) is synaptically released in many areas in the brain involved in regulating social behavior and anxiety. While the neuronal populations releasing this AVP have become better characterized, much less is known about the populations expressing its primary receptor in the brain, the vasopressin 1a receptor (V1aR). Here we examine V1aR-expressing neuronal populations in the brain using RNAscope in situ hybridization to measure V1aR mRNA expression levels in the mouse brain. First, we measured V1aR expression in both sexually-differentiated AVP cell populations (bed nucleus of the stria terminalis (BNST), medial amygdala (MA)), and hypothalamic populations (paraventricular

(PVN), supraoptic (SON) and suprachiasmatic nuclei) to determine if AVP cells are themselves responsive to AVP. In contrast to the hypothalamic AVP cells, BNST and MA AVP cells do not coexpress V1aR. However, V1aR inhibitory neurons are present near BNST and MA AVP cells, suggesting indirect AVP feedback onto these populations is possible. Second, we examined whether V1aR-expressing cells in areas regulating social behavior colocalize with the vesicular transporters Vglut2 or Vgat as markers for glutamate and GABA production respectively. We show that regions predominantly expressing either Vgat such as the lateral septum (LS) or predominantly expressing Vglut2 such as the lateral habenula have V1aR almost exclusively co-labeled with those transporters respectively, while V1aR+ regions more heterogenous for Vglut2 and Vgat expression such as the diagonal band, ventral pallidum, and supramammillary nucleus have V1aR predominantly (~90%) coexpressing with Vgat. Lastly, we quantified the amount of overall V1aR mRNA transcript per cell in these brain regions to determine expression profiles across populations and observed two populations of V1aR+ neurons in the LS that differ significantly in their total V1aR expression. These results lay the groundwork for understanding how AVP acts on V1aR cells to regulate social and emotional behavior.

Disclosures: **N.A. Schappaugh:** None. **G.J. De Vries:** None. **A. Petrulis:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.03/MM16

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: BSN Project support grant
NIH HD090151
NIH HD099084
NIH DK133760

Title: Deciphering the neuronal circuitry controlling vasomotor symptoms.

Authors: *E. TORRES JIMENEZ, R. TALBI, M. S. B. SILVA, S. A. PEREIRA, D. PAULIS, V. M. NAVARRO;
Med., Brigham and Women's Hosp., Boston, MA

Abstract: Most women suffer vasomotor symptoms, also referred to as hot flushes (HFs), derived from the decline in circulating estrogen levels during menopausal transition. HFs are described as a sudden-onset sensation of extreme warmth, cutaneous vasodilation, sweating and cold-seeking behavior as part of an exaggerated heat dissipation response to drop core temperature. HFs are variable in frequency, duration and severity; and can temporally impair the quality of life of the menopausal women affected. The neurokinin 3 receptor (NK3R), the cognate receptor for neurokinin B (NKB), has emerged as a therapeutic target to alleviate HFs. Recent studies have documented that the release of NKB from Kisspeptin-Neurokinin B-

Dynorphin (KNDy) neurons in the arcuate nucleus contributes to HFs via signaling through NK3R in median preoptic nucleus (MnPO) neurons; however, the precise mechanism of action and neurocircuitry of this pathway remain to be fully characterized. Here, we evaluated the role of NK3R neurons located in the MnPO and NK3R-expressing population of the midline thalamus in heat defense response in mice. To this end, chemogenetic manipulation of KNDy and NK3R neurons was performed using cre-dependent adeno-associated viral injections in *Kiss1*-cre or *Tacr3*-cre mice. Temperature recording on free moving mice was used to monitor HFs episodes. Additionally, cold seeking behavior was monitored in a thermocline chamber. We observed that KNDy neurons project to additional neuronal populations of the midline thalamus, which also express NKB and NK3R. Interestingly, the expression of NKB in this area is increased in the absence of sex steroids after OVX, frequently used as a mouse model of menopause. Chemogenetic stimulation of these NK3R neurons outside of the MnPO increases skin temperature and decreases body temperature (measure of hot flush-like symptoms); however, those mice do not display different thermoregulatory behaviors 45 minutes after NK3R neuron activation. These findings suggest the existence of a complex neurocircuitry of NK3R-expressing neurons that may involve MnPO neurons and additional NK3R populations in the response to KNDy-initiated HFs, thus uncovering new potential therapeutic targets to treat HFs.

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Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.04/MM17

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Academy of Finland Fellowship, research cost grant 314104
Academy of Finland Fellowship, research cost grant grant 335396
Academy of Finland Fellowship 310765

Title: Arginine vasopressin increases the excitatory synaptic drive and firing activity of neonatal serotonergic neurons in dorsal raphe nucleus

Authors: E. ORAV, B. KOKINOVIC, H. TEPPOLA-GÜREL, S. LAURI, ***H. HARTUNG**; Neurosci. Center, Helsinki Inst. of Life Sci. (HiLIFE), Univ. of Helsinki, Helsinki, Finland

Abstract: Birth stress, a risk factor for psychiatric disorders, triggers a surge of the stress hormone arginine vasopressin (AVP). The dorsal raphe nucleus (DRN), where serotonergic cell bodies are located, also receives a dense vasopressinergic innervation. However, the effect of AVP on developing DRN serotonergic neurons is largely unknown. **Aims:** To investigate the effect of AVP on the synaptic drive and firing activity of neonatal serotonergic neurons *in vitro* and *in vivo* and to reveal the origin of the vasopressinergic innervation of neonatal DRN.

Methods: Electrophysiological whole-cell patch clamp recordings were performed *in vitro* in acute brain slices containing DRN from P10-12 rat pups and *in vivo* in urethane anaesthetised P10-12 rat pups by juxtacellular recording and labelling as well as multi-channel local field potential recordings. The origin of the vasopressinergic innervation in neonatal DRN was determined by retrograde tracer injections (Fluorogold) combined with immunohistochemistry against AVP. **Results:** We found that 10 nM AVP significantly increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in neonatal serotonergic neurons mainly through $V1_a$ receptors. AVP also strongly augmented the action potential firing of neonatal serotonergic neurons *in vitro*. This effect was independent of $GABA_A$, ionotropic and metabotropic glutamate receptors and was neither mediated by α1-adrenoreceptors. These findings and the faster onset of the effect on action potential firing compared to the increase in sEPSCs suggest a direct excitatory effect of AVP on neonatal serotonergic neurons.*In vivo* single unit recordings of identified neonatal serotonergic neurons revealed a broad range of firing patterns. Injections of AVP into DRN seemed to increase the firing rates of putative serotonergic neurons *in vivo*. Retrograde tracing experiments showed that the vasopressinergic innervation of neonatal DRN emerges from sparse cell groups in medial amygdala and bed nucleus of the stria terminalis. **Conclusions:** AVP is a powerful modulator of neonatal serotonergic activity in DRN. Abnormal AVP release by birth stress may alter the functional development of the DRN serotonin system and contribute to increased susceptibility to psychiatric disorders later on.

Disclosures: E. Orav: None. **B. Kokinovic:** None. **H. Teppola-Gürel:** None. **S. Lauri:** None. **H. Hartung:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.05/MM18

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: IK01HL145339-01A1

Title: Investigation of stress reactivity in two non-traditional animal models: oxytocin and stress responses in the gray short-tailed opossum (Monodelphis domestica) and Syrian hamster (Mesocricetus auratus)

Authors: *E. I. ALANIZ[1], F. DOMINGUEZ[2], M. GARCIA[2], I. PEREZ[1], C. M. BOTELLO[1], V. L. VANDEBERG[3], M. GIL[4];
[1]Psychological sciences, [2]Dept. of Hlth. and Biomed. Sci., [3]Sch. of Med. Dept. of Human Genet. and South Texas Diabetes and Obesity Inst., [4]Sch. of Med. Dept. of Neurosci. and Inst. of Neurosci., The Univ. of Texas Rio Grande Valley, Brownsville, TX

Abstract: Stress is a precipitating factor for anxiety-related disorders. Translational research using animal models helps understand its mechanisms and contributes to the development of

treatments and preventive measures, but there are unique and shared traits across species that can be advantageous. Prolonged stress exposure in rats produces behavioral and neurochemical characteristics resembling post-traumatic stress disorder (PTSD). To address knowledge gaps for non-traditional models, we conducted: behavioral and the neurobiological comparison between the laboratory opossum (*Monodelphis domestic*a) and Syrian hamster (*Mesocricetus auratus*). Our study used restraint stress and open field tests to assess stress and anxiety-like responses in both species. We plan to compare oxytocin (OT), a neuropeptide known to regulate stress-related behaviors, immunoreactivity between hamsters and opossums. For the restraint stress task, behaviors of 12 *M. domestica* (6 males, 6 females) were quantified using JWatcher. Independent t-test results indicate that females had significantly higher duration of head movements ($t(10)=2.940$. $p<0.05$) and males showed higher latency to first head movement ($t(10)=-2.397$. $p<0.05$). Reactions to the restraint stress test were recorded for the *M. auratus* (n=88), using 43 males and 44 females. Independent samples t-test showed significant biological sex differences for overall reactivity ($t(85) = 0.810$, $p<0.05$), with males having higher reactivity. Similar biological sex differences may be present in both species. Previously, when 8 *M. domestica* (4 males and 4 females) were exposed to the open field, the time spent in the center had a significant interaction effect between sex and trial ($f(2,12)=5.023$, $p<0.05$), the time the animals spent in the center increased over time, females spent more time in the center zone than males. We plan to study if the same trial effect is also found in the *M. auratus*. Our team previously used immunohistochemistry (IHC) to characterize oxytocin immunoreactivity in the paraventricular nucleus (PVN) of the *M. domestica*, and found an approach to significance when comparing males to females for the same region, ($t(6)=1.688$, $p=0.142$. We ran IHC to increase our sample and focused on different levels of the hypothalamus. Comparing *M. domestica* with *M. auratus*, the morphological characteristics are similar, with alike structures for the cell bodies that mostly concentrated in the SON and PVN. However, the *M. domestica* doesn't show OT immunoreactivity in the medial preoptic area and the medial septal nucleus. Future studies will compare neural systems and behaviors of non-traditional animal models.

Disclosures: E.I. Alaniz: None. **F. Dominguez:** None. **M. Garcia:** None. **I. Perez:** None. **C.M. Botello:** None. **V.L. VandeBerg:** None. **M. Gil:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.06/MM19

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01 DK069861
NIH R01 DK103676
Dept. of Defense W81XWH-19-1-0015

Title: Oxytocin signaling in the ventromedial hypothalamus as a mechanistic link between social isolation and glucose homeostasis.

Authors: *H. H. LAMONT[1], I. CARCEA[2], V. H. ROUTH[3];
[1]Rutgers Univ., Newark, NJ; [2]Brain Hlth. Inst., Rutgers, Newark, NJ; [3]Pharmacol, Physiol & Neurosci, New Jersey Med. Sch. Dept. of Neurol. & Neuroscienes, Newark, NJ

Abstract: Social isolation and diabetes are emerging public health crises. Human longitudinal studies demonstrate that perceived loneliness is an independent risk factor for metabolic disease. Oxytocin is a neurohormone which modulates both social behavior and energy balance. The oxytocin receptor is highly expressed in the ventromedial hypothalamus (VMH), a brain area critical to glucose homeostasis. The VMH regulates glucose homeostasis via glucose sensing neurons which sense and respond to changes in glucose availability. However, the role of oxytocin in modulating glucose sensing and the extent to which social isolation disrupts this neural circuitry has never been investigated. We find that glucose sensing neurons express the oxytocin receptor. These data support the hypothesis that oxytocin directly modulates VMH glucose sensing and glucose homeostasis. We will further test this hypothesis using slice electrophysiology to determine whether oxytocin directly modulates hypothalamic glucose sensing. We will then determine if social isolation disrupts hypothalamic glucose sensing by modulating the activity of VMH neurons that express the oxytocin receptor.

Disclosures: H.H. Lamont: None. **I. Carcea:** None. **V.H. Routh:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.07/MM20

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Latexin labels vasopressin and oxytocin neurosecretory neurons of hypothalamus in brain of Carollia perspicillata

Authors: R. ORMAN, T. MORELLO, R. KOLLMAR, ***M. STEWART**;
SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY

Abstract: Latexin is a carboxypeptidase A inhibitor that has been identified in multiple mammalian tissues, including brain. Latexin was first shown by Arimatsu and colleagues to label neurons of select brain regions, both cortical and subcortical, with high specificity. As examples, we have used latexin extensively as a marker for claustrum and endopiriform nucleus in rat and bat brain. We have also reported highly specific labeling in retrosplenial cortex. Here we report immunohistochemical evidence of specific latexin labeling of neurosecretory neurons that express either vasopressin or oxytocin in the paraventricular nucleus and supraoptic nucleus in brains from bats (Carollia perspicillata). Confocal microscopy of coronal brain sections labeled with latexin and vasopressin or latexin and oxytocin showed highly specific co-expression of latexin with both nonapeptide neurosecretory hormones. Although the function of latexin in brain is not well understood, its selective labeling suggests certain brain areas may (1) share

similar requirements for controlling carboxypeptidase activity and/or (2) suggest structural and functional neural circuitry relationships, such as a hypothalamic-cingulate-claustral circuit that could serve complex social behaviors.

Disclosures: **R. Orman:** None. **T. Morello:** None. **R. Kollmar:** None. **M. Stewart:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.08/MM21

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01MH116176

Title: Oxytocin receptor positive neurons in the ventral claustrum-dorsal endopiriform nucleus contain neuroanatomical and functional characteristics as a novel default mode network in mice.

Authors: *S. B MANJILA, S. SON, Y.-T. WU, H. KLINE, R. BETTY, K. N. BROWNING, A. PAUL, Y. KIM;
Penn State Col. of Med., Hershey, Pennsylvania, PA

Abstract: The brain contains a set of areas that supports the resting and non-attentive states of the brain, broadly termed the "default mode network" (DMN). Impairment of the DMN has been implicated in neurodevelopmental disorders (e.g., autism), psychiatric disorders (e.g., schizophrenia), and neurodegenerative disorders (e.g., Alzheimer's disease). Despite its importance, we have very limited knowledge of the neurobiological mechanisms of the DMN. Previously, our unbiased brain mapping study identified dense oxytocin receptor (Oxtr) expression in the ventral claustrum-dorsal endopiriform nucleus (vCLA-EPd), one of key DMN areas identified by resting state functional MRI studies. The vCLA-EPd is located deep in the agranular insular and piriform cortex, and exists in both rodent and higher mammal brains. We found that vCLA-EPd Oxtr neurons are mostly excitatory neurons with high expression of neuromodulator receptors (e.g., serotonin). We performed the axonal output and mono-synaptic input mapping of vCLA-EPd Oxtr positive neurons using Oxtr-Cre mice and found extensive bidirectional connections to the ventral half of the brain including the olfactory, limbic, and many neuromodulatory areas. This connectivity pattern differs dramatically from the dorsal claustrum with extensive connection to isocortical areas. In-vivo miniscope recordings in Oxtr-Cre mice further showed that vCLA-EPd Oxtr neurons exhibit high baseline neural activity during non-attentive exploratory behavior, which sharply decreases upon encountering novel stimuli. This suggests that the vCLA-EPd is a crucial component of the DMN involved in regulating the non-attentive state and competing with salient networks in response to environmental novelty. Together, our findings provide foundational knowledge to understand the vCLA-EPd as a part of the DMN in the normal brain and will critically inform future studies to investigate the vCLA-EPd in various pathological conditions.

Disclosures: **S. B manjila:** None. **S. Son:** None. **Y. Wu:** None. **H. Kline:** None. **R. Betty:** None. **K.N. Browning:** None. **A. Paul:** None. **Y. Kim:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.09/MM22

Topic: F.02. Neuroendrocrine Processes and Behavior

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MZ: FCT IF/00787/2014
MK: LISBOA-01-0145-FEDER-030907

Title: Changes in oscillatory power and connectivity in theta, alpha and beta oscillations following administration of oxytocin

Authors: *M. ZELENINA[1], M. KOSILO[2], M. CRADDOCK[3], D. PRATA[4];
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Abstract: Oxytocin (OT) is popular in behavioral neuroscience due to its ability to modulate social cognition, and it is a promising target for treatment of various neuropsychological conditions. To adequately design and interpret intranasal oxytocin (IN-OT) research, it is crucial to know for how long it affects the human brain after administration. Previous studies measures OT indirectly or used uncommon dosages. We measured brain activity directly with EEG and analyzed changes in relative power amplitude and amplitude-amplitude cross-frequency coupling. Nineteen healthy males participated in a double-blind, placebo-controlled, within-subject, cross-over design. We collected EEG data from 15min to 1h 42min after administration of 24 IU of IN-OT.IN-OT group showed an increased amplitude in theta and beta bands and a decrease in alpha band in the open-eyes condition. In terms of cross-frequency coupling, IN-OT, again in open-eyes condition, caused an increase in theta-alpha and a decrease in alpha-beta coupling. In eyes closed recordings we only observed a decrease in alpha-beta coupling. We did not observe any significant drug by time interactions, which suggests an overall stable effect of IN-OT across time.Our results agree with literature. OT increased the amplitude of theta band, which may reflect higher focus and sustained attention, also observed with other anxiolytic

drugs. Beta band is associated with visual perception, attention reorienting, and visual crowding, and alpha band plays an important role in cognitive processing, including attention and memory. We suggest that the functional significance of our observed patterns might reflect “tuning in” towards external environment and preparing for processing of incoming stimulus.Since we did not introduce any social stimulation, it is impossible to differentiate whether there would be any preference towards potential stimuli of social nature, as predicted by the social salience hypothesis of OT; however, this notion cannot be rejected. We also discuss potential clinical implications of our results.

Disclosures: **M. Zelenina:** None. **M. Kosilo:** None. **M. Craddock:** None. **D. Prata:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.10/MM23

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: CIHR Grant FRN 148365

Title: Divergent electrophysiological and morphological properties of hypothalamic paraventricular nucleus neurons between the common marmoset and the mouse

Authors: *J. K. SUNSTRUM[1], S. A. MESTERN[1], R. PRZY[2], Y. QIN[2], F. GAO[2], S. EVERLING[3,4], J. C. MARTINEZ-TRUJILLO[3,4], W. INOUE[3,4];
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Abstract: The release of stress hormones via the hypothalamic-pituitary-adrenal (HPA) axis is preserved across mammals. The apex of the HPA axis is formed by parvocellular neuroendocrine neurons in the paraventricular nucleus of the hypothalamus (PVN) that release corticotropin releasing hormone (CRH). While the functions and structures of PVN-CRH neurons have been extensively studied in rodents, studies in primates remain scarce. It is possible that the diurnal activity cycle and complex social lives of primates have exposed them to different stressors, and PVN-CRH neurons have evolved new features under evolutionary pressures. In this exploratory study, we compared PVN neurons between mice (N=24; age=3-24 months; 14 female) and the common marmoset (Callithrix jacchus; N=24; age=1-10 years; 15 female), a New World monkey that has emerged as a promising primate model in neuroscience. Using patch clamp electrophysiology in acute brain slices, combined with post-hoc morphology reconstruction, immunohistochemistry and *in situ* hybridization, we characterized marmoset PVN neurons (n=374) and compared them to their mouse counterparts (n=184). The stereotyped electrophysiological features for the three major PVN neuron groups (parvocellular, magnocellular and preautonomic) are conserved between marmosets and rodents. However, species-specific features were found: Marmoset parvocellular neurons exhibited substantially

larger voltage sag due to hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Further, blocking HCN channels with ZD7288, increased input resistance and membrane time constant leading to enhanced temporal summation of subthreshold membrane depolarizations and increased firing rate. Conversely, enhancing HCN with cAMP modulators (8-Bromo, IBMX, 5-HT) lowers the input resistance and time constant, suggesting HCN channels enable state-dependent modulation of synaptic integration. Fluorescent in situ hybridization (RNAscope) revealed that two of the four HCN channel subtypes, HCN1 and HCN3, are expressed in marmoset PVN-CRH neurons. Preliminary morphological analysis revealed that marmoset PVN parvocellular neurons exhibit more dendritic spines compared to mice. Overall, marmoset parvocellular neurons have an added layer of regulation, whereby HCN channels allow for dynamic control of the time window for synaptic integration. These distinct features of marmoset PVN parvocellular neurons may reflect fundamental differences in synaptic integration adaptive for the lifestyle and stressors of primates.

Disclosures: J.K. Sunstrum: None. **S.A. Mestern:** None. **R. Przy:** None. **Y. Qin:** None. **F. Gao:** None. **S. Everling:** None. **J.C. Martinez-Trujillo:** None. **W. Inoue:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.11/MM24

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: CIHR Grant (FDN-143337)

Title: Osmotically-induced nanopit formation in actin fenestrae of rat supraoptic magnocellular neurosecretory cells (MNCs)

Authors: *A. MURTAZ[1], C. BOURQUE[2];
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Abstract: Osmosensory transduction (OT) in rat magnocellular neurosecretory cells (MNCs) is mediated by activation of ΔN-Trpv1 channels (N-terminal variant of the transient receptor potential vanilloid 1) via push-force applied by microtubules during hypertonicity-induced cell shrinkage (Prager-Khoutorsky et al., 2014). MNCs also express a thick layer of subcortical actin filaments which is also essential for OT, but how so remains undefined. Here we used immunocytochemistry and proximity ligation assays (PLA) with super resolution (SR) imaging (FV 3000 Olympus confocal with FV-OSR and deconvolution using constrained iterative with cellSens software) to examine the actin cytoskeleton of acutely isolated MNCs. We found the subcortical actin layer of vasopressin MNCs features fenestrae; ~0.5 µm segments of low actin density. PLA confirmed the presence of membrane sites at which TRPV1 interact with α-tubulin in MNCs (n=31) and also in osmosensory neurons of the OVLT (organum vasculosum lamina terminalis; n=31), but not in non-osmosensitive vasopressin neurons of the suprachiasmatic

nucleus (n=39). Moreover, PLA did not reveal interaction sites between TRPV1 and β-actin in MNCs (n=17), suggesting channels are not linked to the actin cortex in these neurons. Interestingly, TRPV1-α tubulin interaction sites were preferentially aligned with actin fenestrae. Moreover, live-cell imaging showed that hypertonicity causes the appearance of submicron membrane pits similar in size to fenestrae in MNCs (n=30). These results suggest that actin fenestrae may favor the formation of nanodomains where the cell surface becomes indented during the early stages of hypertonicity-induced shrinkage and that OT may specifically occur at such sites within MNCs.

Disclosures: A. Murtaz: None. **C. Bourque:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

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Program #/Poster #: PSTR224.12/MM25

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NTU Grant NTU-112L895404
MOST Grant MOST 109-2311-B-002-008-MY3

Title: Synapsin Ia phosphodeficiency regulates the oxytocin peripheral release in adult male rats

Authors: C.-T. HUANG, Y.-C. CHANG, *C.-T. WANG;
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Abstract: In the hypothalamic-neurohypophysial system (HNS), magnocellular neurons (MCNs) release neuropeptides (oxytocin/OT and vasopressin/VP) into the cerebrospinal fluid (CSF) and peripheral plasma, termed central and peripheral release, respectively. The MCNs consist of neuropeptide-laden large-dense core vesicles (LDCVs) and microvesicles (MVs) similar to synaptic vesicles (SVs). We previously found that the SV/MV associated protein, Synapsin Ia (Syn Ia), can also regulate the release from LDCVs, by interacting with the LDCV protein Synaptophysin (Syp) in a phosphorylation-dependent manner. Here, we examined how Syn Ia may regulate the OT central or peripheral release in the HNS of adult male Sprague-Dawley rats. First, we found that Syn Ia was colocalized with Syp in the MCNs of the supraoptic nucleus (SON) and the pituitary nerve terminals, suggesting that Syn Ia may interact with Syp in the HNS to affect neuropeptide release. Further, we transfected the SON-MCNs with the OT neuron-specific plasmids to overexpress Syn Ia or the Syn Ia phosphodeficient mutants such as Syn Ia-S62A (phosphodeficiency at the MAPK site) and Syn Ia-S9,566,603A (phosphodeficiency at the CaMK sites) using *in vivo* electroporation. By performing ELISA, we detected the changes in OT levels in the CSF or plasma before and after 3 or 7 days post transfection. As a result, we found that overexpressing Syn Ia-S62A in the SON-OT neurons significantly decreased the OT release to peripheral plasma compared to wild-type Syn Ia or Syn Ia-S9,566,603A ($p < 0.05$), suggesting that Syn Ia-S62A may preferentially down-regulate OT peripheral release. However,

overexpressing Syn Ia-S9,566,603A did not alter the OT release to CSF or plasma compared to wild-type Syn Ia, suggesting that Syn Ia-S9,566,603A may not affect the central or peripheral OT release. In addition, with OT neuron-specific expression, Syn Ia-S62A cannot further regulate the VP central or peripheral release compared to wild-type Syn Ia. Together, our results suggest that Syn Ia phosphodeficiency at the MAPK site may preferentially decrease the peripheral release of OT from the HNS in adult male rats.

Disclosures: C. Huang: None. **Y. Chang:** None. **C. Wang:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.13/NN1

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF IOS 2238071
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The Alfred P. Sloan Foundation

Title: Neuroendocrine changes in the Russian farm-fox experiment: A stereological assessment of immunohistochemistry-labeled brain tissue

Authors: *J. M. MICHLICH[1], C. ROGERS-FLATTERY[1], M. ABDULLA[1], L. TRUT[2], A. V. KUKEKOVA[3], C. C. SHERWOOD[4], E. E. HECHT[1];
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Abstract: Variation in the organization of neuroendocrine systems is associated with evolved variation in social behavior across several mammalian species. The vasopressin-oxytocin neuroendocrine system has been associated with a continuum of evolved variation in affiliative and aggressive social behaviors in mammals, including in experimentally domesticated rats, and is believed to have been involved in dog domestication. The Russian fox-farm experiment has been a long-running model of the effects of selective breeding for affiliative and aggressive behaviors in the silver fox (Vulpes vulpes). This model has generated discoveries on behavioral, physiological, neural, and genetic changes under this artificial selection paradigm. However, the vasopressin-oxytocin neuroendocrine system in domesticated foxes has yet to be characterized. In this study, we performed a stereological analysis of vasopressin-producing cells in the left hemispheres of 15 1.5-year-old male foxes from the Russian fox-farm experiment including 5 brains from each of the selectively bred populations (tame and aggressive) and 5 controls (unselected). Structural findings from the stereological investigation were paired with behavioral measures previously collected in these individual foxes. Results are discussed in relation to the

social behavior of the fox model and in relation to brain-behavior evolution in domestication more generally.

Disclosures: J.M. Michlich: None. **C. Rogers-Flattery:** None. **M. Abdulla:** None. **L. Trut:** None. **A.V. Kukekova:** None. **C.C. Sherwood:** None. **E.E. Hecht:** None.

Poster

PSTR224. Vassopressin and Oxytocin Expression and Signaling

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR224.14/NN2

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: CIHR Grant PJT-180511

Title: Lateral head impact traumatic brain injury mouse model causes hyponatremia and activates supraoptic nucleus

Authors: *J. O'REILLY, N. J. SIMPSON, Z. S. THIROUIN, P. A. BASTONE, C. W. BOURQUE;
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Abstract: Background: Hyponatremia, an electrolyte disorder characterized by low serum sodium, is commonly reported in the days following a traumatic brain injury (TBI). Because the brain is highly vulnerable to osmotic swelling, hyponatremia following TBI may result in additional cognitive deficits and functional impairments. However, the mechanisms underlying the development of hyponatremia after TBI are not yet understood. One of the most common causes of hyponatremia is inappropriate secretion of vasopressin (VP). VP is released by magnocellular neurosecretory cells of the supraoptic nucleus (SON) and promotes water reabsorption by the kidney. We hypothesize that TBI causes an inappropriate activation of the supraoptic nucleus, resulting in excessive VP release and dilutional hyponatremia. **Methods**: C57Bl/6 male mice aged 2-4 months old were lightly anesthetized with isoflurane and subjected to a lateral head injury using a Gothenburg Impactor (Collision Analysis Inc). This instrument was used to deliver a reproducible, calibrated blow to the side of the head protected by a helmet via a 50 g projectile launched at a velocity of 9 m/s. Mice treated the same way but without the head impact served as controls (shams). The time courses of serum sodium and c-Fos protein expression in the SON after TBI/sham were performed. **Results**: TBI mice showed significantly lower serum sodium compared to sham at 3 h ($p = 0.035$, $n = 8$), 6 h ($p = 0.0231$, $n = 8$) and 12 h ($p = 0.0012$, $n = 8$) after TBI/sham, but not at 24 h or 48 h. 3 h after TBI/sham, SON sections of TBI mice showed significantly more c-Fos positive VP cells ($p = 0.004$, $n = 6$) and c-Fos-positive oxytocin cells ($p = 0.000004$, $n = 6$) compared to sham. **Conclusion**: The SON may be involved in the development of hyponatremia after TBI.

Disclosures: J. O'Reilly: None. **N.J. Simpson:** None. **Z.S. Thirouin:** None. **P.A. Bastone:** None. **C.W. Bourque:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.01/NN3

Topic: F.03. Stress and the Brain

Support: NRF-2021R1F1A1060027
23-BR-02-01

Title: The impact of neonatal maternal separation on adolescent behavior: role of environmental factors and changes in paraventricular thalamus activity

Authors: *S. SHIN[1], S. LEE[2];
[1]Korea Brain Res. Inst. (KBRI), DAEGU, Korea, Republic of; [2]Dev Disord & Rare Dis Res. Group, Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of

Abstract: Neonatal maternal separation (MS) is a well-established model for studying early-life stress in rodents. It involves separating pups from their mothers for several hours daily during the first two weeks of life, mimicking negative early life experiences. This paradigm has been associated with significant impacts on behavior and psychological health, particularly anxiety and depression, during adolescence. However, the specific effects of different conditions and factors during repetitive separation, such as isolated maternal separation (iMS), random dam exchanges (eDam), and olfactory stimulation (OF), remain understudied. This study aimed to investigate the behavioral effects of neonatal MS and explore how these behaviors are influenced by diverse factors during separation. Five groups of adolescent mice (Con, MS, iMS, eDam, OF) underwent periodic neonatal MS for four hours daily from postnatal day 2 to 20 (19 consecutive days). A comprehensive battery of behavioral assessments was conducted to evaluate locomotion, anxiety, recognition, learning, and memory. Our findings revealed that regardless of the conditions, maternal separation led to deficits in recognition memory, motor coordination, and motor skill learning. Specifically, the iMS group displayed anxiety-like behavior in the elevated plus-maze test and enhanced extinction of fear memory in the auditory fear conditioning test. The OF and eDam groups exhibited partial recovery in short-term working memory as assessed by the Y-maze test, but showed contrasting exploratory behaviors, with the OF group spending more time in the center area and the eDam group spending less time. These results highlight the significant impact of environmental factors during maternal separation on adolescent behavior, potentially contributing to the variability in behavioral phenotypes observed within the early-life stress model. Furthermore, our investigation into the underlying neural mechanisms focused on the brain regions of the iMS mice. Using patch clamp and c-fos staining techniques, we observed a decrease in activity within the paraventricular thalamus (PVT) region. Collectively, these findings suggest that repetitive maternal separation during the neurodevelopmental stage induces behavioral alterations in adolescence, which may be associated with reduced activity within the PVT region. In conclusion, this study sheds light on

the effects of neonatal maternal separation on adolescent behavior and highlights the role of environmental factors in shaping these behaviors.

Disclosures: **S. Shin:** A. Employment/Salary (full or part-time):; KBRI. **S. Lee:** A. Employment/Salary (full or part-time):; KBRI.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.02/NN4

Topic: F.03. Stress and the Brain

Support: Research trust for PI

Title: Cognitive and affective consequences of maternal separation co-occur with changes in hippocampal parvalbumin interneuron density

Authors: *A. T. ROPER, V. A. LEWIS, S. ROY, T. R. TERRY-THOMAS, D. Y. BRAVO, S. DONALDSON;
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Abstract: Background A number of studies have found declines in cognitive performance in learning and memory following early life stress (ELS) in clinical and preclinical experiments. Additionally there is evidence of increased anxiety like behavior following ELS. Methods In rodents, a common form of ELS is maternal separation (MS) and this model also demonstrates cognitive and affective changes. In the current study we used a total of three Long Evans dams (Charles River Breeding Lab (MA) and their offspring (N = 39). On PND 1 rat pups were separated from their dam for two hours, five days a week, for three weeks during the pre weaning period (PND 1-21). Adolescent offspring (n = 24 (PND 32-34), n = 15 (PND 45-48) were tested for anxiety-like behavior on the elevated plus maze (EPM) and for novelty seeking using the hole board test (HB) and forced novel object recognition using the novel object test. Immunohistochemistry targeted hippocampal parvalbumin (PV) and post-synaptic density (PSD)-95, indicators of a subset of GABA interneurons and glutamate synaptic signaling, respectively, in MS and no-MS rats.Results The EPM testing showed anxiety differences across both sexes and treatment. Females in both conditions had fewer open arm entries and less time spent in the open arms compared to males. The MS group had less open and closed arm exploration time and fewer closed arm entries. In the novel object test, control males had an increased percentage of time interacting with the familiar object than control females and the MS group spent more time with the familiar object than controls. For the HB test, the MS males had greater ambulatory counts and movement compared to their female counterparts and the no-MS females had more hole entries than control males. Preliminary IHC counts show significant treatment effects in the CA3 and dentate gyrus of the hippocampus, with the MS group having

less PV positive neurons. There were also sex differences seen in the CA3 with males having more positive PV interneurons than their female counterparts in both the MS and no-MS groups. The PSD-95 results trended towards more PSD-95 positive cells for the MS groups. Conclusions These results showed both sex and and treatment effects of MS on anxiety-like and exploratory behavior, and novelty-seeking. MS females were more anxious, explored less, and interacted more with the familiar object in the novel object recognition test. Disruptions in the GABA/glutamate balance in the hippocampus was altered in MS animals, suggesting a possible mechanism of the behavioral deficits observed.
Kew Words Early life stress, maternal separation, parvalbumin, hippocampus, novel object, novelty seeking.

Disclosures: **A.T. Roper:** None. **V.A. Lewis:** None. **S. Roy:** None. **T.R. Terry-Thomas:** None. **D.Y. Bravo:** None. **S. Donaldson:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.03/NN5

Topic: F.03. Stress and the Brain

Support: CSUPERB New Investigator Grant Program

Title: Determining the impact of adolescent stress on opioid use vulnerability

Authors: *R. JAUREGUI OCAMPO, S. BATES;
Psychology, CSU Chico, Chico, CA

Abstract: Uncovering factors that influence the vulnerability of adolescents to stress is imperative to improving treatment strategies. This is vital since adolescents, particularly those with stress related mental illnesses, are more likely to develop substance use disorders. That are often concomitant with stress-related psychiatric illness that disproportionately impact adolescents. Preliminary data (n= 39) has shown an increased in morphine reward in stressed female mice compared to non-stressed mice, with one dose of morphine (20mg/kg). With two exposures to morphine, males (stress and non-stressed) show a significant preference to morphine compared to females. Female mice do not show preference for morphine reward after a second exposure. The present study aims to examine the role of stress experienced in adolescence on opioid reward and dependence and oxytocin activity in adolescent male and female mice. We will examine levels of the neuropeptide oxytocin and the immediate early gene, Fos, in stressed and non-stressed mice. Stress has been shown to dysregulate oxytocin signaling. In rodents, stress has been shown to increase neuronal activity in the amygdala (AMY), locus coeruleus (LC) and prefrontal cortex (PFC). Some studies have demonstrated the effectiveness of oxytocin in treating drug response in humans and rodents by lowering craving and cue-response. Particularly, higher levels of oxytocin neuronal activity in the nucleus accumbens (NAcc) are

associated with a decrease in drug reward. Furthermore, higher levels of oxytocin in the BNST have been associate with higher stress levels, particularly in females. We predict that higher activity in the BNST after stress will be associated with higher drug response. We predict that stress will enhance opioid reward and dependence in male, but not female mice. The results of the project are also of clinical importance because they will identify new behavioral and pharmacological treatment strategies for adolescent mental illness and substance use.

Disclosures: R. Jauregui Ocampo: None. **S. Bates:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.04/NN6

Topic: F.03. Stress and the Brain

Title: An Examination of the Effects of Combined Chronic Early Life Stress During Adolescence

Authors: *C. FORD, M. J. HYLIN, C. RICHARDSON, K. WHEELER, S. UM, R. BARDLE; Psychological and Behavioral Sci., Southern Illinois Univ., Carbondale, IL

Abstract: Stress is pervasive across species, and while acute stressors can allow for the healthy adaptability of an organism to its environment, chronic stressors tend to lead to poor health outcomes that can continue into the long term. This is especially the case when stress occurs during development. The current study attempts to examine how two distinct early life stressors (ELS) (maternal separation and limited bedding/nesting, both combined and separate) impact outcomes during adolescence. The dam's maternal behaviors were analyzed at various points while the pups were undergoing the early life stress, and upon cessation of the stressors, the dams were tested for anxiety-like behavior using the open field (OF) and elevated plus (EPM). Our initial results have shown that dams in the combined stress group exhibit less overall anxiety-like behaviors, even compared to those in the control group. It was expected that the dams who received singular stressors will display less overall anxiety-like behaviors than those who received combined stressors. Pups were weighed at 3 separate time points (P9, P21, P44) and their average weights per group were compared. There were no significant differences in the weight of the pups, suggesting that ELS does not markedly affect adolescent weights. Upon reaching adolescence, pups were tested on the EPM and OFM for anxiety-like behavior, as well as in the Morris water maze (MWM) to assess the formation and retention of long-term spatial memory. Initial analysis of the OF and EPM data suggests those in the combined group exhibit the lowest overall anxiety-like behaviors, whereas the LBN group exhibits the highest. For the MWM, initial analysis indicates that female rats in the combined group have the quickest latency to the platform throughout training trials, whereas male rats have the longest latency. In the reversal trials, the male and female rats have opposing performances. These MWM results indicate sex as a mediating factor in the cognitive-developmental outcome following ELS. The

initial analysis of adrenal and brain weights has shown that combined stress rats have the highest weights overall. Further analysis is being conducted on the volume of the hippocampus and its subregions. Additionally, microglia will be counted to assess the overall impact that early life stress has upon the general neuroinflammatory response. This study is the first to examine combined chronic ELS during adolescence, while simultaneously examining the sexual differentiation associated with ELS outcomes, which will help to develop a better understanding of how combined stressors when encountered early in life impact later development.

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Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.05/NN7

Topic: F.03. Stress and the Brain

Support: AA019884
AA022534

Title: Repeated mild acute stress alters corticolimbic mRNA expression central CRH, circadian clock, and proinflammatory immune factors in female adolescent mice

Authors: *A. K. FERNANDEZ;
Univ. of New Mexico Dept. of Neurosciences, Albuquerque, NM

Abstract: Title: Repeated mild acute stress alters corticolimbic mRNA expression central CRH, circadian clock, and proinflammatory immune factors in female adolescent mice.**Authors:** A.K. Fernandez-Oropeza[1], J. Zimmerly[2], M.S. Sun, C. Milliken[3], N. Mellios, E.D. Milligan[1,].
Affiliations: Department of Neurosciences, School of Medicine1, University of New Mexico, Albuquerque, New Mexico, 87131-0001, United States of America.
Abstract: Anxiety disorders are highly prevalent worldwide, with a lifetime prevalence of approximately 32% in the US. The adolescent brain undergoes significant developmental changes in corticolimbic regions involved in cognition and mood, making it highly sensitive to stress. Adolescent stress may reprogram mood responses both immediately and in the long term. Psychological stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of corticosterone (CORT) and inducing sterile inflammation in the central nervous system (CNS). CRH and proinflammatory factors such as IL-1β, TNFα, CCL2, and NFκB are produced in the CNS, regulated by diurnal expression of Clock and Bmal1, which control the core clock genes, Per1 and Per2. Dysregulation of clock genes in corticolimbic areas like the prefrontal cortex (PFC), amygdala (AMG), and hypothalamus (HYP) has been associated with clinical anxiety. The impact of stress-induced CNS immune factors and clock genes on corticolimbic stress responses during adolescence remains poorly understood. Our hypothesis is

that repeated mild stress during adolescence alters the expression of proinflammatory and clock genes in the corticolimbic region. Female C57BL/6 mice (postnatal day 38-44) were exposed to 1-hour isolation stress for 3 consecutive days. Tail vein blood was collected before and after the first day of stress, and the PFC, AMG, and HYP were dissected 24 hours after the last stress session. Plasma CORT levels were measured using ELISA, and mRNA expression of Crh, Il-1β, Tnfα, Ccl2, Nfκb, Clock, Bmal1, Per1, and Per2 was analyzed using RT-qPCR. Repeated isolation stress significantly increased CORT levels in all mice. Blunted mRNA levels of Ccl2 and Nfκb were observed in the HYP, and Il-1β was reduced in the PFC. Conversely, elevated levels of Il-1β, Tnfα, and Ccl2 were found in the AMG, while Bmal1, Clock, Per1, Per2, and Nfκb were decreased. Mild stress-induced changes in proinflammatory factors and clock gene expression suggest that adolescent corticolimbic regions are particularly vulnerable to stress reprogramming, potentially contributing to the development of mood disorders such as anxiety.

Disclosures: A.K. Fernandez: None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.06/NN8

Topic: F.03. Stress and the Brain

Title: Early life stress endures: both ten and twenty days of maternal separation alter the rodent acoustic startle response in adolescence and adulthood

Authors: *R. A. CAUCHON, L. E. GRANATA, A. E. R. PARAKOYI, C. R. CODY, M. M. FANIKOS, A. K. REKAPALLI, H. C. BRENHOUSE;
Northeastern Univ., Boston, MA

Abstract: Both prenatal and early life stress (ELS) cause anxiety-like behavior in adulthood, including altered threat responsivity. In rats, maternal separation (MS) during the pre-weaning period causes age- and sex-specific changes in the Acoustic Startle Response (ASR). This test measures baseline startle in response to white noise bursts, and the potentiation of startle in response to playback of an ethologically-relevant social signal of threat, a 22 kHz Ultrasonic Vocalization (USV). We have found by late adolescence (P55) males startle more than females for either rearing condition, and that USV-potentiated startle is blunted in MS-reared males. MS is a common ELS model, but there is a lack of consensus on the duration necessary to elicit pathological adult behavior. We directly compared two "doses" of MS by rearing litters under control (CON), full MS (P2-P20), and late MS ("LMS," P11-P20) conditions, and testing offspring ASR at P35 (early adolescence), P55 (late adolescence), and P70 (adulthood). At P55, we replicated a main effect of sex for startle amplitude; however, MS-reared females, not males, had blunted USV-potentiation of startle. LMS did not impact baseline or USV-potentiation of startle at P55. At P70, LMS but not MS yielded higher baseline startle than CON rearing. CON rats had an expected potentiated startle response to the USV, while MS males, but not LMS

males, displayed a blunted response. Importantly, the cohorts tested in this study were offspring of dams shipped while pregnant, compared to those bred in-house previously. Our results indicate that both “full” and “late” doses of early life stress have enduring impacts on behavior. There were no significant differences between MS and LMS conditions, which suggests compatibility between the two doses of ELS; however, the notable differences in whether MS or LMS yielded differences from CON should be further explored. Additionally, the limited replication of our previous findings may be understood as a consequence of the gestational stress experienced by the pregnant dams used in this study. As prenatal stress has been shown to impact the development of anxiety-related circuits, follow-up experiments will directly compare ASR in offspring with or without prenatal stress.

Disclosures: **R.A. Cauchon:** None. **L.E. Granata:** None. **A.E.R. Parakoyi:** None. **C.R. Cody:** None. **M.M. Fanikos:** None. **A.K. Rekapalli:** None. **H.C. Brenhouse:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.07/NN9

Topic: F.03. Stress and the Brain

Support: NIMH Grant R21MH119090

Title: Impact of pubertal ovarian hormones on parvalbumin interneuron activation in stress-sensitive brain regions

Authors: *E. M. WOODWARD, J. ACKERMAN, L. COUTELLIER;
The Ohio State Univ., Columbus, OH

Abstract: Stress-induced neuropsychiatric disorders, including anxiety disorders, are over twice as common in women than men, however this sex difference in prevalence of anxiety disorders does not emerge until pubertal onset. This suggests that there may be a role for ovarian hormones in organizing sex-specific vulnerability to anxiety. Prefrontal parvalbumin (PV) interneurons, which mature during adolescence in a process mediated by estradiol, are sensitive to stress and mediate anxiety-like behavior in female mice only. We recently found that prepubertal, but not adult, ovariectomy protects against stress-induced anxiety-like behavior in adult female mice. We further showed that prepubertal ovariectomy prevents the stress-induced increase in prefrontal PV+ interneuron hyperactivity previously causally related to an anxious phenotype. Therefore, we hypothesize that ovarian hormones at puberty contribute to the adolescent maturation of PV+ interneurons and organize their stress-sensitive properties responsible for sex-specific responses to chronic stress. Here, we aim to further investigate the role of pubertal ovarian hormones on PV+ interneurons in other stress-sensitive brain regions, including basolateral amygdala (BLA) and ventral hippocampus (vHC). Female mice were subjected to prepubertal or adult ovariectomy or sham surgery followed by four weeks of chronic mild stress.

We then used immunohistochemistry to assess markers of PV+ interneuron activation, including total number of PV+ interneurons and PV+ interneuron activity through co-labeling with FosB. First, we found that adult ovariectomy did not prevent the hyperactivity phenotype of prefrontal PV cells induced by stress. Furthermore, we found that adult ovariectomy lead to an overall decrease in the number of PV+ interneurons and the level of PV/FosB labeled cells in the PFC, independent of stress condition. On the contrary to the PFC, the vHipp is not sensitive to prepubertal ovariectomy as chronic stress increases number of PV cells in both sham and surgery group. Ongoing analysis will reveal if PV+ neurons in the BLA are sensitive to ovariectomy. Altogether, our results provide new insight into the role of ovarian hormones in the organization and activation of inhibitory circuitry in stress-sensitive brain regions at puberty, providing a new understanding of increased vulnerability of females to stress-related disorders.

Disclosures: E.M. Woodward: None. **J. Ackerman:** None. **L. Coutellier:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.08/NN10

Topic: F.03. Stress and the Brain

Support: Northeastern PEAK Award 2023

Title: Maternal response to infant calls in adverse rearing environments: who gets mom's attention, and when?

Authors: *A. K. REKAPALLI, L. GRANATA, C. CODY, M. FANIKOS, A. PARAKOYI, R. CAUCHON, H. C. BRENHOUSE;
Northeastern Univ., Boston, MA

Abstract: Motherhood changes women's perception of threats, often perceiving potential dangers as more threatening, especially when their children are potentially at risk. Rat pups use a variety of ultrasonic vocalizations (USVs) to help their dam locate them and provide care/nursing. Adult rats also use USV calls to communicate information about the environment such as potential threat (22kHz) or an opportunity to play, eat or mate (50kHz). Importantly, infant USV repertoires are dependent on both the age and early life experiences of the pups. We have shown that maternal separation (MS), a type of early life adversity where pups are separated from their dam and littermates for 4 h/day from postnatal day (p)2-20, changes dams' caregiving behavior, but there have not been studies looking at how maternal behavior changes in response to threatening stimuli or pup USVs. We used a rodent model to investigate the impact of potential threats and postpartum experiences on the drive to retrieve offspring in response to their calls. In the first experiment, MS and control (Con) dams were exposed to 22 kHz or 50 kHz USV playback during a retrieval task, and their latency to retrieve the litter was assessed. This behavioral paradigm was conducted at p9 (before pup thermoregulation) and 19

(before weaning) with the testing repeated in silence as a control condition. The data suggests that at p9, the 22kHz USV stimulus provokes a faster retrieval response in MS dams compared to Con, whereas the 50 kHz vocalization elicited a similar pace of retrieval in both groups. At p19, there did not appear to be a difference in retrieval time. Additionally, to determine whether pup USVs elicit maternal attention, MS and Con dams' preference for retrieving a vocalizing pup over a silent pup was tested in a Y-maze apparatus at a similar developmental time point of p10 and 20. This was repeated using female and male pup pairs to identify potential sex differences in eliciting maternal care. The pup USVs were recorded and analyzed using DeepSqueak to confirm and identify vocalization characteristics. At the p10 time point, when pups vocalize more than at p20, Con dams spend a longer duration near the vocalizing pup than the anesthetized pup, an effect driven largely by female pups. As expected at p20, dams did not show a preference for either the vocalizing or silent pups regardless of rearing environment, however, MS dams spent less time near the vocalizing pup than the Cons. Notably, at both p10 and 20, both MS and Con dams entered the male vocalizing pup's section more frequently than any of the other groups. These findings can aid in understanding dam and pup interactions and how threat communication plays a role in maternal care.

Disclosures: A.K. Rekapalli: None. **L. Granata:** None. **C. Cody:** None. **M. Fanikos:** None. **A. Parakoyi:** None. **R. Cauchon:** None. **H.C. Brenhouse:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.09/NN12

Topic: F.03. Stress and the Brain

Support: FNS Grant 310030_185192

Title: Fecal Microbiota Transfer reduces alcohol preference in stressed rats

Authors: *L. AESCHLIMANN[1,2], K. S. JADHAV[3], F. GILARDI[4], C. BERTELLI[5], S. NASSIRNIA[5], A. THOMAS[4], G. GREUB[5], B. BOUTREL[6];
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Abstract: Alcohol use represents a significant health concern, accounting for 4.5% of global disease burden. Only a small proportion of individuals develop persistent alcohol used disorder though. With current pharmacotherapies largely unsatisfying, discovering novel alternatives to prevent alcohol use disorder becomes a priority. Hence, identifying biological markers predicting vulnerability to develop excessive alcohol consumption may lead to real improvement of clinical

care. Converging evidence suggests that gut microbiota is capable of influencing immunity, brain and behavior. We thus investigated gut microbiome and signs of peripheral inflammation in stressed rats exhibiting uncontrolled alcohol seeking behaviors defined as: 1) Inability to abstain during a signaled period of reward unavailability, 2) Increased motivation and 3) Persistent alcohol seeking despite aversive foot shocks. Compared to controls, rats exposed to chronic stress during adolescence exhibited impulsive, inattentive and disinhibited behaviors. After 33 sessions of daily alcohol (10% weight/volume) self-administration, all rats were screened according to the 3criteria defined above. Majority of the vulnerable group was composed of stressed rats, and most of the resilient group was composed of controls, confirming that stress during adolescence increases the vulnerability to develop AUD-like behavior. All rats were then given access to two sources of reward: 10% w/v ethanol and saccharine (0.2 %, 0.00625%, 0%), 2consecutive sessions for each concentration, during which stressed rats exhibited a clear-cut preference for alcohol compared to controls. Strikingly, we identify a long-lasting peripheral inflammation in stressed rats (Rantes (CCL5) and Interleukin-4 (IL-4)). Not only fecal microbiota transfer lowered stressed rats' preference for alcohol but it restored inflammation modulators levels to those observed in controls.

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Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.10/Web Only

Topic: A.09. Adolescent Development

Support: Grants-in-Aid for Scientific Research (B) 17H04232 of Japan Society for the Promotion of Science (JSPS)
Grants-in-Aid for Scientific Research (B) 20H03649 of Japan Society for the Promotion of Science (JSPS)
Grants-in-Aid for Scientific Research (C) 19K08306 of Japan Society for the Promotion of Science (JSPS)

Title: Maternal diet restriction causes anxiety in adolescent offspring through cerebral dysgenesis

Authors: F. GOTO[1], *T. MITSUHASHI[1], S. SHIBATA[2], K.-I. KUBO[3], T. TAKAHASHI[1]; [1]Pediatrics, [2]Physiol., [3]Anat., Keio Univ, Sch. Med., Tokyo, Japan

Abstract: Multiple reports have described a continuous decrease in the body weights of mothers and newborns over the past 30 years in Japan. Here, we show that mild diet restriction (DR) in utero in mice alters the differentiation patterns of neural stem/progenitor cells (NSPCs) that resulted in thinning of cerebral cortex. In detail, the probability of differentiation of NSPCs was

higher in the DR group than in the control group (0.119 vs. 0.223, control group, n = 3; DR group, n = 3) during the early phase of neurogenesis. In addition, the thickness of the cerebral cortex was decreased by 10.9% in the DR group on post-natal day 21 (801.5 ± 13.3 vs. 714.3 ± 9.8 μm; $P < 0.001$, control group, n = 7; DR group, n = 13), compared with that in the control group. To investigate whether diet restriction in utero disturbs the gene expression profile of NSPCs, we conducted RNA-seq analysis using total RNA from the cerebral walls on embryonic day 12. We detected 169 differentially expressed genes (DEGs): 110 genes had increased expressions and 59 genes had decreased expressions in the DR group, compared with the expression levels in the control group. A gene ontology cluster analysis indicated that the genes with reduced expressions in response to diet restriction in utero were involved in pathways for cerebral cortical histogenesis, including neurogenesis, axonogenesis, neuron differentiation, neuron projection morphogenesis, and neuron projection guidance. Moreover, a level of anxiety was increased in adolescent offspring exposed to diet restriction. The duration of the stay in the center of the open field was shorter in 4-week-old mice in the DR group, compared with the control group (7.84 ± 0.48 vs. 6.54 ± 0.37 %; $P = 0.034$, control group, n = 37; DR group, n = 38). In the elevated-plus maze test, the duration of time spent in the open arms was shorter in the DR group than in the control group (16.14 ± 1.68 vs. 11.86 ± 1.12 %; $P = 0.024$, control group, n = 36; DR group, n = 30). These results demonstrate that mild diet restriction in utero may affect the cerebral development, resulting in increased susceptibility to anxiety during adolescence.

Disclosures: F. Goto: None. **T. Mitsuhashi:** None. **S. Shibata:** None. **K. Kubo:** None. **T. Takahashi:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.11/NN13

Topic: A.09. Adolescent Development

Support: National Institute of Mental Health R01MH098348 (D.C.K. and S.M.)

Title: Neural reactivity to stress varies with trajectories of adolescent discrimination exposure

Authors: *D. K. GREY[1], J. B. PURCELL[1], S. MRUG[1], M. A. SCHUSTER[2], M. N. ELLIOTT[3], S. TORTOLERO EMERY[4], D. C. KNIGHT[1];
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Abstract: Discrimination is a long-term stressor linked to the disruption of stress reactivity processes associated with emotional distress (e.g., depression, anxiety, posttraumatic stress). Discrimination exposure may be particularly detrimental during adolescence, an important period of both neural and emotional development. Specifically, emotional processes (e.g., stress

reactivity) are mediated by a neural network that includes the prefrontal cortex (PFC), inferior parietal lobule (IPL), hippocampus, and amygdala. Adolescent exposure to discrimination is associated with emotional distress. Further, dorsolateral PFC, IPL, and hippocampal reactivity to stress varies with discrimination exposure. However, it remains unclear whether distinct trajectories of adolescent discrimination exposure have a differential impact on stress-related brain function. Thus, this project investigated the relationship between trajectories of adolescent discrimination exposure and stress-elicited brain activity. Latent growth curve modeling (LGCM) estimated trajectories of adolescent discrimination exposure at ages ~11, 13, 16, and 19 years in participants (N = 1594) from the Birmingham cohort of the Healthy Passages study. A subset of these participants (n = 301) then completed the Montreal Imaging Stress Task during functional magnetic resonance imaging to assess neural reactivity to stress in young adulthood (Age 20 years). LGCMs estimated trajectories representing early exposure to discrimination, progression of adolescent discrimination exposure, and acceleration of adolescent discrimination exposure. Multivariate modeling then compared stress-elicited brain activity to the trajectories of discrimination exposure. Early discrimination exposure was positively associated with dorsolateral PFC and IPL activity and negatively associated with dorsomedial PFC, ventromedial PFC, and hippocampal activity. Further, the progression of discrimination exposure was positively associated with dorsolateral PFC and dorsomedial PFC activity, while the acceleration of discrimination exposure was positively associated with hippocampal activity. The present results suggest that the trajectory of adolescent discrimination exposure (early exposure, progression, and acceleration) might alter the neural response to future stressors within brain regions that support emotional function. These findings might have important implications for understanding the interrelationships among discrimination exposure, adolescent neural and emotional development, and mental health outcomes.

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Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.12/NN14

Topic: A.09. Adolescent Development

Support: NIDA R01 DA037911
CIHR FRN156272
Douglas Foundation and Bombardier

Title: Microrna expression profiles from peripheral blood may serve as biomarkers for depression risk in children and adolescents

Authors: *A. MORGUNOVA[1], N. O'TOOLE[3], G. TURECKI[2], P. P. SILVEIRA[2], A. J. GIFUNI[2], I. H. GOTLIB[4], C. NAGY[2], M. J. MEANEY[2], T. C. HO[5], C. FLORES[2];

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Abstract: Background: Identifying reliable molecular indicators for psychiatric risk, especially when combined with longitudinal clinical data, can significantly contribute to early intervention and prevention strategies. Given the emerging significance of microRNAs in neurodevelopment and mental health disorders, it is crucial to explore non-invasive approaches during early stages. This study aimed to analyze microRNA profiles in peripheral blood samples from children and adolescents, comparing those with and without clinical depression, using a high-throughput approach. **Methods**: A total of 60 dried blood spots samples from the Teen Inflammation Glutamate Emotion Research (TIGER) cohort and 170 blood plasma samples from the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) were sequenced using small RNA protocol. Trimmed reads were processed following exceRpt small RNA-Seq pipeline and differential expression analyzed using DESeq2 package. Categorical groups were assigned based on the clinical diagnosis and Reynolds Adolescent Depression Scale (RADS) or threshold based Child Depression Inventory (CDI) measures in TIGER and GUSTO cohorts, respectively. **Results**: We identify several differentially expressed (DE) microRNAs (padj<0.05), with upregulation in individuals diagnosed with MDD compared to controls, and mainly downregulation in children with low versus high CDI. A common microRNA (p<0.05) was upregulated in both cohorts. Gene ontology based on predicted DE microRNA targets confirmed association with MDD, as well with cardiomuscular biological processes. **Conclusions**: To our knowledge, this is the first investigation of blood microRNA expression signatures of depression in youth. The identified circulating miRNAs linked to early vulnerability to depression allow us to probe into ongoing developmental processes that may be shaping lifelong psychiatric risk.

Disclosures: A. Morgunova: None. **N. O'Toole:** None. **G. Turecki:** None. **P.P. Silveira:** None. **A.J. Gifuni:** None. **I.H. Gotlib:** None. **C. Nagy:** None. **M.J. Meaney:** None. **T.C. Ho:** None. **C. Flores:** None.

Poster

PSTR225. Early-Life Stress: Adolescence

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Program #/Poster #: PSTR225.13

Topic: A.09. Adolescent Development

Support: NIH RO1 (5R01MH129732-02, A-CM)

Title: Early life stress induces strain-specific object recognition memory deficits and increased oxidative stress in the prefrontal cortex

Authors: *A. BRACK[1], R. PHADKE[1], L. FOURNIER[2], M. BOBERG[3], D. LÓPEZ-SOTO[3], M. SALGADO[3], S. ALI[3], A. CRUZ-MARTÍN[1];
[1]Mol. & Cell. Biol. and Biochem., [2]Neurobio., [3]Biol., Boston Univ., Boston, MA

Abstract: Early life stress (ELS) is a well-established risk factor for neuropsychiatric disorders, but its impact on neurobiology remains inadequately understood. Cognitive disruptions, including memory deficits and altered activity patterns in the prefrontal cortex (PFC), a region crucial for cognitive function, have been observed in adults exposed to ELS. A robust mouse model of ELS is the limited bedding model, which restricts bedding from the dam and litter during the early postnatal window, P2-P9. We found memory deficits in ELS-exposed adult mice using a modified form of the novel object recognition task. Over three trials with 5 minute inter-trial intervals where mice interacted with two identical objects, standard reared mice decreased interaction time across trials, indicating memory of the objects and decreased novelty. ELS mice, on the other hand, interacted with the identical objects the same amount of time all three trials, suggesting that they did not remember the objects across trials. In a final trial, ELS mice showed no novel object preference, further suggesting impaired object recognition memory. Oxidative stress (OS) in the PFC has been implicated in schizophrenia (SCZ) patients and SCZ mouse models. We found increased levels of 8-oxo-dG, an oxidized form of DNA commonly used as a marker of OS, in the PFC following ELS. High levels of OS can impair typical neuronal function, possibly acting as the mechanism behind the impaired object recognition memory observed in our ELS mice. Lastly, we found that these changes were specific to the C57BL6/J mouse line, as the CD-1 mouse line did not exhibit either the object recognition deficit or the increase in OS in the PFC. Overall, our modified NORT protocol shows strain-specific impaired object recognition memory in ELS mice in two different ways, and preliminary results suggest that OS regulation may be impaired in the PFC of C57BL6/J ELS mice, hinting at a possible mechanism.

Disclosures: A. Brack: None. **R. Phadke:** None. **L. Fournier:** None. **M. Boberg:** None. **D. López-Soto:** None. **M. Salgado:** None. **S. Ali:** None. **A. Cruz-Martín:** A. Employment/Salary (full or part-time):; Department of Neurobiology, Boston University, Boston, MA, USA, Department of Biomedical Engineering, Boston University, Boston, MA, USA, Center for Systems Neuroscience, Boston University, Boston, MA, USA, Center for Systems Network Biology, Boston University, Boston, MA, USA, Department of Pharmacology and Experimental Therapeutics, Boston University, Boston, MA, USA.

Poster

PSTR225. Early-Life Stress: Adolescence

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR225.14/NN15

Topic: A.09. Adolescent Development

Support: NINDS T-32
NIMH R21MH127486

Schmitt Program for Integrative Neuroscience (SPIN)
DHHS/ PHS/NIH P50 HD103536

Title: Alterations in microglia in primate amygdala paralaminar nucleus after maternal separation

Authors: *D. P. KING[1], C. R. IBE[2], D. M. DECAMPO[4], A. K. MAJEWSKA[3], J. L. CAMERON[6], J. L. FUDGE[5];
[1]Neurosci., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY; [3]Univ. of Rochester Med. Ctr., [2]Univ. of Rochester, Rochester, NY; [4]Neurosci., [5]Univ. of Rochester Med. Ctr., Rochester, NY; [6]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The primate amygdala's paralaminar nucleus (PL) is uniquely composed of immature post-mitotic glutamatergic neurons. We previously showed that the PL slowly expands in volume and gains more mature neurons between infancy and adolescence. We also showed that this change in the mature: immature neuron ratio in the PL is accompanied by shifts in the morphology of microglia, which become more ramified and complex by adolescence. This shift suggests an increased capacity for synaptic pruning by microglia as neurons mature. Here, we examine whether early maternal separation that alters lifetime social interaction alters PL microglia morphology and function.All macaques were born and reared in group pens at the University of Pittsburgh. Three cohorts of monkeys (maternally reared, maternally separated at 1 week or 1 month of age) were sacrificed at either 3 months (infancy) or 4 years (adolescence) of age (n=4 infants, n=4 adolescents per group). At sacrifice, one hemisphere was flash frozen and the other was fixed in 4% paraformaldehyde. Microarray studies were performed on laser captured fresh frozen PL tissue from the infant groups. Microarray pathway analysis was done in BioCarta. Microglia morphology is being assessed on immersion fixed tissue through the PL in all groups in infancy and adolescence. Preliminary results of microarray pathway analysis revealed a significant upregulation of several complement-associated pathways in both separated infant groups. The lectin complement pathway was significantly upregulated in both 1 week and 1 month maternally separated PL versus maternally reared PL as indicated by their enrichment scores of 0.514 $p<0.0001$, and 0.518, $p<0.001$ respectively. Complement is expressed by both neurons and glia and plays a crucial role in identifying and tagging weak or underutilized neuron terminals and spines for elimination by microglia. Upregulation in PL of both maternally separated infant groups suggests that maternal separation may alter synaptogenesis in the PL, through excessive pruning or elimination of synaptic elements. Although our analysis is still in progress, we hypothesize that in the maternally separated animals, microglia will have a phenotype consistent with increased synaptic pruning. Specifically, we expect that maternal separation will result in increased density, clustering, and process extension of PL microglia between maternally reared infants and adolescents, and their maternally reared controls.

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Poster

PSTR225. Early-Life Stress: Adolescence

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Topic: F.03. Stress and the Brain

Support: Virginia Tech startup funds to Tae-Ho Lee
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Government Scholarships to Study Abroad, the Ministry of Education, Taiwan, to Ya-Yun Chen
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Title: The association between family conflict and children's emotional capacity is mediated by parent-child neural similarity during unpredictable aversive events in youth

Authors: *Y.-Y. CHEN, T.-H. LEE;
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Abstract: Heightened susceptibility to stress that is increased by uncertainty in adolescents is a significant issue, specifically regarding potential impacts on their psychological well-being. Literature suggests that dyadic similarity in psychological and neural processing between parents and their child has a protective effect on children's adjustment as it can increase psychological resilience in children. Thus, positive family interactions that increase shared psychological processes between parents and their child is vital for emotional adjustment in children. In this context, the current study examines how parent-youth dyadic neural similarity during uncertainty-induced stress play roles in family relationship and emotional adjustment in youth by focusing on the amygdala reactivity to stress and the level of family conflict. Twenty-five parent-youth dyads (parents: M_{age} = 43.48 years, 72% female; children: M_{age} = 11.28 years, 44% female) completed fMRI scans with a cue-based stress regulation task in which various cues predict forthcoming aversive events with different probabilities. Amygdala responses to three types of cues (uncertain aversive, certain aversive, neutral cues) were extracted, vectorized, and computed using pairwise Pearson correlations for generating parent-youth neural similarity value. Subsequent analyses, using 5,000 bootstrapped samples, investigated the association of this neural similarity with youth emotional adjustment and family conflict. Results revealed a trend toward a positive association between amygdala activity in the uncertain condition and anxiety (r = .27, 95%CI: [-.06 .68]), as the prior finding, but did not correlate with the neural similarity (r = -.05, 95%CI: [-.50 .43]). However, higher neural similarity in the uncertain condition was linked to better emotional adjustment in youth: lower anxiety (r = -.34, 95%CI: [-.59 -.05]) and emotion nonacceptance (r = -.49, 95%CI: [-.73 -.13]). Regarding family conflict, results showed that high family conflict was associated with higher anxiety (r = .40, 95%CI: [-.08 .73]) and emotional nonacceptance (r = .45, 95%CI: [.01 .73]) in youth. Notably, the neural similarity mediated the relationship between family conflict and youth's anxiety (B=2.03, 95%CI: [-0.04 7.43]) and emotional nonacceptance (B=0.56, 95%CI: [0.06 1.75]). Our findings emphasize that increased parent-youth neural alignment in amygdala reactivity during uncertain stress not only predicts children's emotional adjustment but also mediates family conflict's

impact on youth emotional acceptance, thus highlighting the importance of neurobiological congruence in youth mental health.

Disclosures: **Y. Chen:** None. **T. Lee:** None.

Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.01/NN17

Topic: C.01. Brain Wellness and Aging

Title: The H63D HFE variant confers neuroprotection through altered structural integrity and neurotransmission

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Abstract: The H63D variant of the homeostatic iron regulator (HFE) gene is carried by up to 30% of the population and is the most common variant carried by neurodegenerative disease (ND) patients. H63D HFE is known to cause systemic iron overload, but with low clinical penetrance. Iron overload in the brain can exacerbate pathological hallmarks of NDs such as increased reactive oxygen species (ROS), neuroinflammation, and protein misfolding and aggregation. Data from our lab has shown increased neuroinflammation and oxidative damage in the ventral midbrain (VM) of 3-month-old mice carrying H67D HFE (the mouse homolog of H63D). Despite this evidence suggesting a detrimental effect of H67D HFE, both human and mouse studies of this variant have demonstrated protection against neurotoxins that cause NDs. In fact, in 6-month-old H67D HFE mice, we have seen increased antioxidant expression and reversed oxidative damage that was seen at 3 months, suggesting an age-dependent adaptation to the stress caused by this variant. Our hypothesis is that H63D HFE initially increases iron and consequential oxidative stress, but during aging, the brain, and particularly the regions involved in NDs, develop adaptive mechanisms leaving them with heightened neuroprotection compared to those without the variant. I have studied the neurodevelopment of H67D HFE mice, specifically in the brain regions that are vulnerable to NDs; namely the VM, which contains the dopaminergic neurons affected in Parkinson's disease, and lumbar spinal cord (LSC), which contains motor neurons affected in ALS. From immunohistochemical analysis, I have found dopaminergic neurons in the VM have lower survival in H67D HFE mice, while motor neurons in the LSC do not have altered viability. Additionally, protein analysis shows significantly lower expression of the structural proteins vinculin and actin at birth, but these are no longer altered after one month of age. nCounter transcriptome analysis of the 3-month-old VM and LSC of H67D and WT mice demonstrates differential expression of regulators of cellular structure components including cell-cell and cell-matrix adhesion and cytoskeletal organization. Both

regions also showed altered transcripts of neurotransmitter receptors as well as enzymes involved in neurotransmitter synthesis, storage, and release between H67D and WT mice. This data suggests regulation of structural integrity and neurotransmission are implicated in neuroprotection warranted by this common HFE variant. Further investigation into this mechanism of neuroprotection can help elucidate novel therapeutic pathways for more personalized treatment of ND patients.

Disclosures: S. Marshall Moscon: None. **E.B. Neely:** None. **J.R. Connor:** None.

Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.02/NN18

Topic: C.01. Brain Wellness and Aging

Title: Diffusion tensor imaging measures reveal impaired glymphatic functions in Gulf War veterans with chronic multisymptom illness

Authors: *Y. ZHANG[1], M. MOORE[3], D. CLARK[1], P. J. BAYLEY[2], J. W. ASHFORD, Jr.[4], A. J. FURST[2];
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Abstract: Background: Chronic multisymptom illness (CMI), which includes chronic fatigue, sleep difficulty, pain, neurological, respiratory, and gastrointestinal problems is common in veterans after returning from the Gulf War (GW). There is currently no knowledge about glymphatic system functions underlying GW veterans who present CMI. Diffusion tensor imaging-derived analysis along the perivascular space (DTI-ALPS) is an increasingly popular approach for evaluating activity of the glymphatic system in individuals. The objective of this study is to identify impairment of glymphatic system functions using DTI-ALPS measurement in GW veterans. Methods: In this cross-sectional study, ALPS-indices were measured from imaging data of 204 GW veterans from the War Related Illness and Injury Study Center (WRIISC), and 224 age-matched healthy controls (HC) from multiple public research databases. Clinical definition of CMI, severities of chronic fatigue syndromes (CFS), sleep difficulty, and pain intensity were evaluated for GW veterans. Out of the entire 204 veterans, 203 met CMI criteria, 12 had minimal severity of CFS, while the other 192 veterans presented mild to severe CFS symptoms. MRI scanner and site variations were harmonized. Statistical analyses were performed adjusting for age, sex, and years of education as confounding factors. Results: Both HC and GW veterans showed significantly reduced ALPS-indices associated with increased age. Compared with control (HC + veterans with minimal CFS), the symptomatic GW veteran group still had substantially lower ALPS-indices (Cohen's $d = 0.71$; $p < 0.001$) after adjusting for age,

sex, and education. Across the entire GW veterans, significant negative correlations were observed between ALPS-indices and severities of CFS as well as pain intensities. Conclusion: This is the first study analyzing glymphatic function in a large cohort of GW veterans. DTI-ALPS index analysis revealed substantially impaired glymphatic clearance in Gulf War veterans with CMI which may be an important feature of Gulf War illness pathology.

Disclosures: Y. Zhang: None. **M. Moore:** None. **D. Clark:** None. **P.J. Bayley:** None. **J.W. Ashford:** None. **A.J. Furst:** None.

Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.03/NN19

Topic: C.01. Brain Wellness and Aging

Support: Zumberge Individual award (Rajagopalan)
Alzheimer's Association International Clinician Scientist Award (Rajagopalan)
ADNI U01AG024904 (Thompson)

Title: Bmi and sex associated gray matter differences in 3,321 healthy individuals from the uk biobank

Authors: *E. J. GLEAVE[1], M. J. KEMPTON[2], P. M. THOMPSON[3], P. RAJAGOPALAN[4]; [1]USC, Los Angeles, CA; [2]Inst. of Psychiatry, Psychology & Neurosci., King's Col., London, United Kingdom; [3]Mark and Mary Stevens Neuroimaging & Informatics Inst., Imaging Genet. Center, USC, Los Angeles, CA; [4]Radiology, Keck Sch. of medicine, Imaging Genet. Center, USC, Marina del Rey, CA

Abstract: A cardiovascular and metabolic (CM) risk factor, higher body mass index (BMI) increases risk of brain atrophy in the normal population, and in people with a range of psychiatric conditions; we have shown lower brain volumes to be associated with levels of leptin, a marker of obesity. In adults, elevated body mass index (BMI) has been consistently associated with lower gray matter volumes (GMV) in frontal, temporal and cerebellar regions. BMI may mediate higher ventricular volumes in psychiatric illnesses such as bipolar disorder. Further, BMI-associated differences in GMV have been shown to differ between men and women, emphasizing the importance of evaluating sex differences when exploring BMI-related effects to GMV changes. Here we used voxel-based morphometry (VBM) to map brain signatures of BMI in relation to sex after accounting for related risk factors such as high blood pressure. The ENIGMA VBM (https://sites.google.com/view/enigmavbm) pipeline was used to perform a large-scale analysis of 3D T1-weighted brain MRI data from 1,512 male (45.5%; 70.6 (7.3 SD) years old) and 1809 female (54.5%; 72.1 (6.3 SD) years old) participants from the UK Biobank (UKBB). Within the analyzed subset of UKBB participants, effects of BMI on brain

volumes were tested using a linear regression VBM analysis adjusting for mean arterial blood pressure, pulse wave arterial stiffness, age, sex, intracranial volume, ancestry and education, assessed at the time of scan; subjects without these parameters were excluded. After correcting for multiple comparisons by controlling the false discovery rate at 5%, significantly lower gray matter volumes were associated with higher BMI in the majority of the cerebral and cerebellar cortex in the whole cohort and in females-only cohort (standard-FDR critical P-value=0.03; q=0.05), but associations were not detected in bilateral superior and inferior parietal lobules, regions important for higher order visuocognitive and visuospatial tasks. Within males, gray matter volumes in primary visual cortex, amygdala, hippocampus, fusiform gyrus and cerebellum were significantly lower with higher BMI (standard-FDR critical P-value=0.01; q=0.05). We found significantly lower gray matter volumes related to elevated BMI, a modifiable cardiovascular risk factor, throughout the brain. These results support prior findings that BMI is associated with neurostructural alterations in gray matter architecture in healthy adults, even after adjusting for the effects of blood pressure and age.

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Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.04/NN20

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant GM103418
NIH Grant AG035982

Title: Resistance exercise in young and middle-aged rats: effects of sex and age

Authors: *A. LEE[1], J. A. STANFORD[4], P. MOREFIELD[1], J. K. MORRIS[2], F.-C. YANG[3], P. KUECK[1], O. VEATCH[1];
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Abstract: Preclinical studies can reveal mechanisms underlying the effects of exercise. Most animal studies have focused on aerobic exercise protocols. The goal of the current study was to determine effects of a progressive overload resistance exercise (RE) protocol in young adult and middle-aged rats. We trained young adult male and female rats (3-5 month-old) to climb a ladder with weights attached to their tails. Rats then underwent a protocol that determined maximum weight loads (MAX) on Mondays and repetitions with increasing percentages of MAX on Wednesdays and Fridays for eight weeks. MAX was initially greater in the males, but females surpassed the males by week 6. The ratio of MAX to body weight was greater in the females

across all testing sessions. Lean mass (measured non-invasively by echoMRI) increased in both male and female rats, with a greater increase in the males. Fat mass was relatively stable within the two groups, but the males exhibited an increase at the final time point. One year later we repeated the protocol with half of the previously-trained male rats and the remaining half as sedentary controls. Although MAX was similar at the two ages, the ratio of MAX to body weight was greater when rats were young than when they were older. Surprisingly, the rats reached the goal box quicker when they were older than they did the year before. The increase in lean mass with RE was greater when the rats were younger. Fat mass increased slightly in the young rats but decreased with RE when they were older. Blood levels of neurofilament light (NFL; neurodegeneration maker) and glial fibrillary acidic protein (GFAP; inflammation marker) increased from young adult to middle-age. RE had no effect on NFL and GFAP in the older rats. Fresh tissue from the striatum and hippocampus of the RE and sedentary middle-aged male rats were processed for proteomic analysis. In the striatum, the strongest effects of RE included greater expression of proteins related to DNA integrity, circadian processes, protein stability, cell motility, and cell survival. Conversely, processes that inhibit Ras/ERK-MAPK signaling, and proteins related to lipid binding, fatty acid oxidation, glutathione, blood-brain barrier integrity, mRNA degradation, G-protein coupled receptors, and trophic support were downregulated. Our results reveal sex- and age-related differences in RE performance and effects of RE in rats. Proteomic data provide targets for future studies to determine the functional significance of up- and downregulated genes and how RE can prevent sarcopenia and improve brain health.

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Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.05/OO1

Topic: C.01. Brain Wellness and Aging

Support: I2021A006

Title: Potential role of apelin mediating the antidepressant effects of physical exercise

Authors: *J. YU, S. YAU;
Hong Kong Polytechnic Univ., Hong Kong, Hong Kong

Abstract: Sarcopenia is muscle ageing condition comprising progressive decline in muscle strength, mass and function. Epidemiology data have shown that sarcopenia is highly associated with depression in geriatric patients, suggesting an impact of skeletal muscle on brain health. However, the mechanisms underlying this muscle-brain crosstalk resulting in depression is yet to be explored. Apelin, a novel myokine linked to sarcopenia, can elicit antidepressant effects and influence hippocampal neuroplasticity in rodent studies. Physical exercise in humans is effective

in preventing both sarcopenia and depression. A recent study has shown that physical exercise counteracts sarcopenia by restoring decrease in apelin levels of aged mice and humans, suggesting the important role of apelin in both sarcopenia and depression. By using a mouse model of depression induced by chronic unpredictable stress (CUS), here we found that stressed mice showed significantly decreased protein expression of apelin and its receptor (APJ) in the hippocampus, whereas 4-weeks voluntary exercise rescued depression-like behaviour in association with increased Apelin/APJ levels in the hippocampus and lower limb muscles including gastrocnemius (Gas), and tibialis anterior (TA) of stressed mice. Specific knockout of Apelin in skeletal muscle attenuated the antidepressant effect of voluntary exercise, suggesting the key role of apelin in mediating the antidepressant effect of physical exercise. In contrast, overexpression of apelin in Gas and TA muscles mimicked antidepressant effects of physical exercise. These data suggest that the potential role of apelin mediating the crosstalk of the muscle-brain axis on sarcopenia-associated depression, and provide a novel potential therapeutic target for treating geriatric depression.

Disclosures: **J. Yu:** None. **S. Yau:** None.

Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.06/Web Only

Topic: H.05. Working Memory

Support: NIDDK Grant Number P30DK072476

Title: Effect of a Mediterranean Diet Fecal Microbiota Transplantation on Cognitive Function

Authors: *R. OTTAIANO, C. HARPER, E. ENGLER-CHIURAZZI, Y. ZHAO, C. LIU, G. BIX, D. MARAGANORE;
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Abstract: Objective: Diet quality has been associated with cognitive health; the Western diet (WD) is associated with cognitive impairment while the Mediterranean diet (MeDi) is associated with reduced dementia risk and improved cognitive performance. The effects of these diets on cognitive health may be mediated by the gut microbiota. The objective of this pilot study was to explore the efficacy of fecal microbiota transplantation (FMT) in a model of diet-induced cognitive impairment.
Methods: Male Fischer344 rats (4 months) were randomly assigned to 1 of 5 groups (n=9/group): MeDi, WD (control groups); and WD-MeDi FMT, MeDi-WD FMT, or MeDi-MeDi FMT (FMT groups). After 3 months, all groups received antibiotics for three days and received once-weekly FMTs or PBS (control groups) for 2 months. Fresh fecal samples were provided by donor animals (n= 9-18/ diet). At month 5, cognitive performance was assessed using the Y-maze, Morris water maze (MWM), and radial arm water maze (RAWM). Data were

analyzed using a One- or Two-Way Repeated Measures ANOVA. RAWM was analyzed in two periods: 1) learning phase (D1-D6) and 2) testing phase (D7-D12).
Results: There were no differences between groups for Y-maze (p=0.22) or MWM latency (p=0.68). For the RAWM, there were no differences between groups during the learning phase. During the testing phase, MeDi-WD FMT committed significantly more errors compared to WD-MeDi FMT (p=0.01) and MeDi-MeDi FMT (p=0.01) groups. MeDi-MeDi FMT had a trend to commit fewer errors compared to the MeDi group (p=0.06). There were no other differences between either of the three FMT groups and their matching diet control group. All groups ($p \leq 0.02$) reduced total errors across days except for MeDi-WD FMT (p=0.62).
Conclusion: RAWM demonstrated subtle differences between FMT groups as determined by the RAWM. MeDi-WD FMT resulted in worse cognitive performance compared to FMT transplant groups and did not improve across days indicating an inability to learn the cognitive task. The absence of difference between control groups may be due to the lasting impact of antibiotics on cognitive function. Future analyses will determine the effect of diet FMTs on taxon-specific differences in microbiota.

Disclosures: **R. Ottaiano:** None. **C. Harper:** None. **E. Engler-Chiurazzi:** None. **Y. Zhao:** None. **C. Liu:** None. **G. Bix:** None. **D. Maraganore:** None.

Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.07/OO2

Topic: H.06. Social Cognition

Support: RF1AG062166

Title: Role of adipose PPARγ in behavioral regulation

Authors: *J. GARZA, M. GUO, M. MALEK, L. GAN, Q. DU, Y. LEI, X.-Y. LU;
Dept. of Neurosci. and Regenerative Med., Augusta Univ., Augusta, GA

Abstract: Adipose tissue is complex tissue that acts as an endocrine organ and secretes factors which can influence brain function and behavior. Recent data suggests that metabolic state is strongly associated with cognitive functions and may be a contributing factor to certain dementias. The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-dependent transcription factor that is known as the “master regulator” of metabolism and adipose tissue development. PPARγ has an important role in regulating metabolic activity, and our previous work identified a role for adipose PPARγ in the susceptibility to stress and emotional behaviors. In the current study we characterized behavioral phenotypes caused by adipocyte-specific deletion or overexpression of PPARγ. Our results indicate that adipose PPARγ is involved in mediating social recognition. Taken together, these findings suggest a novel role for PPARγ signaling in behavioral regulation.

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Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.08/OO3

Topic: C.01. Brain Wellness and Aging

Support: NRF-2020R1A6A1A0304328
NRF-2023K1A4A3A02057280
RS-2023-00220408
NRF-2022R1F1A1062944

Title: Synergistic effects of photobiomodulation and clostridirum butyricum on the Gut-Brain Axis of aging mouse

Authors: *H. GOO, S.-H. CHOI, S. MO, J.-C. AHN;
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Abstract: Recent research has witnessed a growing interest in studying the gut-brain axis, a mechanism through which the gut and the central nervous system (CNS) communicate via biochemical signals. The intricate community of gut microbiota plays a crucial role in influencing our overall well-being by exchanging signals with the brain. Although current research on the gut microbiome primarily focuses on obesity and diabetes, our understanding of the gut-brain axis and its relationship with gut microbes remains incomplete. The complex communication network among the intestinal microbiota serves as one of the contributing factors. Photobiomodulation (PBM), specifically the use of light, has the potential to modify this communication by stimulating the microbiome. Additionally, PBM is known to reduce oxidative stress, influence the inflammatory process, and prompt cells stimulated by PBM to transmit protective signals to damaged areas such as the brain and gut. A nascent field known as photobiomics, which combines the gut microbiome with PBM, has recently emerged worldwide. Researchers are in the early stages of investigating its role in maintaining homeostasis and slowing down the aging process.In a recent study, an experiment was conducted to explore the effects of combining PBM with 'Clostridirum butyricum (CB),' a gut microbiome known for its potential protective effects in gut diseases, neurodegenerative diseases, and various other conditions. Ten-week-old rats were given CB for nine months while simultaneously receiving PBM irradiation on their heads. At 59 weeks, the rats' brains were removed and examined using tissue staining. The brain hippocampus of the mice that received CB (CB group), mice that were irradiated with PBM (LED group), and mice that underwent PBM after CB intake (CB+LED group) were compared to control mice at 59 weeks. The staining results revealed a higher number of cv-positive cells in both the CB group and the LED group compared to the 59-week-old mice. Notably, a significant increase was observed in the CB+LED group. Furthermore, FJB

staining and TUNEL staining, which selectively stain degenerating neurons and apoptotic cells, respectively, exhibited decreased activity in the CB group, LED group, and CB+LED group compared to the 59-week-old mice.The findings from our study confirmed the influence of CB on the brain hippocampus, with the combination of CB and PBM yielding even more pronounced effects. In conclusion, the development of a technology that combines the gut microbiome with PBM holds promise as a foundation for future advancements in brain research.

Disclosures: **H. Goo:** None. **S. Choi:** None. **S. Mo:** None. **J. Ahn:** None.

Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.09/OO4

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01 AG071686-01

Title: Aged Plasma Drives Sex-Specific Inflammatory Gene Expression and Cognitive Changes

Authors: *E. A. LEITSCHUH[1], S. L. RODRIGUEZ[1], C. M. CARVER[1], P. T. GOMEZ[1], M. J. SCHAFER[2];
[1]Dept. of Physiol. and Biomed. Engin., [2]Dept. of Physiol. and Biomed. Engin. and Neurol., Mayo Clin., Rochester, MN

Abstract: Heterochronic parabiosis enables circulatory exchange between a young and old mouse. Aged blood confers brain dysfunction in young parabionts, whereas young blood confers rejuvenative outcomes in the aged brain. Similar effects have been observed following heterochronic intravenous plasma administration. The mechanisms by which blood factors drive accelerated brain aging outcomes related to distinct cognitive domains are not well characterized. Additionally, the majority of prior plasma administration experiments focused on males. Here, we addressed these knowledge gaps by exploring the influence of aged versus young plasma on gene expression and cognitive function in young wild-type female and male mice. We treated young mice (3 months) with old (~22 months) or young (~2.5 months), sex-matched plasma twice a week for four weeks. We implemented a nest building task to probe cortical-integrated executive function and a contextual fear conditioning (CFC) task to probe hippocampal- and amygdala-integrated spatial memory. We conducted RT-PCR expression profiling of inflammatory and senescence genes in brain and peripheral tissues, including lung (LNG), kidney (KID), hippocampus (HIP), and white matter (WM). Males receiving old plasma demonstrated reduced freezing in CFC, suggestive of impaired associative spatial memory. Females receiving old plasma exhibited poorer nest building, suggestive of impaired executive function. Males receiving old plasma had significant or trend increases in expression of inflammatory and senescence-related markers in LNG, KID, and HIP, which were distinct from expression patterns in females receiving old plasma. Our findings reveal distinct sex-dependent

impacts of aged plasma on cognitive function and inflammatory gene expression across peripheral tissues and brain. Sex-specific cognitive outcomes suggest that aged plasma may confer important differences in distinct brain regions. Our future experiments will enable advanced cell and molecular profiling of distinct brain regions, expanded behavioral testing, and mechanistic investigation of the influence of aged plasma factors on brain and systemic aging.

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Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.10/OO5

Topic: C.01. Brain Wellness and Aging

Support: P30AG062677

Title: Human plasma proteins as biomarkers for mild cognitive impairment and Alzheimer's disease

Authors: *N. C. ASMUSSEN[1], T. A. WHITE[2], K. KANTARCI[3], M. M. MIELKE[6], N. K. LEBRASSEUR[4], M. J. SCHAFER[5];
[2]Robert and Arlene Kogod Ctr. on Aging, [3]Dept. of Radiology, [4]Dept. of Physical Med. and Rehabil., [5]Dept. of Physiol. and Biomed. Engin., [1]Mayo Clin., Rochester, MN; [6]Dept. of Epidemiology and Prevention, Wake Forest Univ. Sch. of Med., Winston-Salem, NC

Abstract: Human plasma proteins as biomarkers for mild cognitive impairment and Alzheimer's disease
Niels C. Asmussen[1], Thomas A. White[2], Kejal Kantarci[3], Michelle M. Mielke[5], Nathan K. LeBrasseur[2,6], Marissa J. Schafer[1,2,4]
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Alzheimer's disease (AD) and mild cognitive impairment (MCI) are highly prevalent among older adults, and biomarkers are increasingly important for predicting which individuals will develop MCI and AD. Circulating proteins arising from dysfunctional organ systems are a possible mechanism by which age-related systemic pathology is propagated to the brain. These

factors could serve as novel prognostic or diagnostic biomarkers to identify individuals at greatest risk of MCI or AD. To address this critical knowledge gap, we cross-sectionally analyzed 224 sex matched human plasma samples from the Mayo Clinic Study of Aging (MCSA) and AD Research Center (ADRC) cohorts using SomaScan aptamer assays to profile 7,289 proteins. We developed multivariate models to explore relationships between plasma proteins and clinical diagnosis, cognitive function, or brain imaging variables with adjustment for age, sex, BMI, and/or education. We discovered that proteins involved in neurological (e.g. neurotensin, acetylcholinesterase, and leucine-rich repeat neuronal protein 1) and metabolic (e.g. gastric inhibitory polypeptide, glucagon, and pancreatic hormone) processes were associated with diagnosis of MCI or AD. Several proteins with established links to neuropathology (e.g. neurofilament light polypeptide, amyloid beta precursor protein binding family B member 1, and stathmin-4) were positively associated with multiple cognitive and imaging outcomes. We are continuing to examine these datasets through additional multivariate models and gradient boosted machine learning algorithms. Our ultimate goal is to better understand relationships between circulating proteins and diagnosis, cognitive decline, and brain imaging variables across the AD clinical spectrum in older adults.

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Poster

PSTR226. Impact of Systemic Factors on Brain Function

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR226.11/OO6

Topic: C.01. Brain Wellness and Aging

Support: Swedish Medical Research Council
The Swedish Brain Foundation

Title: Impact of systemic inflammation on the hippocampal metabolome of a mouse model of surgery-induced cognitive decline.

Authors: *M. GOMEZ-GALAN[1,2], A. EBBERYD[2], D. SCHREY[3], S. ERHARDT[2], L. ERIKSSON[2];
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Abstract: Surgery is known to induce a profound systemic response affecting the body physiology during the following hours and days after the surgery. Conventionally, the body's response to surgery has been described as a stress response consisting of two main components: a neuroendocrine-metabolic response, centrally coordinated by the hypothalamus and affecting the glucose and lipid metabolism of organs such as the liver and the skeletal muscle, and a systemic inflammatory-immune response involving the innate and cell-mediated adaptive immune systems (reviewed by Cusack and Buggy, 2020). However, how this stress response to surgery affects other brain areas and the consequences in higher brain functions such as cognition is not well understood. We previously identified morphological, metabolic, and functional alterations in the hippocampus of mice subjected to orthopedic surgery. Moreover, these alterations associated with cognitive impairments (Terrando et al., 2013; Femenía et al., 2018). Here we expanded our previous finding by mapping the hippocampal metabolic state after orthopedic surgery in male mice by metabolomic and gene expression analysis. We found an early (6h) hippocampal dysregulation of metabolites related to fatty acid metabolism and oxidation (increase of acylcarnitines and decrease of medium- and long- chain fatty acids) accompanied by an increase of metabolites involved in the tryptophan pathway, including kynurenine and other related metabolites. All these changes were accompanied with a reduction of key essential and non-essential amino acids. Hippocampal gene expression analysis confirmed the dysregulation in the expression of key genes related to fatty acid and glucose and kynurenine metabolism. Mitochondria function and integrity remained intact. The levels of the altered metabolites returned to normal by 72h post-surgery while the expression of lipid related genes was downregulated at that time point compared to control. We conclude that the hippocampus responds to an external injury (i.e., orthopedic surgery) by adapting its metabolic needs to the energy substrate availability which, under pathological circumstances might have a detrimental effect for cognitive functions.

Disclosures: M. Gomez-Galan: None. **A. Ebberyd:** None. **D. Schrey:** None. **S. Erhardt:** None. **L. Eriksson:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.01/OO7

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Oxytocin decreases evoked dopamine in the mouse nucleus accumbens core in ex-vivo fast-scan cyclic voltammetry

Authors: *N. REUVENI, S. BARISELLI, Y. MATEO, I. P. ALONSO, D. LOVINGER; NIH/NIAAA, Rockville, MD

Abstract: Oxytocin receptor expression and input into the nucleus accumbens (NAc) is involved in a variety of social behaviors such as social reward and the reinforcing effects of maternal

behavior. The ventral tegmental area and it's mesolimbic projections to regions such as the NAc which are known to be involved in reward are primarily composed of dopaminergic neurons with less than 10% of these neurons also expressing oxytocin receptors. However, the mechanism in which oxytocin modulates mesolimbic dopamine release is still an open area of investigation. Therefore, this work aims to investigate the effects of oxytocin along with an oxytocin receptor specific agonist (WAY-267464) and antagonist (L-368,899) on dopamine release in the nucleus accumbens core (NAcc) of C57BL/6J mice using *ex-vivo* fast-scan cyclic voltammetry. In both virgin females and dams (euthanized immediately after birth to up to five days after birth), 10 µM of WAY-267464 induced a significant depression of electrical stimulation-evoked dopamine in the NAcc. Follow-up experiments suggest that the effect of WAY-267464 at this concentration might be due off-target effects because this decrease in evoked dopamine was not blocked by 5 µM of L-368,899 with or without pre-incubation nor 50 µM of L-368,899. However, in virgin females, 1 nM of oxytocin induces a significant depression of stimulus-evoked dopamine release in the NAcc that appears to be blocked by 10 µM of L-368,899. These studies provide insight into oxytocin's potential role in the modulation of NAcc dopamine which may be relevant in the rewarding elements of social behaviors and maternal behavior.

Disclosures: **N. Reuveni:** None. **S. Bariselli:** None. **Y. Mateo:** None. **I.P. Alonso:** None. **D. Lovinger:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.02/OO8

Topic: G.02. Reward and Appetitive Learning and Memory

Support: McNair Scholars Program
Brain and Behavior Research Foundation-NARSAD Young Investigator Award
Mary E. Groff Foundation

Title: Oxytocin Modulates Excitatory and Inhibitory Inputs to Dopamine Neurons in the Ventral Tegmental Area

Authors: *C. VILLARREAL[1], A. ALLEN[2], K.-C. LEONG[3], G. M. BEAUDOIN, III[2];
[1]Engin. Sci., [2]Biol., [3]Psychology, Trinity Univ., San Antonio, TX

Abstract: The mesolimbic circuit is regulated in part by dopaminergic neurons located in the Ventral Tegmental Area (VTA). This circuit is greatly implicated in reward seeking behavior and in particular are critical for substance use disorder. While dopaminergic neurons can fire tonically through the interaction of ion channels found on the neuron, rewards and cues predictive of rewards induce changes in dopamine release via inputs on dopamine neurons. Recent evidence has shown that the neuropeptide oxytocin (OXT) decreases drug seeking

behavior. This decrease in drug seeking may be due to changes in the activity of inputs onto dopaminergic neurons through activation of OXT receptors on dopaminergic neurons or their inputs.
Whole-cell electrophysiological recordings were performed on DA neurons in the VTA to investigate the effects of bath-applied OXT on excitatory and inhibitory synaptic inputs. Horizontal brain slices from adult male Sprague-Dawley rats containing the VTA were used. The recordings were conducted using glass pipettes filled with an internal solution including cesium chloride and QX-314. Miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) were recorded in the presence of tetrodotoxin.
Excitatory postsynaptic currents (EPSCs) were isolated by GABA-A receptor blockade, while inhibitory postsynaptic currents (IPSCs) were isolated with AMPA and NMDA receptor blockade. Cumulative probability plots were generated to analyze inter-event intervals and amplitude.
Our results demonstrate that OXT significantly decreases the frequency of mEPSCs, indicating a probable reduction in release probability. The amplitude of mEPSCs did not exhibit significant changes across experimental conditions. Furthermore, the application of an OXT receptor antagonist, reversed the effects of OXT, returning the mEPSC frequency to baseline levels. This data suggests OXT's role in reducing the output of excitatory inputs.
These findings contribute to our understanding of the neuromodulatory effects of OXT on reward-related behaviors. Our electrophysiological data supports the hypothesis that OXT decreases excitatory inputs to dopaminergic neurons in the VTA.
By elucidating the mechanisms underlying OXT's effects on reward-related maladaptive behaviors, this research highlights its potential as a pharmacological and therapeutic intervention for addiction to natural rewards and drugs of abuse.

Disclosures: **C. Villarreal:** None. **A. Allen:** None. **K. Leong:** None. **G.M. Beaudoin:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.03/OO9

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Striatal dopamine in a high risk schizophrenia mouse model

Authors: *S. SRIRAMOJI;
New Jersey Med. Sch., Newark, NJ

Abstract: Striatal dopamine in a high-risk schizophrenia mouse model Sindhu Sriramoji, Mariam Mahboob, Miriam Bocarsly
Schizophrenia is a mental disorder affecting behavior, cognition, and emotion. While schizophrenia is characterized by the dysregulation of dopamine in the brain, specifically the striatum, its physiology has not been determined. In humans, the 3q29 microdeletion presents a

40>fold increased risk of schizophrenia. This deletion has been recapitulated in a CRISPR mouse model. Mice with this deletion have reduced brain volume and behavioral impediments including social interaction, cognition, and increased sensitivity to amphetamines. Utilizing fast scan cyclic voltammetry, an electrochemical technique used to examine neurotransmitter activity in brain slices, we examined dopamine levels in the striatum of 3q29 deletion mice and littermate controls. Levels of electrically evoked dopamine were similar in both strains at a range of stimulus intensities. Dopamine levels in the striatum are dependent on two mechanisms. (1) Firing of midbrain dopamine neurons leads to an increase in striatal dopamine levels. (2) Activity at striatal cholinergic interneurons leads to acetylcholine release, which acts of dopamine neuron terminals to trigger action potential independent dopamine release. The addition of Dihydro-β-erythroidine hydrobromide (DhβE), an acetylcholine antagonist, blocks acetylcholine-dependent dopamine release, allowing us to isolate these two mechanisms. When DhβE was applied to striatal brain slices, dopamine was elevated in the 3q29 deletion mice compared to littermate controls. Given differences in striatal dopamine release in the 3q29 deletion mice, we will be looking into altered behavior (depressive-like and anxiety-like behaviors) in the 3q29 deletion mice and littermate controls. Further, we will be looking into the effects of Olanzapine, an atypical antipsychotic commonly prescribed to patients with schizophrenia, on striatal dopamine release in the 3q29 deletion mice.Together, this data will allow us to characterize the striatal dopaminergic activity 3q29 deletion mice compared to littermate controls and assess how this is altered with antipsychotic treatment.

Disclosures: S. Sriramoji: None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.04/OO10

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: BWF CASI 1019469
T32 MH082174
R01 DA056543
R01 MH112729

Title: Arrestin-3 recruitment to the D3 dopamine receptor by select second-generation antipsychotics causes changes in calcium activity and drug tolerance

Authors: *E. LEWIS[1,2], S. W. GOODING[1,2], J. MUIR[1], C. K. KIM[1,3], J. L. WHISTLER[1,4]; [1]Ctr. for Neurosci., Univ. Of California at Davis, Davis, CA; [2]Neurosci. Grad. Group, Univ. of California at Davis, Davis, CA; [3]Neurol., [4]Physiol. and Membrane Biol., UC Davis Sch. of Med., Davis, CA

Abstract: Second generation antipsychotics (SGAs) are widely used for the treatment of schizophrenia, bipolar disorder, and other serious mental illness. Despite being regularly prescribed for decades, the effectiveness of SGAs in patients is unpredictable. SGAs can also cause negative side effects including catatonia, movement disorders, and depressive states, and many of these side effects can outweigh the benefits of the drug. SGAs bind the D2 and D3 dopamine receptors (D2R & D3R, both Gi-protein coupled receptors) and are traditionally considered antagonists for dopamine receptor signaling. However, we have previously shown that some SGAs are arrestin-3-biased (beta-arrestin) agonists at D3R, but not D2R. We can thus separate SGAs into two classes: those that recruit arrestin-3 to D3R versus those that do not. We hypothesize that some of the variability in effect/side effect profiles could be mediated through variable activity at D3R. Here, we show that in mice, both classes of SGAs cause suppression of cocaine-induced locomotion (1-way ANOVA, $p<0.05$, $n=16$). However, only mice chronically pre-treated with an SGA that causes arrestin-3 recruitment to D3R develop tolerance to this locomotor suppressive effect (1-way ANOVA with multiple comparisons, $p<0.05$, $n=18$). Similarly, we demonstrate that both classes of SGAs lead to Conditioned Place Aversion (CPA) (paired t tests, $p<0.05$, $n=10$, $n=15$), but mice pre-treated with an arrestin-recruiting SGA no longer show CPA to the drug (paired t test, $p=0.58$, $n=15$). We hypothesized that this tolerance to arrestin-recruiting SGAs reflected arrestin-3-mediated D3R internalization and degradation. To examine this, we assessed tolerance in mice lacking the D3R degradation protein GASP1 (D3-Cre GASP1 cKO) and saw no tolerance to the locomotor (paired t test, $p<0.05$, $n=18$) or the CPA effects (paired t test, $p<0.05$, $n=13$). To assess what D3R cells might be responsible for these phenotypes, we used fiber photometry with a Cre-dependent gcamp6f in D3-cre mice and monitored calcium activity specifically in D3R-expressing neurons. In the lateral nucleus accumbens, a region associated with aversion, we saw that an arrestin-recruiting SGA inhibits activity of these cells ($n= 3$). These results implicate D3R-neurons in the mechanism of action and tolerance, as well as side effects of SGAs. They also suggest that differences in SGAs' abilities to recruit arrestin-3, both acutely and by promoting downregulation of D3R, could explain the variable effect/side-effect profiles of these drugs. Our results highlight the importance of considering D3R-expressing circuits and arrestin-3 signaling in future drug development of SGAs.

Disclosures: E. lewis: None. **S.W. Gooding:** None. **J. Muir:** None. **C.K. Kim:** None. **J.L. Whistler:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.05/OO11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R44NS117201

Title: Molecular mechanisms of Agonist dependent biased signaling of Dopamine D3 Receptors

Authors: *B. NEPAL, J. BARNETT, W. XU, F. BEAROFF, S. KORTAGERE;
Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The dopamine D3 receptor (D3R) is a class A G-protein coupled receptor (GPCR) known to play a significant role in cognition, mood, motor and reward-based behaviors. Hence it is an attractive drug target for several neurological and neuropsychiatric diseases. D3R agonists can selectively trigger G-protein or β-arrestin-mediated signaling pathways and may promote higher therapeutic efficacy with lower side effects than the unbiased against. We previously designed a selective D3R agonist, SK609 that biases D3R to signal primarily through the G-protein mediated pathway with minimal β-arrestin recruitment. Further, SK609 activated D3R undergoes time-dependent internalization without prolonged receptor desensitization further validating the lack of β-arrestin recruitment. However, the molecular mechanisms by which SK609 activated D3R triggers the G-protein mediated pathway over β-arrestin is not well understood. In the present study, we used microsecond-level all-atom molecular dynamics (MD) simulations in a membrane environment to understand the conformational dynamics of D3R in complex with β-arrestin2 or G-protein in the presence of SK609 or unbiased agonist pramipexole (PRX). Conformational analysis of the trajectories from the MD simulation suggested conserved outward movement of the TM6 helix to stabilize an active D3R conformation that interacts with G-protein. Further, in the D3R-SK609 complex at the cytoplasmic region, TM7 moves inwards towards the cytoplasmic region by ~2Å relative to the D3R-PRX complex. This conformation in the D3R-SK609 complex sterically hinders the finger loop region of the β-arrestin from interacting with the D3R at the cytoplasmic cleft. Proteome-wide phosphorylation analysis of D3R-overexpressing SH-SY5Y neuroblastoma cells revealed SK609 and PRX induce phosphorylation in the Intracellular loop 3 and TM4 respectively suggesting different phosphorylation signatures. Immunoblotting of treated cells showed higher levels of tyrosine residue phosphorylation in D3R by SK609 while there was an increase in serine/threonine residue phosphorylation by PRX treatment. These results confirm that biased agonists of D3R promote unique conformational states and phosphorylation signatures that can be exploited for developing better therapeutics.

Disclosures: B. Nepal: None. **J. Barnett:** None. **W. Xu:** None. **F. Bearoff:** None. **S. Kortagere:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.06/OO12

Topic: G.04. Emotion

Support: NIDA DA047233
NIDA DA007359

Title: Epigenetic control of endogenous deltaFOSB expression in nucleus accumbens medium spiny neurons regulates their activation by salient stimuli

Authors: *T. MARKOVIC[1], A. GODINO[2], L. HOLT[3], A. MINIER-TORIBIO[2], T. M. GYLES[4], E. PARISE[5], F. MARTINEZ-RIVERA[2], R. LINGALA[1], K. CHOUDHRY[1], N. RAHIM[1], E. J. NESTLER[6];
[1]Icahn Sch. of Med., New York, NY; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Neurosci. Dept., Mount Sinai Sch. of Med., New York, NY; [4]Friedman Brain Inst., [5]Icahn Sch. of Med. At Mount Sinai, New York, NY; [6]Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: ΔFOSB is a key transcription factor that mediates gene expression changes in the nucleus accumbens (NAc) in response to chronic exposure to stress, drugs of abuse, or other emotional stimuli. The NAc is composed predominantly of GABAergic medium spiny neurons (MSNs) that express either dopamine receptor 1 (D1) or dopamine receptor 2 (D2). Previous work in rodents showed that chronic exposure to diverse stimuli induces ΔFOSB in the NAc in a cell-type-specific manner: cocaine induces ΔFOSB in D1 MSNs, chronic stress induces the protein in D2 MSNs in stress-susceptible but in D1 MSNs in stress-resilient animals, while natural rewards induce ΔFOSB in both cell types. This cell-type-specific regulation of ΔFOSB expression in the NAc correlates with differential effects of the protein on synaptic properties of MSNs: ΔFOSB decreases excitatory synaptic strength and increases silent synapses onto D1 MSNs, with opposite effects seen for D2 MSNs. However, no studies have investigated how changes in ΔFOSB expression levels in the NAc alter the in vivo activity of these MSN subtypes. To address this gap in knowledge, we injected D1-Cre and D2-Cre mice with Cre-dependent adeno-associated viral vectors that express a calcium sensor and epigenome-editing tools that either induce or repress endogenous ΔFOSB in the NAc. We recorded in vivo neuronal activity of D1 and D2 MSNs using fiber photometry in response to social reward, saccharin reward, foot shock, and drug rewards. We found that manipulation of ΔFOSB primarily altered MSN responses to salient stimuli such as foot shock and cocaine conditioned place preference (CPP). In fact, decreasing ΔFOSB in D1 MSNs attenuated foot shock-induced calcium transients, while decreasing ΔFOSB in D2 MSNs enhanced them. Similarly, in a cell-specific manner, decreasing ΔFOSB in D1 MSNs and increasing ΔFOSB in D2 MSNs decreases social interaction. In addition, decreasing ΔFOSB in D1 MSNs (but not D2 MSNs) blocks cocaine CPP and attenuates neuronal activity aligned with entrance to the cocaine-paired side. These findings of opposite in vivo modulation of D1 vs. D2 MSN activity by ΔFOSB demonstrate how ΔFOSB influences circuit activity and shed light on cell-autonomous mechanisms controlling behavioral responses.

Disclosures: **T. Markovic:** None. **A. Godino:** None. **L. Holt:** None. **A. Minier-Toribio:** None. **T.M. Gyles:** None. **E. Parise:** None. **F. Martinez-Rivera:** None. **E.J. Nestler:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.07/OO13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA NRSA F31 DA054781-01
Foundation of Hope Grant
NIH T32 NS007431

Title: Allopregnanolone regulation of spontaneous dopamine transient frequency and amplitude in freely-moving male and female rats

Authors: *M. H. MCFARLAND[1], A. MORROW[1], D. L. ROBINSON[2];
[1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Bowles Ctr. for Alcohol Studies, Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Neurosteroids are compounds that are synthesized de novo in the brain and influence neuronal activity. Allopregnanolone (ALLO), a neurosteroid that is a potent, positive allosteric modulator of gamma-aminobutyric acid type A (GABA-A) receptors has emerged as a drug with considerable potential in the treatment of mental and affective disorders, including substance use disorders and premenstrual dysphoric disorder. Moreover, ALLO is considered to have a better safety profile than other drugs that target GABA-A receptors, such as benzodiazepines. Previous work in our lab has shown that ALLO dose-dependently reduces electrically-evoked dopamine release in the nucleus accumbens (NAc) in anesthetized male and female rats, with female rats being less sensitive to ALLO than males during the proestrus stage of the cycle when progesterone levels are high. However, it is possible that the dopamine measurements were impacted by anesthesia effects on GABAergic neurotransmission in addition to ALLO. Thus, the present study tested the hypothesis that systemic administration of ALLO in awake rats will dose-dependently decrease the amplitude of spontaneous dopamine transients, while concurrently increasing their frequency. To test this hypothesis, we used in vivo fast scan cyclic voltammetry. We measured spontaneous, phasic dopamine transients in the NAc of freely-moving male and female rats before and after systemic administration of 0.0 (vehicle), 7.5, and 15mg/kg ALLO. We systematically presented unexpected, novel stimuli to increase the probability of dopamine release. We found that high dose ALLO reduces the frequency and amplitude of dopamine transients in female rats more than in male rats. In females, vehicle increased transient frequency in 3/5 rats, 7.5mg/kg ALLO reduced transient frequency in 3/5 rats, and 15mg/kg ALLO reduced transients in 6/6 rats. In males, vehicle increased transient frequency in 5/7 rats, 7.5mg/kg ALLO reduced the frequency of transients in 4/7 rats, and 15mg/kg reduced the frequency of transients in 4/6 rats. As release of dopamine transients in the NAc is often associated with motivational events, a lack of significant ALLO effect in males suggests that motivation and reward processes remain intact at these doses. The specific effect of 15mg/kg ALLO in females suggests a sensitivity in females that must be clarified with further experiments. The results from this study clarify the regulation of dopamine neurotransmission by ALLO, which has clinical implications for the use of ALLO as an alternative therapeutic to benzodiazepines to treat various psychiatric disorders.

Disclosures: M.H. McFarland: None. **A. Morrow:** None. **D.L. Robinson:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.08/OO14

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Grant-in-Aid for Scientific Research

Title: Effects of multistory enriched environment on physical activity and brain monoamine

Authors: *S. YANAGITA[1], N. KUBOTA[2];
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Abstract: Numerous studies have shown that enriched environments (EE) could be effective for experimental rodents to improve some brain functions related to stress response and anxiolytic effect, and speculating that playfulness in EE might influence it. On the other hand, it is well known that increasing levels of physical activity could have beneficial effects as well as EE. Taken together with this evidence, the question arises: Which is effective for the improvement of brain function between playing or physical activity? To answer the question, we made a multistory enriched environment (Multi-EE) that can increase physical activity in rats. In this study, we examined whether the amount of daily physical activity increases when the enriched environment is multileveled. We also aimed to examine whether the biological effects of this increase in physical activity were different from wheel running. We originally made Multi-EE, which are consisted of three stories. The male Wistar rats housed the Multi-EE or normal- EE for 4weeks in group housing conditions (2 rats per cage). The rats housed in Multi-EE allow access to the three stories freely by ladders. Daily physical activity was recorded using a mobile accelerometer and compared Multi-EE and Normal-EE. Following 4weeks, brain monoamine levels, which are involved with increasing physical activity-induced- psychological effects, were measured by High-Performance Liquid Chromatography (HPLC) in several brain regions. Muscle and fat volume were also measured. In this study, we have been successful to analyze voluntary physical activity in both normal-EE and Multi-EE. The Multi-EE significantly changed physical activity compared to normal-EE. The voluntary physical activity in Multi-EE significantly increased the volume of soleus muscle compared to normal-EE, indicating that Multi-EE might be effective to increase the physical load. However, the Multi-EE housing was not able to change the brain monoamine levels, which are known to be increased by running wheel. The changing levels of these monoamines are known to have some beneficial effects on brain health. Therefore, the results of the present study suggest that increasing levels of physical activity by Multi-EE have a different physiological effect compared to running wheel.

Disclosures: S. Yanagita: None. **N. Kubota:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.09/OO15

Topic: F.07. Biological Rhythms and Sleep

Support: AMED 22gm1510007h0001

Title: Dopamine receptor type 2-expressing medium spiny neurons in the ventral lateral striatum have a non-REM sleep-induce function

Authors: *T. KATO[1,3], K. F. TANAKA[2], A. NATSUBORI[4];
[1]Keio Univ. Sch. of Med., Tokyo, Japan; [2]Keio Univ. Sch. of Med., Shinjuku, Japan; [3]Fac. of Sci. and Technol., Keio Univ., Yokohama, Japan; [4]Tokyo Metropolitan Inst. of Med. Sci., Setagaya-Ku, Tokyo, Japan

Abstract: Dopamine receptor type 2-expressing medium spiny neurons (D2-MSNs) in the medial part of the ventral striatum (VS) induce non-REM (NREM) sleep from the wake state in animals. However, it is unclear whether D2-MSNs in the lateral part of the VS (VLS), which is anatomically and functionally different from the medial part of the VS, contribute to sleep-wake regulation. This study aims to clarify whether and how D2-MSNs in the VLS are involved in sleep-wake regulation. Our study found that specifically removing D2-MSNs in the VLS led to an increase in wakefulness time in mice during the dark phase using a diphtheria toxin-mediated cell ablation/dysfunction technique. D2-MSN ablation throughout the VS increased dark phase wakefulness time. These findings suggest that VLS D2-MSNs may induce sleep during the dark phase with the medial part of the VS. Next, our fiber photometric recordings revealed that the population intracellular calcium (Ca^{2+}) signal in the VLS D2-MSNs increased during the transition from wake to NREM sleep. The mean Ca^{2+} signal level of VLS D2-MSNs was higher during NREM and REM sleep than during the wake state, supporting their sleep-inducing role. Finally, optogenetic activation of the VLS D2-MSNs during the wake state always induced NREM sleep, demonstrating the causality of VLS D2-MSNs activity with sleep-induction. Additionally, activation of the VLS D1-MSNs, counterparts of D2-MSNs, always induced wake from NREM sleep, indicating a wake-promoting role. In conclusion, VLS D2-MSNs could have an NREM sleep-inducing function in coordination with those in the medial VS.

Disclosures: T. Kato: None. **K.F. Tanaka:** None. **A. Natsubori:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.10/OO16

Topic: F.03. Stress and the Brain

Support: Korean Government Research Fund, Grant No. NRF-2022R1I1A3063177

Title: Cortisol-induced dysregulation of prenatal neuroendocrine impairs neurodevelopment of prefrontal cortex via downregulating dopaminergic and PKA-mediated signaling cascades in rats

Authors: *H.-J. KIM[1], K. AMARSANAA[1], E.-A. KO[1], O.-B. KWON[2], S.-C. JUNG[1];
[1]Jeju Natl. Univ., Jeju National University, 102 Jejudaehak, Korea, Republic of; [2]New Drug Develop. Ctr., Kmedihub, Daegu, 41061, Korea, Republic of

Abstract: We previously reported that rat pups (Corti.Pups) born from rat mothers that were repetitively injected with corticosterone (s.c., 20 mg/kg/day, 21 days) during pregnancy, exhibited ADHD-like behaviors and delayed cognitive functions. In this study, we investigated the cellular mechanisms underlying ADHD-like phenotypes observed in Corti.Pups, targeting the neurodevelopmental impairments of their prefrontal cortex (PFC). In results, we confirmed that using the enzyme-linked immunosorbent assay, both BDNF and cAMP levels were significantly reduced in the PFC of Corti.Pups, compared to that of the control group (Nor.Pups). mTOR and PKA, which are dominant factors for neurodevelopmental signaling, were also less expressed in Corti.Pups. This signaling downregulation clearly affected the neuronal development, showing lower expression of PSD-95 in PFC of Corti.Pups. Furthermore, in electrophysiological studies, cortical neurons of Corti.Pups exhibited higher excitability of plasma membrane in supra- and subthreshold ranges. This BDNF-mediated downregulation of neurodevelopmental signaling observed in Corti.Pups, seemed to be attributed to the dopaminergic dysregulation in the PFC, because these pups showed the lower level of dopamine and higher expression of dopamine D1 receptor. Our results clearly provide evidence that prenatal exposure to high cortisol disrupts the neurodevelopment of PFC neurons via downregulating dopaminergic and PKA-mediated signaling cascades, possibly triggering the pathogenesis of neuropsychiatric disorders such as ADHD.

Disclosures: H. Kim: None. **K. Amarsanaa:** None. **E. Ko:** None. **O. Kwon:** None. **S. Jung:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.11/OO17

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA035432

Title: Sex-dependent effects of chronic stress on future context-induced relapse to palatable food seeking after punishment and involvement of dopamine D_1-like receptors

Authors: *K. T. BALL, A. CHIKHALENKO, D. DITILLO, A. LEHMAN;
Commonwealth University, Bloomsburg Campus, Bloomsburg, PA

Abstract: The long-term success of dietary treatments for overweight/obesity are low because most individuals relapse to unhealthy eating habits within months of starting treatment. Although chronic stress has long been associated with relapse vulnerability in the clinical literature, relatively few pre-clinical studies have used models of relapse that incorporate a chronic stressor. Using classical animal models of relapse that incorporate forced abstinence procedures, we have shown that a history of chronic stress impacts future relapse vulnerability in a sex-dependent manner. Moreover, SCH-23390, a dopamine D1-like receptor antagonist, combined with daily stress prevented chronic stress effects in males, but not in females. In the present study we tested the effect of chronic restraint stress on future context-induced relapse to palatable food seeking after voluntary (punishment-induced) abstinence. Thus, male and female rats were trained to self-administer highly palatable food pellets in Context A and were then exposed to punishment training for 8 days in Context B. During punishment, 50% of food-reinforced lever-presses produced an aversive footshock of increasing intensity (0 - 0.7 mA). Following each punishment session, stress was manipulated (0 or 2 hr restraint/day X 8 days). To assess dopaminergic involvement, we administered either SCH-23390 (10.0 μg/kg; i.p.), a dopamine D_1-like receptor antagonist, or vehicle prior to daily treatments. Following 6 days of home-cage abstinence, rats were tested for relapse to food seeking in the absence of food or shock in Contexts A and B. Results showed that, for females, a history of chronic stress increased relapse in Contexts A *and* B, whereas in males, a history of chronic stress *decreased* relapse in Context A only. SCH-23390 combined with stress attenuated those effects in both males and females. These results establish that 1) chronic stress has lasting effects on context-induced relapse after punishment-induced abstinence, 2) those effects are dependent on biological sex, and 3) they are mediated by activation of dopamine D1-like receptors in both males and females. Such findings should inform the development of sex-specific interventions for dietary relapse and other stress-related health problems.

Disclosures: K.T. Ball: None. **A. Chikhalenko:** None. **D. Ditillo:** None. **A. Lehman:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.12/OO18

Topic: H.05. Working Memory

Support: COFAA-IPN Fellowships
BEIFI-IPN Program

Title: Gabaergic stimulation of thalamic reticular nucleus reverts the effect of dopaminergic lesion in globus pallidus on short-memory in rats

Authors: F. RIVERA-SORIANO[1], C. EVANGELISTA-ARZATE[1], M. MARTINEZ-RUIZ[1], K. MERAZ-JUAREZ[1], H. GUTIÉRREZ-GUERRERO[1], M. GARCIA-RAMIREZ[2], *E. CHUC-MEZA[2];

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Abstract: We have reported that anxiety and cognitive dysfunctions can be elicited by dopaminergic lesion in the Globus Pallidus (GP) of the rat. Reticular Thalamic Nucleus (TRn) is innervated by GP, a GABAergic nucleus, so is possible that cognitive disfunction, could be linked to hypofunction of GP after dopaminergic lesion. The purpose of this research is to analyze the influence of GABAergic agents in the thalamic reticular nucleus on short memory in dopaminergic lesioned rats in GP. All animal procedures were performed in accordance with national and international guidelines for care and use of laboratory animals (NOM-062-ZOO-199 & NIH Guide). Cognitive dysfunction was evaluated by means of the new object recognition memory test (NOR) in adult male Wistar rats (n=8/group, 5 groups) in normal and 6-OHDA lesioned in GP. GABAergic agents muscimol, or bicuculline, were intracerebrally injected in TRn and as well as a combination. It was found that, in rats with 6-OHDA lesion, there was a decrease in the novel object recognition index (NORI) without affecting the motor performance of the rats in open field. When muscimol (0.539 µM) was administered in TRn, the NORI was increased. By contrary, bicuculline (0.216 µM), decreased it. In the case of the combination of drugs no effect was observed. In any case locomotor activity in open field was affected. So, is possible that effects over memory produce by dopamine denervation in GP is due to decreased GABAergic activity on TRn.

Disclosures: F. Rivera-Soriano: None. **C. Evangelista-Arzate:** None. **M. Martinez-Ruiz:** None. **K. Meraz-Juarez:** None. **H. Gutiérrez-Guerrero:** None. **M. Garcia-Ramirez:** None. **E. Chuc-Meza:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.13/OO19

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant R35GM142490
NIH Grant R00NS101065
Whitehall Foundation
BrightFocus Foundation

Title: Serotonin regulates protein hunger-dependent dopaminergic axonal activity in Drosophila

Authors: *E. PAUL, M. TABUCHI;
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Abstract: Serotonin and dopamine are both neurotransmitters involved in the modulation of synaptic plasticity. Serotonin can affect the release of dopamine, resulting in the modulation of dopaminergic synaptic strength. In addition, serotonin can affect the expression and availability

of dopamine receptors, thereby influencing the responsiveness of dopaminergic signaling. Recently, four dopamine neurons, termed DA-WED, were identified as the first neural circuit encoding protein-specific hunger in Drosophila. In addition to promoting protein feeding, DA-WED neurons also suppress sugar intake. Two distinct axonal branches of DA-WED have two distinct functions: The "protein branch," which promotes protein feeding, and the "sugar branch," which suppresses sugar intake. These two branches of DA-WED neurons are modulated by synaptic plasticity, depending on the nutritional balance between sugar and protein. Here, we found that DA-WED neurons and neurons downstream of DA-WED neurons receive serotonergic modulation and that this serotonergic modulation affects nonspiking membrane potential dynamics underlying branch-specific plasticity of DA-WED neurons. These results suggest a context-dependent serotonin-dopamine interplay in modulating synaptic plasticity to regulate the internal state of the protein hunger drive.

Disclosures: E. Paul: None. **M. Tabuchi:** None.

Poster

PSTR227. Dopamine

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR227.14/OO20

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Oral L-dopa disrupts behavioral self-control in male Siamese Fighting Fish (Betta splendens)

Authors: *A. J. VELKEY, II[1], K. WATSON[1], P. HARRIS[1], E. HOFFMAN[2], J. MARTIN[1]; [1]Neurosci. Program, [2]Mol. Biol. and Chem., Christopher Newport Univ., Newport News, VA

Abstract: In their natural habitat, *B.splendens* demonstrate sex differences in foraging; males are territorial and feed primarily as ambush predators using a “sit-and-wait” strategy while females are non-territorial and feed primarily as opportunistic foragers. This ecology suggests that males, but not females, may be capable of delaying gratification, at least for food. Previous research has examined impulsive choice in a variety of species; no previous research has explored preference between Larger-Later (LL) and Smaller-Sooner (SS) rewards in *B.splendens*. The current study consists of two experiments. Experiment I addressed sex differences in instrumental choice behavior in male and female *B.splendens*. Using a submerged T-maze, thrice-daily instrumental-choice trials were conducted in which subjects were presented with a choice between a SS reward (1 food pellet delivered immediately) and a LL reward (3 pellets delivered after a 15-s delay). 70 percent of males displayed a stable preference for the LL reward option over the SS option, whereas females were just as likely to stabilize on the SS option as the LL option (48% vs 52%, respectively). These results indicate that a majority of the males in Exp. I displayed spontaneous behavioral self control for food reward without any specialized training, while females were collectively indifferent. Reward valuation is determined, in part, through mesolimbic dopaminergic activity, and previous research has demonstrated that orally-

administered L-dopa increases impulsivity in humans; again, no such research exists regarding *B.splendens*. Experiment II investigated the potential for L-dopa to disrupt self-control in male *B.splendens*. The same instrumental-choice procedures from Exp.I were used for Exp. II, but with only male subjects. Subjects in the treatment group received oral L-dopa (60mg/kg) in a customized "fish pill" 30-minutes prior to each trial, while subjects in the control group received an inert "fish pill". Male subjects in the control group were as likely to stabilize on the SS option as they were to stabilize on the LL option (48% vs 52%, respectively). Furthermore, only 30% of subjects receiving oral L-dopa demonstrated stable preference for the LL reward, with 70% of male subjects in the treatment group demonstrating stable preference for the SS. Administration of L-Dopa prior to instrumental choice between a LL reward and a SS reward increased the likelihood of impulsivity in males. Future researchers could examine dopamine agonists or antagonists (rather than a dopamine precursor) in males and/or females to further explore the role of dopamine in immediate/delayed reward valuation in *B.splendens*.

Disclosures: A.J. Velkey: None. **K. Watson:** None. **P. Harris:** None. **E. Hoffman:** None. **J. Martin:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.01/OO21

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R34NS122050

Title: Conserved hierarchies of single-unit timescales in frontal and limbic regions in mice, macaques, and humans

Authors: Z. R. ZEISLER[1], J. MINXHA[2], U. RUTISHAUSER[3], F. M. STOLL[4], *P. RUDEBECK[5];
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Abstract: A neuron's intrinsic timescale is thought to reflect the amount of time over which it integrates information, estimated based on the autocorrelation of spike times. These single neuron timescales are hierarchically organized across the cortical surface, such that neurons in higher-order association cortex like the anterior cingulate cortex have longer timescales compared to sensory cortex. These conclusions, however, are primarily based on analyses of spiking and fMRI activity recorded from the macaque brain. Thus, it remains unclear whether hierarchical variation of single neuron timescales is a general principle of brain organization that is present in species from rodents to humans.
Here we took a cross-species approach and applied a task-agnostic inter-spike interval-based

timescale estimation approach to neuronal activity recorded from cortical and subcortical structures in mice (n = 5,990 neurons), macaque monkeys (n = 15,124), and humans (n = 3,595). We identified largely consistent hierarchies of timescales in frontal and limbic regions across species: hippocampus and amygdala had the shortest timescale whereas anterior cingulate cortex had the longest.
Despite these similarities, there was variability across species most notably in amygdala and orbitofrontal cortex - potentially because these areas show the greatest anatomical variation between species. Because one key difference between orbitofrontal cortex in humans, macaques and mice is the presence of an internal granule cell layer, we next set out to determine whether this feature of cortex shaped intrinsic timescales. We focused our analysis on a dataset of single neurons that extensively surveyed the macaque ventral frontal cortex and where granular, dysgranular and agranular cortex are all present. We found no clear relationship between cortical granularity and intrinsic timescale. Instead, we observed strong variation within ventral frontal cortex along the anterior-posterior and medial-lateral axes. Thus, hierarchically organized timescales are a consistent feature across species, and variation in single neuron timescales are not directly shaped by cytoarchitecture. Instead, single neuron timescales appear to be shaped by a combination of intrinsic and extrinsic factors.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR228.02/OO22

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01DA036534
R01AG060778
K99DA041493
5T32AG061892-04

Title: Investigating the functional role of ventral tegmental area dopamine neurons in decision making under risk of punishment

Authors: *W. S. PYON[1], S. L. BLAES[2], C. ORSINI[5], M. FARAJI[1], O. VIERA[1], L. CAO[1], S. M. BETZHOLD[1], S. ATHAVALE[1], B. BERRIOS[1], J. BARRETT[1], S. SINGHAL[1], C. J. FRAZIER[3], J. L. BIZON[6], B. SETLOW[4];
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Abstract: A prevailing theory regarding the role of dopamine in reward-motivated behaviors is that dopaminergic neurons of the ventral tegmental area (VTA) signal discrepancies between predicted and actual outcomes of a particular event. This theory of reward prediction error signaling further implies that VTA dopamine neurons also play a role in cost-benefit decision-making in which outcomes can include reward alongside the potential for punishment. To elucidate the functional role of VTA dopamine neurons in decision making under risk of punishment, male and female tyrosine hydroxylase (TH)-Cre transgenic rats were injected with Cre-dependent GCaMP and trained on a risky decision making task in which they made discrete choices between a small, "safe" food reward and a large food reward accompanied by ascending risk of mild footshock (0%, 25%, 75%). *In vivo* fiber photometric recording of VTA dopamine neurons revealed an increase in neuron activity during receipt of the large reward in the absence of probabilistic punishment, and this increase was greater in blocks of trials in which there was some chance of punishment (25% and 75%) vs. no chance of punishment (0%). In contrast, VTA dopamine neuron activity was suppressed during receipt of the large reward when it was accompanied by punishment. To confirm that this change in VTA dopamine neuron activity was causal to risky decision making, Cre-dependent halorhodopsin was expressed in dopamine neurons of TH-Cre rats. After stable behavior was established, these neurons were then selectively inhibited during delivery of the large reward. Relative to baseline sessions, inhibition of VTA dopamine neurons during receipt of the large reward when it was unpunished reduced preference for the large reward. In contrast, inhibition of VTA dopamine neurons during receipt of the large reward when it was punished had no significant effect on behavior. These findings support a causal link between VTA dopamine neuron activity and decision making under risk of punishment through signaling errors in outcome predictions. These results also provide evidence for a "negative prediction error floor", whereby further inhibition of VTA dopamine neurons during an already suppressive event does not result in further shifts in choice preference.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.03/OO23

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Future Leaders in Canadian Brain Research
BBRF Young Investigator Award
FRQS Salary Award (#324009)
NSERC Discovery Grant (RGPIN-2021-03284)
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Title: Dopamine release encodes contrasting reinforcement signals across the dopaminergic system

Authors: S.-J. BOUCHARD[1], J. BOUTIN[1], C. DESBIENS[1], L.-M. GAUTHIER[4], Y. ZHUO[5], Y. LI[5], M. LEVESQUE[2], ***V. BRETON-PROVENCHER**[3];
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Abstract: The dopaminergic system facilitates associative learning and motivated behaviors by signaling reward expectation and reinforcement valence - appetitive versus aversive - within the brain. According to previous studies, this reinforcement signaling is heterogeneously represented by dopaminergic neurons, suggesting a distributional coding of reinforcement within the dopaminergic system. However, it remains unclear how the dopamine signals for expectation and valence interact together within this code in various brain regions. Moreover, the extent by which fluctuations in dopamine levels track reinforcement signals, previously measured with somatic and axonal activity, is poorly understood, especially due to increasing evidence for target-specific mechanisms that control dopamine release. Here, we used an improved fluorescent dopamine sensor to record dopamine signals associated with reward predictions and stimulus valence in multiple pathways of the dopaminergic system in mice. Using this approach, we compared dopaminergic signals across various locations of the mesolimbic pathway (including subregions of the nucleus accumbens, olfactory tubercle, amygdala), the nigrostriatal pathway (dorsal and tail striatum), and mesocortical pathway (medial prefrontal cortex). Our preliminary findings indicate that the delivery of a rewarding stimulus triggered dopamine release in all the output regions. Importantly, the encoding of reward expectation is heterogeneously distributed across the various targets. Specifically, the relationship between reward prediction error and dopamine release was the strongest in various regions of the mesolimbic pathway, while it was the weakest in the mesocortical pathway. Additionally, when measuring the change in dopamine levels following an aversive stimulus, we observed that dopamine release peaked in regions where the encoding of reward expectation by dopamine was the lowest, except for dopamine signals in the basolateral amygdala. Together, our findings provide evidence of contrasting reinforcement signals across the primary targets of dopaminergic pathways through which dopamine release supports learning.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.04/OO24

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NS094754
DA040701
NS121253

Title: Direct and indirect pathways precisely modulate action parameters in licking behavior

Authors: *K. BAKHURIN, M. ROSCHINA, G. R. LEE, H. YIN;
Psychology & Neurosci., Duke Univ., Durham, NC

Abstract: The striatum has two output pathways: the striatonigral (direct) and the striatopallidal (indirect). Recent work has shown that activity in these pathways may regulate movement kinematics, but the underlying mechanisms remain unclear. In this study, we used high speed video combined with electrophysiology and optogenetics to study the roles of direct and indirect pathways to the continuous regulation of goal-directed licking behavior in mice. D1-Cre+ and A2A-Cre+ mice expressing channelrhodopsin in the orofacial striatum (VLS) were head restrained and trained to receive rewards on a fixed-time schedule, receiving sucrose reward from a lick spout every 10 s regardless of their movements. With training, after consuming a reward, mice pause for a few seconds before generating robust anticipatory licking prior to receiving the next reward. By varying the angle of the spout relative to the head, we found that mice generate anticipatory licking in the direction of the spout, indicating that they maintain an internal heading in the direction of expected reward. In trained mice, unilateral VLS direct pathway activation during the pause in licking generates licking in the direction of expected reward. When activating the direct pathway during anticipatory licking, we found that licking is redirected contraversively away from the spout. The degree of deviation increased gradually over the duration of the laser stimulation epoch. Moreover, unilateral indirect pathway stimulation resulted in incremental licking redirections in the ipsiversive direction. The rate of redirection during stimulation of both pathways was dependent on laser frequency. Together these findings show that the activity of the direct pathway and indirect pathways can exert a continuous push-pull influence on the intended direction of currently active goal-directed movements.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: T32-MH018399
NIMH R01MH123650

Title: On-demand electrical brain stimulation to modulate beta oscillations during reward processing

Authors: *M. J. FRANCOEUR[1,2], M. SALIMI[1,2], N. LEE[1,2], A. TERRY[1,2], D. RAMANATHAN[1,2];
[1]Psychiatry, Univ. of California San Diego, San Diego, CA; [2]Mental Hlth., VA San Diego Med. Ctr., San Diego, CA

Abstract: Reward processing comprises a set of systems related to motivation, value assignment and action-outcome associations. Deficits in reward processing are linked with decision-making impairments and likely contribute to anhedonia, amotivation, and substance abuse problems observed in various psychiatric conditions. Although several cortical and striatal regions are implicated in reward processing, less is known about how these separate regions participate in extensive functional networks. Therapies, such as neuromodulation, that aim to change functional networks would be most successful if applied in a temporally and spatially precise manner to selectively bring brain networks of interest "online". Thus, preclinical models are essential for identifying brain-based markers operating in reward processing networks. Here, we use multi-site local field potential (LFP) recordings to identify electrophysiology markers and test "on-demand" electrical stimulation modulating reward-related brain signals in a behaviorally precise manner to influence decision-making.16 Long-Evans rats (n=10 male; 6 female) were used for this study. First, LFPs were recorded from 32-brain regions simultaneously as rats performed a temporal discounting task (n=10 rats; 124 sessions). Subjects chose between a low reward (1µL) delivered immediately, or a high reward (3µL) delivered at a variable delay. Between sessions, high reward delays ranged from 500ms to 20s. As animals' preference shifted from high to low reward choices at longer delays ($F_{(5,45)}$ =30.9, p =*<0.001,* delay x choice), beta-frequency oscillations also reflected this shift in value assignment. In cortico-striatal electrodes (prefrontal cortex, orbitofrontal cortex, insula, ventromedial striatum, nucleus accumbens, and amygdala), beta power scaled with the value of high-reward choice based on delay length ($F_{(11,2553.80)}$=27.6, *p<0.001,* delay x choice). We predicted beta oscillations may represent an electrophysiological marker of reward value that could be applied to an "on-demand" neuromodulation approach to influence decision-making. To test this hypothesis, we applied 1s of electrical stimulation (80uA; 20Hz) to ventromedial striatum time-locked to high reward outcomes (n=6 rats). Stimulation significantly increased the number of high reward choices compared to pre-stimulation sessions ($t_{(5)}$= 3.89, *p=0.004*). Thus, beta oscillations in the cortico-striatal network may reflect reward value and represent a physiological signature that can be modulated "on-demand" to shape value-based decision-making.

Disclosures: M.J. Francoeur: None. **M. Salimi:** None. **N. Lee:** None. **A. Terry:** None. **D. Ramanathan:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: MEXT/JSPS KAKENHI JP19K16299 (to T.Y)
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JST CREST JPMJCR1751 (to Y.I)
AMED Brain/MINDS JP19dm0207089 (to Y.I)

Title: Parallel implementation of working and reference memory-based reward prediction in a dual dopamine pathway to the striatum

Authors: *T. YOSHIZAWA[1,2,3], Y. MIYAMURA[2], Y. OCHI[4], R. HIRA[2], M. FUNAHASHI[1], Y. SAKAI[3], Y. CUI[4], Y. ISOMURA[2,3];
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Abstract: Humans and animals can learn new behaviors in unfamiliar environments by exploring and memorizing sensory cues or actions that lead to good or bad outcomes. Such learning would require some form of working memory (WM) and reference memory (RM). Learning by trial-and-error, which can yield positive or negative consequences, is known as reinforcement learning using RM on past rewards, whereas in the WM-based reward prediction, forthcoming rewards can be directly predicted from latest trials. For instance, if you win in rock-paper-scissors game, you will tend to choose the same option in the next game, and if lose, switch to others. Such WM-based behaviors are referred to as “Win-Stay-Lose-Switch (WSLS)”. We recently reported that spike activity of dorsal striatal neurons, which receive dopaminergic inputs from the midbrain, encoded previous actions, rewards and their interactions when rats behaved according to the WSLS strategy in a choice task (Yoshizawa, Ito and Doya, *eNeuro*, 2023). In the present study, we investigated how the midbrain dopamine (DA) system contributes to WM-based and RM-based (WM-free) reward prediction. We compared rat's neural activity during two different tasks: one in which an operant behavior was alternately rewarded (WM-based task) and another in which the same operant behavior was randomly rewarded with a 50% probability (RM-based task). The ^{18}F-FDG-PET imaging revealed that the ventral tegmental area (VTA) was more strongly activated by the WM-based task than by the RM-based task. We recorded neuronal responses to alternate rewards from head-fixed rats performing a similar task to the PET experiment by using an electrophysiological technique. The lateral VTA neurons tended to show weaker responses to alternate rewards in the WM-based task than the medial VTA. The lateral and medial VTA neurons mainly project to the dorsomedial striatum (DMS) and the nucleus accumbens (NAc), respectively, thus we measured DA dynamics in these areas from head-fixed mice performing a similar task to rat's experiments by using fiber photometry recording of dLight1.1. In the DMS, phasic DA release to alternate rewards was weaker than to random rewards, whereas there was no difference in the NAc, indicating that DA dynamics in the DMS more strongly reflect reward prediction error based on the alternation nature than in the NAc. Contrary to the reinforcement learning theory, the reward, rather than no reward, caused a “dip” of DA release in the DMS once the reward alternation was learned well. These results

suggest that dual DA pathway in the striatum processes WM-based and RM-based reward prediction in parallel.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.07/PP1

Topic: G.02. Reward and Appetitive Learning and Memory

Support: HHMI Hannah Gray Fellowship

Title: Striatal Dopamine-Acetylcholine Interactions During Pavlovian Cue-Reward Learning

Authors: *A. GAMAM, H. WEHELIE, *A. HAMID;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Reward-evoked striatal dopamine release exhibits a wave-like pattern with spatiotemporally heterogeneous trajectories regulated by task demands. This finding is hard to reconcile with the prevailing theories that assume dopamine is broadcast to all recipient brain regions to drive reinforcement learning. To help revise how the spatial and temporal patterns of dopamine release affect reward-credit learning in the dorsal striatum, we measured dopamine responses together with Acetylcholine levels during Pavlovian cue-reward learning. We will present results demonstrating that striatal DA and ACh exhibit dynamic changes in cue-induced and reward-induced responses in a region-specific manner and the impact of pharmacological interventions on DA-ACh dynamics.

Disclosures: **A. Gamam:** None. **H. Wehelie:** None. **A. Hamid:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR228.08/PP2

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH T32DK128782 (MKL)
R01DA025634 (MFR)

Title: Conditioned taste aversion suppresses phasic dopamine responses to sucrose in the nucleus accumbens in correlation with behavioral reactivity

Authors: *M. LOH[1], P. BAZZINO[2], R. DONKA[4], S. HURH[3], M. F. ROITMAN[2]; [1]Medicine/Psychology, [2]Grad. Program in Neurosci., [3]Psychology, Univ. of Illinois at Chicago, Chicago, IL; [4]Psychology, Univ. of Illinios, Chicago, Chicago, IL

Abstract: To minimize harm, ingested stimuli resulting in illness must be remembered and subsequently avoided. Phasic dopamine release in the nucleus accumbens (NAc) is crucial in the encoding of food reward and is involved in associating tastes with positive post-ingestive outcomes. How dopamine release encodes tastes associated with negative post-ingestive outcomes remains unclear. We expressed the fluorescent dopamine sensor GRABDA2h in different subterritories of the NAc shell and conducted real-time dopamine release using in vivo fiber photometry. In naïve rats, we delivered brief (5s; 200µl) intra-oral infusions (30 trials/session; 35-55s variable inter-trial interval) of sucrose, during which dopamine dynamics and behavior were captured. Immediately following sucrose infusions, half of the cohort was injected (i.p,) with malaise-inducing lithium chloride (Paired) or equimolar sodium chloride (Unpaired). The subsequent day, rats received the counterbalanced injection in their homecage and were untreated the following day. This 3-day conditioning process was repeated two more times, followed by eight consecutive daily sucrose infusion (extinction) sessions without further injections. In initially naïve Paired rats, dopamine release in the lateral NAc shell increased to sucrose. However, following pairing with LiCl, dopamine responses were significantly suppressed. No significant changes were observed Unpaired rats. We tracked forepaw and nose movements using DeepLabCut. In initially naïve Paired rats, intra-oral sucrose suppressed movement relative to a 5s pre-infusion period. However, with repeated pairings, behavioral reactivity to intra-oral infusions significantly increased. Dopamine responses were negatively correlated with the magnitude of behavioral reactivity to intra-oral sucrose. The pattern of dopamine signaling in the ventromedial, though, was qualitatively different than the lateral shell. These results implicate plasticity in select dopamine systems in the formation, expression and extinction of a condition taste aversion and pauses in dopamine release in the NAc lateral shell may guide food-rejection behaviors. Research Supported by NIH T32DK128782 (MKL) and R01DA025634 (MFR).

Disclosures: **M. Loh:** None. **P. Bazzino:** None. **R. Donka:** None. **S. Hurh:** None. **M.F. Roitman:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.09/PP3

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Intramural Research Program at the National Eye Institute, 1ZIAEY000415

Title: Predictive Activation of Amygdala Neurons during Sequential Environmental Changes

Authors: *K. MAEDA[1], O. HIKOSAKA[2];
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Abstract: Anticipating future situations is an essential cognitive process that relies on learned sequences, memories, and past experiences. Animals depend on assessing their present and future environments to discern beneficial from detrimental actions. Similarly, humans require a precise understanding and prediction of social environments to exhibit appropriate behaviors. Nevertheless, mental disorders frequently present challenges to this abstract form of anticipatory cognition. Our research extensively delves into the role of the basal ganglia and limbic system in facilitating the selection of rewarding and valuable objects while avoiding harmful and irrelevant ones to gain insights into the brain circuits responsible for regulating behavior in different environmental contexts.Our recent findings indicate that neurons in the amygdala encode the environmental context and influence choice-related neural activity through projections to the basal ganglia. However, it remains unclear whether these neurons only respond to conditioned visual stimuli or encode environmental contexts independently of specific visual cues. The latter is crucial for retaining environmental context information in memory. To investigate this question, we conducted an experiment to examine whether amygdala neurons exhibit responses to environmental contexts prior to visual cues when presented in a sequential manner. In our experimental task, monkeys participated in a Pavlovian conditioning task that involved sequentially presented environmental contexts. Specifically, we utilized four environmental contexts resulting from the combination of two dimensions: High or Low reward and Dangerous or Safe. These contexts were presented in a predetermined order. Following multiple training trials, we observed responsive behaviors from the subjects' neurons that corresponded to the subsequent environmental contexts. Among the 230 neurons recorded, 91 neurons responded specifically to the high-reward and safe environmental contexts. Interestingly, many neurons showed increased responses before the actual presentation of the environments (33 / 91 neurons). This indicates that these neurons encode the environmental contexts in memory and suggest that the monkeys may be able to predict upcoming environments based on the amygdala information. In conclusion, our findings provide evidence that amygdala neurons encode and retain environmental context information, enabling the anticipation of future situations based on repeated sequences. This research contributes to our understanding of how the brain processes and utilizes environmental cues to guide behavior.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.10/PP4

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Glutamatergic Stimulation in the Medial Prefrontal Cortex Modulates Reward-Related Learning

Authors: *R. NISANOV[1], G. SAFIER[2], N. PATEL[2], K. PERSAUD[2], R. RANALDI[2,3]; [1]Psychology, Grad. Sch. City Univ. of New York, NYC, NY; [2]Psychology, Queens Col. - City Univ. of New Neuroscience, Flushing, NY; [3]Psychology, Grad. School, CUNY, Nyc, NY

Abstract: This study aimed to investigate the role of glutamatergic stimulation in the dorsal and ventral regions of the medial prefrontal cortex (mPFC) on the acquisition and expression of reward-related learning using the conditioned approach paradigm in rats. Cannula-implanted rats underwent Pavlovian conditioning sessions, three days for acquisition testing or seven days for expression testing. Each 60-minute conditioning session involved random presentations of a 3-s light stimulus (CS), each followed by the delivery of a food pellet (US). Following a two-day interval, rats underwent a session without either of the CS or US, followed by a CS-only test session the next day. Acquisition experiments involved blocking NMDA receptors with AP5 prior to each of the three conditioning sessions, while expression experiments involved blocking AMPA receptors with CNQX before the CS-only test session. The results of the acquisition experiments demonstrated significantly greater responding to the CS (conditioned approach) in rats with inhibited NMDA receptors in both the dorsal and ventral regions of the mPFC in comparison to control groups. On the other hand, in the expression experiments, inhibiting AMPA receptors in the dorsal region led to a significant impairment of conditioned approach, while in the ventral region, it resulted in a significant enhancement of conditioned approach for the highest dose group, relative to the control group. These findings suggest that NMDA receptor stimulation in the dorsal or ventral mPFC is not necessary for acquisition of conditioned approach but stimulation of AMPA receptors in the dorsal, but not ventral, mPFC is critical for the expression of this learned behavior. To explore the neuronal activation patterns associated with the CS, an in-situ hybridization experiment was conducted on two groups of rats. The results showed significantly higher activation of glutamatergic neurons in response to the CS compared to a non-CS. These findings highlight the crucial role of glutamatergic signaling in the mPFC in mediating reward-related learning processes in rats. The divergent effects of receptor inhibition on acquisition and expression provide valuable insights into the complex mechanisms underlying reward-based behaviors. Further investigation of the precise neuronal circuits involved, and their connectivity patterns may help unravel the intricate interplay between glutamatergic transmission and reward-related learning in the mPFC.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: BRAIN initiative grant R01MH117040
Takeda Science Foundation Overseas Research Fellowship
Brain and Behavior Research Foundation #28979

Title: Contrasting role of dopamine receptor subtypes during learning in macaque monkeys as revealed by resting-state functional MRI

Authors: *A. FUJIMOTO[1], C. ELORETTE[1], S. H. FUJIMOTO[1], L. FLEYSHER[1], B. E. RUSS[1,2,3], P. H. RUDEBECK[1];
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Abstract: Dopamine plays a critical role in learning, with D_1 and D_2 receptors heavily implicated in behaviors related to action generation, decision-making, and motivation. However, the regional specificity and specific role of these receptor subtypes in learning remains unknown. To answer these questions, we conducted parallel fMRI and behavioral experiments in non-human primates. Seven rhesus macaques (Macaca mulatta, ages 3-7 yo, 4 F) underwent a resting-state fMRI with pharmacological manipulation to pinpoint the patterns of functional interaction that are altered by D_1-R and D_2-R antagonism. The animals participated in at least one of the three treatment conditions: D_1-R antagonist (SCH23390), D_2-R antagonist (Haloperidol), and saline I.V. administration. Functional resting-state scans were obtained before and after drug injection, and the normalized difference between them was computed for each session. In separate sessions, four of the imaging animals participated in a behavioral experiment under identical pharmacology challenge. They were trained to perform a probabilistic learning task, in which they were required to choose one of two visual stimuli (90, 50, or 30% reward probability) using eye movements to obtain a juice reward. In the learning task, novel stimuli were used every block, while the same previously learned stimuli were repeated in the control task. Functional connectivity (FC) analysis using dorsal striatum as a seed revealed that fronto-striatal FC decreased after SCH administration, while haloperidol increased FC ($p < 0.001$, uncorrected). Whole-brain connectome analysis using cortical and subcortical atlases showed that SCH administration significantly decreased fronto-temporal as well as temporo-thalamic FCs, while haloperidol robustly increased cortico-cortical FCs. In the behavioral experiment we found that SCH deteriorated correct performance only in the learning task. In contrast, haloperidol increased correct performance in the learning task. Taken together, our result suggests a complementary role of D_1-R and D_2-R-mediated dopaminergic systems to control learning. Specifically, we found the fronto-striatal and thalamo-fronto-temporal networks are mediating contrasting behavioral effects of dopamine receptor types. These findings provided a receptor type specific mechanism for behavioral outcomes that could have implications in a variety of mental health disorders.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

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Program #/Poster #: PSTR228.12/PP6

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH grant MH127377
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BBRF NARSAD Young Investigator grant

Title: Enhanced cognitive flexibility, goal-directed behavior and phasic dopamine signals in Norepinephrine transporter knockout mice

Authors: J. DELANEY[1], S. NATHANI[1], V. TAN[1], C. CHAVEZ[1], A. ORR[1], B. SETLOW[2], ***N. URS**[3];
[1]Pharmacol., [2]Psychiatry, [3]Univ. of Florida, Gainesville, FL

Abstract: Catecholamine neuromodulators dopamine and norepinephrine are implicated in locomotion, motivation, and cognitive behaviors. Although striatal dopamine signaling and circuitry are well established, the specific role of cortical catecholamines in regulating striatal dopamine dynamics and cognitive behavior is not clear. We recently showed that cortical dopamine but not norepinephrine inhibits hyperactivity of DAT-KO mice. Additionally, microdialysis studies have shown that the norepinephrine transporter (NET) knockout mice are a unique model as they have elevated extracellular cortical catecholamines but reduced striatal dopamine levels. We asked the question whether altered catecholamine levels in the NET KO mice affect motivated behavior and striatal dopamine dynamics. We used a probabilistic reversal learning (PRL) task, a devaluation variable interval reinforcement (VI30/60) task, progressive ratio task, nestlet shredding and a Y-maze task to test effects of NET KO on reinforcement learning and goal directed behavior. The NET KO mice show enhanced reversals per session in the PRL task, were sensitive to devaluation in the VI task compared to WT littermates, and had a shorter latency to shred nestlets compared to controls but no chnage in the progressive ratio task. Lesion of cortical norepinephrine did not change reversal learning but reduced average nosepokes per session and increased number of omissions, suggesting that cortical norepinephrine might predominantly regulate impulsivity, whereas cortical dopamine might regulate reinforcement learning via regulation of striatal dopamine. dLight 1.3b based measurements in the dorsomedial striatum showed that NET KO mice had lower baseline dopamine activity but significantly larger cue-dependent or reward-dependent phasic dopamine spikes. These observations suggest that cortical dopamine tone might regulate striatal dopamine tonic and phasic dopamine release. Our studies show a novel mechanism that alters motivated behavior and phasic dopamine signals, which could lead to a better understanding of dopamine neurotransmission and help develop novel therapeutic strategies to counter multiple CNS disorders.

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Poster

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Program #/Poster #: PSTR228.13/PP7

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Swedish Research Council
LUA/ALF
Swedish brain foundation

Title: Unraveling the role of paraventricular thalamic glucagon-like peptide-1 receptors on alcohol-related behaviours in rodents

Authors: *C. ARANÄS, C. E. EDVARDSSON, S. WITLEY, E. JERLHAG;
Pharmacol., Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Alcohol use disorder (AUD) is a complex and severe neuropsychiatric disorder with high mortality and morbidity rates. Insight into the complex mechanisms that underlie alcohol-related responses would contribute toward novel treatment options for AUD. Glucagon-like peptide 1, a gut brain peptide that regulate appetite, has lately been suggested as such candidate. For instance, the GLP-1 receptor agonist, exendin-4(Ex4), prevents alcohol-induced reward, alcohol intake, relapse drinking and the motivation to consume alcohol in rodents. However, the brain circuits that participate in the ability of GLP-1 to regulate alcohol-related responses is unknown. The paraventricular nucleus of thalamus (PVT) might serve as one candidate as it connects to nucleus accumbens, an area well known for its involvement in AUD processes. Additionally, PVT expresses GLP-1 receptors and their activation suppresses food intake. The hypothesis that activation of GLP-1 receptors, by means of local infusion of Ex4 into PVT, attenuates alcohol-related responses was therefore tested in the present study. We showed that Ex4 into PVT attenuated alcohol-induced locomotor stimulation and dopamine release in nucleus accumbens of male mice. We further found that Ex4 into PVT decreased alcohol intake in male and female rats. Interestingly, female rats exhibited a higher dosage requirement than male rats, pointing to a gender-based variation in the GLP-1 response within the PVT. On the contrary, the expression of GLP-1 receptors showed no difference between high- and low-alcohol preferring rats. Taken together, these findings indicate that GLP-1 receptors expressed in the PVT play a role in alcohol-related responses, providing further evidence that targeting GLP-1 receptors could be a promising approach for AUD treatment.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: F30 MH126615
T32 DA050558
T32 GM008620
P50 MH096889

Title: The paraventricular nucleus of the thalamus contributes to early-life adversity-induced disruptions in reward behaviors in a sex-dependent manner

Authors: *C. L. KOOIKER[1], Y. CHEN[2], Q. DING[3], N. THIAGARAJAN[3], M. BIRNIE[2], T. Z. BARAM[1,2];
[1]Anat. and Neurobio., [2]Pediatrics, [3]Sch. of Biol. Sci., Univ. of California, Irvine, Irvine, CA

Abstract: Background: Early-life adversity (ELA) is associated with poor emotional health and increased risk for a variety of affective disorders, including depression and substance use disorders. Many of these disorders are characterized by impairments in reward behaviors, and we find that these same disruptions are provoked by rodent models of ELA. However, the brain regions and processes underlying these long-term consequences of ELA remain largely unknown. The paraventricular nucleus of the thalamus (PVT) is an important component of the reward circuit that encodes remote emotionally salient experiences to influence future motivated behaviors. We hypothesize that the PVT encodes adverse experiences as remote as the early postnatal period in mice, and that ELA-engaged PVT neurons subsequently contribute to disruptions in adult reward behaviors in a sex-dependent manner. **Methods**: We identified and manipulated neurons activated during early-life adversity by using TRAP2 mice. We induced the TRAP2 system using tamoxifen on P6, triggering Cre-dependent recombination in neurons activated during P6-P8, in the middle of an ELA period lasting from P2-P10. This leads to permanent DREADD expression in neurons activated during this epoch. We then chemogenetically inhibited typical-rearing-engaged or ELA-engaged PVT neurons during adult reward tasks. **Results**: ELA robustly and selectively activated PVT neurons to a degree much higher than typical rearing conditions, and a large proportion of these ELA-engaged PVT neurons expressed the receptor for the stress-sensitive peptide CRH (CRFR1). We then tested the role of the ELA-activated neurons in ELA-induced aberrant reward behaviors, which differ by sex (males become less motivated (‘anhedonic’), while females have excessive reward motivations). Upon silencing ELA-engaged PVT neurons during reward-related tasks in adult females, the observed ELA-induced changes in reward behaviors are ameliorated. Upon silencing PVT neurons engaged during typical rearing in adult males, the ELA reward phenotype is recapitulated. **Conclusions**: The PVT is robustly and almost uniquely activated in response to ELA in neonatal mice, and inhibition of these ELA-engaged neurons ameliorates ELA-induced changes in reward behavior, indicating the PVT as an important contributor to the long-term consequences of ELA on behavior. Supported by NIH F30 MH126615, T32 DA050558, T32 GM008620, and P50 MH096889.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.15/PP9

Topic: G.02. Reward and Appetitive Learning and Memory

Title: The role of microRNAs in nicotine-mediated behaviors in C. elegans

Authors: A. L. HOBBLE, M. M. DEVINE, A. J. KALLARACKAL;
Psychology, Mount St. Mary s Univ., Emmitsburg, MD

Abstract: Nicotine use is prevalent in many countries, and affects the lives of millions of people. Within recent decades research has turned to the effect that nicotine has on people and their community. It has been estimated that 40% of children will be exposed to nicotine products in their childhood and that there is a relationship that contributes to them using nicotine products later in their life. There is also a heritability factor of addiction in humans, which ranges from .4-.6. This has led to increased research in animal models to provide insight to the mechanisms and pathologies behind addiction to provide future directions and interventions for work in humans. *C. elegans* can be used in chemotaxis assays and various behavioral experiments to determine the impact of nicotine on the nervous system. Research has included the study of micro-RNAs as modulators of gene expression, possibly giving insight to the heritability of nicotine preference and behaviors. In this research *C. elegans* were tested for transgenerational inheritance of nicotine preference when the F0 generation was exposed to nicotine during the L4 stage of development for 24 hours. After analyzing data, it was found that there was no significant difference in nicotine preference between those exposed during the prenatal development phase and controls. However, follow-up experiments with mir-232, mir-235, mutants demonstrated effects on nicotine preference. This allows insight on how genetic factors can play an integral role in nicotine use.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

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Topic: G.02. Reward and Appetitive Learning and Memory

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South Carolina Clinical & Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina CTSA, NIH/NCATS Grant Number UL1TR001450

Title: Essential role of a novellong-non-coding enhancer RNA (eRNA) in activity-dependent development of maladaptive depressive- and addiction-related behaviors

Authors: *R. AKIKI[1], R. CORNBROOKS[1], K. MAGAMI[1], K. SNYDER[1], A. GREIGE[4], D. WOOD[2], B. HUGHES[4], P. MACE[1], N. KOIKE[5], S. BERTO[1], C. COWAN[1], M. TANIGUCHI[3]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]Med. Univ. of South Carolina, North Charleston, SC; [3]Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC; [4]Med. Univ. of South Carolina Neurosci. Grad. Program, Charleston, SC; [5]Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Background: Exposure to pathological stimuli can promote maladaptive plasticity disrupts typical function of the brain-reward circuitry and development of mental health conditions, including anhedonia and substance use disorder. We showed previously that the activity-regulated gene, *Npas4*, is rapidly and transiently induced by stress or cocaine in brain reward-related regions, and it's required for the development of several depression- and addiction-related behaviors. However, the gemomic mechanisms by which pathological stimuli rapidly activate Npas4 expression remains unknown. **Methods:** We utilized total RNA-Seq, bioinformatic analyses, custom adeno-associated viruses (AAVs) designed to reduce (shRNA) or overexpress a novel *Npas4* enhancer RNA (eRNA), Lentiviral-mediated sgRNA/dCas9-RNAseH1 to digest R-loops, R-loop-specific antibodies for genomic DNA immunoprecipitation, cultured primairy neurons or neural-derived cell lines, quantitative PCR, and C57BL/6J male and female mice. We also examined anhedonia-like behavior following chronic social defeat stress (CSCS) and drug-context associations in the cocaine conditioned place preference (CPP) test.**Results:** We show here that a novel long non-coding enhancer RNA (eRNA) is produced from a conserved enhancer region of *Npas4* (*Npas4*^{eRNA}). Using viral-mediated approaches we show that the eRNA is necessary and sufficient for the activity-dependent expression of Npas4 mRNA in neurons *in vitro* and *in vivo*, and we show that the eRNA is necessary in mPFC for CSDS-induced anhedonia-like behavior, and in the NAc for drug-context associations through cocaine CPP assays. In the genomic corresponding to the eRNA seqeunce, we also discovered the presence of a DNA-RNA hybrid R-loop structure. Using viral-mediated sgRNA-targeted dCas9-RNAaseH1, we show that the basal and activity-regulated R-loops at the *Npas4* enhancer are required for activity-dependent induction of Npas4 mRNA. **Conclusions:** Our findings reveal a novel mechanism by which a long-non-coding eRNA, produced constitutively from an activity-sensitive enhancer, forms a DNA:RNA three-stranded R-loop structure to enable activity-dependent induction of an essential immediate-early gene, Npas4, involved in maladaptive drug- and stress-induced plasticity.

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Poster

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Program #/Poster #: PSTR228.17/PP11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: HHMI Hannah Gray Fellowship

Title: Spatiotemporal and Circuit Constraints on Dopamine Waves in Dorsal Striatum

Authors: *D. FLINK, A. GAMAM, A. HAMID;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Dopamine release in striatum is vital for reinforcement learning and flexible behavioral control. Our group has recently discovered wave-like dopamine dynamics across the dorsal striatal regions related to instrumental learning and credit assignment. The circuit and computational bases of spatiotemporal dopamine activation patterns remain poorly understood. Here we set out to investigate the local striatal circuit and midbrain cell body basis of initiating, propagating and terminating dopamine waves across the striatum using optical, pharmacological and electrophysiological methods. We will provide preliminary evidence for coordinated, multi-locus regulation of striatal release relevant for value learning.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: Israel Science Foundation (770/17)
NIH-BSF CRCNS (2019793)

Title: Mapping whole-brain dynamics underlying associative learning in behaving mice using functional magnetic resonance imaing (fMRI) and fiber photometry

Authors: *A. LAWEN[1], I. K. SUCCI[2], D. LICHTMAN[3], D. S. PETERKA[4], I. KAHN[4]; [1]Columbia Univ. Program In Neurobio. And Behavior, New York, NY; [2]Biol. Sci., Zuckerman Inst. at Columbia Univ., New York, NY; [4]Zuckerman Inst., [3]Columbia Univ., New York, NY

Abstract: Associative learning involves multiple neural circuits. Our understanding of how information in brain regions such as primary sensory areas, ventral striatum, and frontal and cingulate cortices is incorporated to subserve learning and modification of value assignment remains incomplete. To gain a more complete understanding of associative learning, it is necessary to measure activity from various regions simultaneously. We therefore developed an fMRI setup enabling longitudinal whole-brain mapping and high-resolution behavioral monitoring (n=16 males, 16-24 sessions/mouse). Mice performed an associative learning task with varying water reward volumes as an unconditioned stimulus (US). Rewards were preceded by a conditioned stimulus (CS) in the form of a light cue in CS-US trials or delivered unexpectedly (NoCS-US trials). Variable rewards were delivered interchangeably (low: 1 µl, medium: 3 µl, high: 6 µl) to evaluate reward prediction error's impact on learning. We optimized an MR-compatible camera-based lick detection system to monitor licking behavior. Lick rates progressively increased during CS-US intervals across sessions. Moreover, we found a correlation between nucleus accumbens (NAc) blood oxygenation level dependent (BOLD) signal and anticipatory licking across sessions. NAc responses were modulated by reward volume and anticipation (p<0.05, FWE correction), suggesting the formation of stimulus-driven reward prediction in the NAc. We distinguished naïve and expert stages based on individual mouse behavior, with the expert stage exhibiting higher lick rates during CS-US intervals compared to NoCS-US intervals (n=16; p<10e-05 paired t-test). Task-based functional connectivity (FC) analysis revealed increased NAc to anterior cingulate area (ACA) FC in the expert compared to naïve stage, highlighting their critical role in learning. Moreover, NAc exhibited a gradual buildup of a delayed secondary BOLD response which was not present in early learning stages and was influenced by reward volume. To investigate the molecular mechanism underlying this secondary response, we are currently measuring dopamine release in the NAc using fiber photometry of the dopamine sensor $GRAB_{DA}$. We are testing the hypothesis that each trial's reward volume influences the subsequent trial's dopamine dynamics. In summary, our study provides a novel perspective on longitudinal brain activity and connectivity during associative learning. Advanced imaging techniques are used to shed light on the intricate networks involved in learning and ultimately unravel the role of the novel dynamics we observe in the NAc.

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Poster

PSTR228. Neural Mechanisms of Reward Processing

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Topic: G.02. Reward and Appetitive Learning and Memory

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Title: A brain fingerprint deep learning approach for distinguishing subjects with obesity and normal weight

Authors: H. QI[1], W. ZHANG[1], J. CUI[1], M. ZHANG[1], J. XU[1], G. LI[1], Y. HU[1], J. LI[1], W. JI[1], X. FAN[1], P. MANZA[2], N. D. VOLKOW[2], G.-J. WANG[2], *Y. ZHANG[1];
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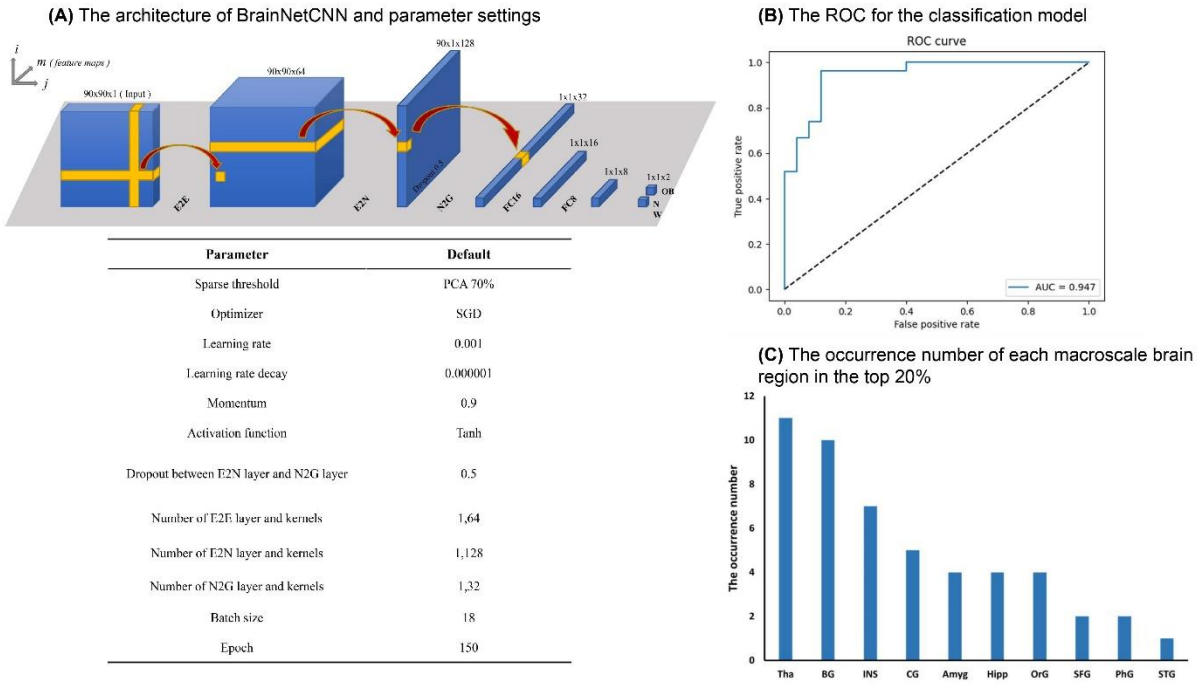
Abstract: Neuroimaging studies showed that obesity is associated with abnormal functional connectivity in brain regions involved in reward processing, cognitive control, emotional regulation and obtained differences between patients with obesity (OB) and normal weight (NW) using traditional group level statistics. Few studies utilize newly developed deep learning approaches which benefit from the ability to represent individual-level information. Thus, the current study employed a deep learning approach with resting-state functional magnetic resonance imaging (fMRI) to classify OB and NW individuals based on whole-brain functional connectivity in 143 OB and 149 NW. Firstly, the Brainnetome atlas was used to divide the whole brain into 246 regions of interest (ROI). Next, regional mean time series were obtained by averaging the fMRI time series. Pearson correlation coefficients of time series between each ROI pair were calculated, resulting in a 246 × 246 correlation matrix for each subject and principal component analysis was employed to eliminate redundant features. Then, BrainNetCNN (**Fig 1A**), a convolutional neural network designed for graph data, was trained to learn the topological characteristics of brain networks and tested with five-fold cross-validation. Results showed that BrainNetCNN successfully distinguished OB and NW individuals with an accuracy of 92.31% and an area under the curve of 0.947 (**Fig 1B**). Gradient-weighted class activation mapping was performed to evaluate the importance of each brain region, showing that the thalamus, basal ganglia, insula, cingulate gyrus, which are brain regions involved with emotion processing and salience attribution have the highest contribution to classification (**Fig 1C**). These results are consistent with previously reported differences in functional brain organization between OB and NW, and the reproducibility of these regions was also verified. These findings demonstrate that deep learning of brain functional connectivity patterns can classify OB and NW, and provide new insights in obesity-related neural mechanisms.



Parameter	Default
Sparse threshold	PCA 70%
Optimizer	SGD
Learning rate	0.001
Learning rate decay	0.000001
Momentum	0.9
Activation function	Tanh
Dropout between E2N layer and N2G layer	0.5
Number of E2E layer and kernels	1,64
Number of E2N layer and kernels	1,128
Number of N2G layer and kernels	1,32
Batch size	18
Epoch	150

Figure 1. Classification model structure, performance and brain regions occurrence statistics.
Abbreviation: Tha, thalamus; BG, basal ganglia; INS, insular gyrus; CG, cingulate gyrus; Amyg, amygdala; Hipp, hippocampus; OrG, orbital gyrus; SFG, superior frontal gyrus; PhG, parahippocampal gyrus; STG, Superior Temporal Gyrus.

Disclosures: **H. Qi:** None. **W. Zhang:** None. **J. Cui:** None. **M. Zhang:** None. **J. Xu:** None. **G. Li:** None. **Y. Hu:** None. **J. Li:** None. **W. Ji:** None. **X. Fan:** None. **P. Manza:** None. **N.D. Volkow:** None. **G. Wang:** None. **Y. Zhang:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.20/PP14

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant R01 DA045639

Title: Genetic deletion of CB1 receptors in serotoninergic neurons causes sexual dimorphism in reward-seeking and behavioral flexibility

Authors: ***F. MORGADO**, S. M. RYAN, I. GILDISH, J. F. CHEER;
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Abstract: Serotonin (5HT) deficits are common in several psychiatric disorders. Endocannabinoids (eCBs) through their presynaptic activity on cannabinoid type 1 receptors (CB1) modulate 5HT transmission, impacting mood states. The dorsal raphe nucleus (DRN), one the largest group of 5HT neurons in the brain, projects to the ventral tegmental area (VTA)

which regulates reward-seeking motivated behavior through its dopaminergic (DA) projections to the nucleus accumbens (NAc). Prior work shows eCBs modulation of VTA-NAc pathway but how 5HT regulation impacts this circuitry is understudied. Thus, we sought to explore the consequences of CB1 deletion in 5HT cells in reward-seeking behavior. By crossing mice expressing cre recombinase under the control of the 5HT specific promoter Pet1 (ePetcre) to $CB1^{flox/flox}$ mice, we generated constitutive conditional knockouts (KO) of CB1 receptor on 5HT cells. After reaching adulthood, CB1 deletion was confirmed through RNAScope. Control ($CB1^{fl/fl}$) and KO (ePetcre:$CB1^{fl/fl}$) mice underwent open-field test to assess their physical and mental condition. Later mice were trained in a fixed-ratio 5 (FR5) schedule to associate cue light and lever press with reward delivery. Following, mice enter a progressive ratio (PR) schedule of reinforcement for 8 days (one session/day) to assess motivation. To shape for a 2-choice task, we employed a FR1 with a fixed timeout of 10 seconds (FTO10) test and a reverse FTO10 (3 sessions each). Finally, to assess reward-related decision making, mice were then placed in a 2-choice task where the lever associated with a cue light provided 90% probability of reward delivery and the one without cue light 10%. Cue light alternated randomly between the two levers to evaluate behavioral flexibility. We found that KO mice spent less time in the center of the open field, an indication of anxiety-like behavior. In the PR task, KO female mice took the same amount of time to complete sessions when compared to the other groups but performed less active lever presses. This is due to higher latency per breakpoint and lower response rate. KO female mice in FTO10 showed fewer active lever presses and high latency. Unexpectedly, KO female mice didn't differ from control mice in the 2-choice task. KO male mice, however, presented greater behavioral flexibility. This model proved to be a great resource to study sexual dimorphism in eCB modulation of 5HT neurons. Our work continues with nuclei-specific (VTA) re-expression of CB1 and assessment of DA and 5HT release in both VTA and NAc to further unravel the sexual dimorphic nature of this understudied brain reward node (DRN-VTA-NAc) and evaluate its pathophysiological impacts in motivation.

Disclosures: **F. Morgado:** None. **S.M. Ryan:** None. **I. Gildish:** None. **J.F. Cheer:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.21/PP15

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01 135294

Title: Examining mechanisms of multiple memory encoding of both cocaine- and fear-associated memories

Authors: *M. HAFENBREIDEL, R. H. COLE, M. M. TORREGROSSA;
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Abstract: Maladaptive memories, such as those associated with substance use disorder (SUD) or post-traumatic stress disorder (PTSD), are long-lasting and resistant to treatment. These memories link environmental stimuli (cues) to associated outcomes, such as drug effects or a threatening event(s). When the cues are encountered, it can lead to recall of the memories and resumption of substance use or presentation of anxiety- or fear-like behaviors. These disorders are often comorbid. However, the interplay between them is understudied. We previously reported that projections from the medial geniculate nucleus (MGN) to the lateral amygdala (LA) when optically stimulated to induce long-term depression (LTD) disrupts cue-induced restatement following cocaine self-administration. Here we trained male and female rats to self-administer cocaine as previously published and conditioned the same rats to associate a different cue with foot shock (i.e., classical fear conditioning). Given that both types of memories are encoded in the lateral amygdala, we hypothesized that opto-LTD at MGN-LA synapses would disrupt both memories. We found that again cue-induced reinstatement of cocaine seeking is disrupted following LTD induction, but the fear-conditioned memory was intact. One possibility is that different neural circuits encode these different types of memory. To explore the brain regions contributing to expression of these memories, rats underwent cocaine self-administration followed by fear conditioning, while control groups underwent saline self-administration and no-shock exposure. Rats then underwent reactivation of the cocaine, fear, or both memories (control rats were placed in both the self-administration and fear context) and then were euthanized 90 minutes later to examine cFos expression. The number of cFos positive cells was counted in a number of reward and learning-related brain regions including the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), amygdala, central amygdala (CeA), dorsal hippocampus (dHPC), hypothalamus, and retrosplenial cortex (RSC). Preliminary analysis suggests general differences in regional activation and quantitative differences in the amount of activation between groups. Examining the mechanisms underlying both cocaine- and fear-associated memories, and how they might interact, is not well explored. Determining unique or overlapping mechanisms could lead to novel therapeutic options.

Disclosures: **M. Hafenbreidel:** None. **R.H. Cole:** None. **M.M. Torregrossa:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.22/PP16

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant T32 MH119049
NIH Grant P50 DA044118
NIH Grant R01 DA055849
NIH Grant U01 DA053826

Title: Does acute psychedelic "therapy" persistently reverse reward-related abnormalities induced by early-life adversity?

Authors: *K. LAWSON, C. RUIZ, M. MARTINEZ, N. WEEMS, D. COATES, S. MAHLER; Dept. of Neurobio. & Behavior, UC Irvine, Irvine, CA

Abstract: Depression, addiction, and disordered eating are common mental health disorders that affect millions of people worldwide, and all involve dysregulated reward circuitry leading to abnormally low reward pursuit (e.g. anhedonia), or abnormally high-reward pursuit (e.g. addiction, binge eating disorder). Risk of developing these disorders is greater in those with a history of stress or parental neglect during childhood, but it is difficult to determine the specific causal impacts of developmental adversity in humans. Therefore, translationally relevant animal models are required. In our well-validated limited bedding and nesting model of early life adversity (ELA) in rats, we found that ELA causes profound, sex-dependent changes in reward and stress circuits—ELA induces excessive seeking of drugs and palatable food in females, while ELA in males instead induces anhedonia for a range of natural and drug rewards. Therefore, our rat model of ELA is useful for establishing the mechanisms of ELA-induced psychopathology, including depression and addiction. In the last few years, psychedelic drugs like LSD, psilocybin, and DMT have re-emerged in psychiatry, with promise for treating depression, addiction, and other disorders. Yet animal models of therapeutic psychedelic effects are underdeveloped at present, inspiring us to develop a new rat model of "psychedelic therapy," and to test whether this treatment ameliorates ELA-induced alterations in reward seeking relevant to depression, addiction, and disordered eating. Our preliminary data suggests that a single dose of the psychedelic serotonin 2A (5-HT2A) receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) may reverse ELA-induced anhedonia in male rats, and we are currently replicating and extending these findings. We hope these studies will contribute to the sorely-needed body of basic research on therapeutic psychedelic drug effects, and provide new insights that could be capitalized upon when developing maximally effective, but minimally disruptive therapeutic strategies for people with psychiatric disorders.

Disclosures: K. Lawson: None. **C. Ruiz:** None. **M. Martinez:** None. **N. Weems:** None. **D. Coates:** None. **S. Mahler:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.23/PP17

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CA TRDRP T31R1767
NIH grants R01 DA055849
NIH Grant U01 DA053826
NIH grants P50 DA044118

Title: Does Vaping Nicotine, THC, or the Combination Have Long-Lasting Effects on Voluntary Nicotine Vaping in Male or Female Rats?

Authors: *Y. XIE[1], C. RUIZ[2], E. CASTILLO[2], C. D. FOWLER[2], S. V. MAHLER[2];
[2]Neurobio. and Behavior, [1]Univ. of California, Irvine, Irvine, CA

Abstract: Nicotine and THC are two of the drugs most commonly used by adolescents, and "vaping" is the fastest-growing method of use in this population. One possibility is that exposure to these drugs in adolescence, when the brain is still developing into its adult configuration, may have long-lasting effects such as increasing the propensity of individuals to develop nicotine addiction later in life. Here we tested this proposition in male and female Wistar rats. Rats received 10, 5-sec vapor puffs at regularly spaced intervals during daily 60min sessions, conducted from postnatal days 39-49. Vapor contained nicotine, THC, nicotine+THC, or vehicle only. After allowing 21 days for these drugs to wash out, and for rats to grow to adulthood, they were then trained to press a lever to self-administer 5% sucrose in a vegetable broth in daily 1hr sessions held in vapor chambers, by pressing a lever on a FR1, 2, 3, then 5 schedule. Following this pre-training, rats self-administered nicotine vapor in daily 1hr sessions, also delivered on an FR5 schedule. Nicotine doses were 2.5, 5 7.5, 10mg/mL (7 days at each dose), and stable self-administration was determined in the last 3 days of training at each dose. Following behavioral testing, perfused brains were examined for cortical thickness, and for c-Fos expression elicited by noncontingent nicotine vapor. Impacts of adolescent treatment, sex, and dose were observed, and several methodological issues are examined and discussed.

Disclosures: Y. Xie: None. **C. Ruiz:** None. **E. Castillo:** None. **C.D. Fowler:** None. **S.V. Mahler:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.24/PP18

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NASEM Ford Fellowship
NIH NIDA Grant 1R01DA055849-01A1
NIH NIDA 1U01DA053826-01A1
NIH NIDA 1U01DA053826-01A1

Title: The Social Desire: How ventral pallidal GABAergic neurons regulate social behaviors

Authors: *E. M. RAMIREZ, K. LAWSON, R. ROCKERYA, S. V. MAHLER;
Univ. of California, Irvine, Irvine, CA

Abstract: During COVID-19 lockdowns and the countless Zoom get-togethers that came with it, people across the world were desperate for social contact and yet the brain mechanisms underlying this basic human craving to be together are not well understood. The ventral pallidum (VP) is an intriguing target for this research due to its role in motivated responses to both food and drug rewards where work in mice and rats has shown that GABAergic VP neurons are

specifically important for reward seeking and approach. Do VP GABA neurons also play a role in social reward? Here we investigate the role of GABAergic VP neurons in social behaviors using Cre-dependent inhibitory and excitatory DREADD (hM4Di and hM3Dq) vectors injected into the VP of adult GADiCre transgenic and wildtype rats. Animals were socially isolated from their same-sex littermate for one week before being undergoing behaviors designed to assess 1) social reward preference and 2) social interactions through behavior and ultrasonic vocalization (USV) recording. First, rats were trained to form a conditioned place preference (CPP) for their former cagemate, versus a distinct chamber with no rat. On test day, rats explored both chambers without another rat, following injection of either clozapine-n-oxide (CNO) or vehicle. Following CPP, rats remained socially isolated until again being reunited with their cagemate in two 60 min social interaction tests, following counterbalanced CNO and vehicle. Social interactions and ultrasonic vocalizations were recorded.Preliminary results show that chemogenetic manipulation of VP GABA neurons bidirectionally regulate social reward, social behavior, and ultrasonic vocalizations, and may do so in a sex-dependent manner.

Disclosures: **E.M. Ramirez:** None. **K. Lawson:** None. **R. Rockerya:** None. **S.V. Mahler:** None.

Poster

PSTR228. Neural Mechanisms of Reward Processing

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR228.25/PP19

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NSF-GRFP DGE-1839285
P50 DA044118

Title: Assessment of microglia after adolescent Δ^9-tetrahydrocannabinol and adulthood heroin exposure

Authors: *M. X. MARTINEZ, ***M. MARTINEZ**, H. RAMIREZ RAMIREZ, C. M. RUIZ, E. CASTILLO, V. C. INSHISHIAN, Y. XIE, S. V. MAHLER;
Univ. of California, Irvine, Irvine, CA

Abstract: Adolescence is a critical developmental window for prefrontal cortex (PFC), and exposure to drugs of abuse during this period can have long-term consequences on PFC and connected neural circuits. Accordingly, studies in humans and rodents have shown that cannabinoid drug exposure during adolescence may lead to alterations in cognitive function, potentially including enhanced susceptibility to the addictive effects of “harder” drugs like opioids. Microglia are the resident immune cells of the brain, but they also play other roles in synaptic plasticity and pruning, at least during development. Might microglia play a role in the long-lasting changes in brain function caused by adolescent THC exposure? Here, we exposed female and male Long-Evans rats to THC (5mg/kg i.p.) or vehicle daily during adolescence

(postnatal day 30 to 43). After they grew into adulthood (PD70+), rats underwent multiple behavioral tasks measuring addiction-relevant responses to opioid drugs, including heroin conditioned place preference (CPP) and sensitization, as well as a behavioral economic assessment of demand for self-administered doses of the highly reinforcing opioid drug remifentanil. We found that adolescent THC caused changes in opioid reward in both sexes, with an especially pronounced pro-opioid phenotype in females. To determine the potential involvement of microglia in these persistent adolescent THC effects, we used structural analyses of microglia morphology and co-expression of function-relevant markers, and found effects of both acute adolescent THC treatment, as well as changes in opioid-induced structural characteristics that persisted long into adulthood. To further query whether microglia retain a long-lasting "memory" of THC exposure that is relevant to later-life responses to opioid drugs, we administered the CSF1R inhibitor PLX 5622, or vehicle to adult rats with a history of adolescent THC exposure—a manipulation that can ablate existing microglia. After cessation of PLX 5622 treatment microglia will repopulate in the brain with THC-naïve microglia. We predict that in the absence of THC-experienced microglia, abnormal opioid drug behavioral responses will be corrected, indicating a role for these "immune cells" in addiction-relevant plasticity in the adult brain. Implications and mechanisms of these findings will be discussed.

Disclosures: **M.X. Martinez:** None. **M. Martinez:** None. **H. Ramirez Ramirez:** None. **C.M. Ruiz:** None. **E. Castillo:** None. **V.C. Inshishian:** None. **Y. Xie:** None. **S.V. Mahler:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.01/PP20

Topic: G.08. Other Psychiatric Disorders

Support: New York State Office for People with Developmental Disabilities

Title: The effects of context on sociability in the BTBR mice

Authors: *K. K. CHADMAN[1], A. VARGHESE[1,2], S. SAYAKKARA[1,3,4];
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Abstract: Autism spectrum disorder (ASD) is characterized by deficits in social communication and repetitive behaviors/restricted interests. A mouse model of ASD is the BTBR T+ ItprTF/J (BTBR) mice which display low levels of social behavior in several tests. The social approach test has been commonly used to examine the preference for social interaction between a stranger mouse or a novel object. BTBR mice generally do not show a preference in the social approach test, though this behavior is less consistent under certain conditions. The current experiment tested different contexts for the social approach test using BTBR subject mice to determine the

effect of context on social behavior. Contextual variables included modified lighting and novel object following a saline injection. Once the context with the lowest sociability level was determined, it was used to test whether saline or drug vehicle (80% saline:10% EtOh:10%Kolliphor) affected sociability. The results show that the injection of saline led to similar levels of sociability in all contexts. However, the vehicle led to increased sociability in some contexts, but not others. These results suggest that the sociability of the BTBR mice in three chambered social approach test is affected both by context and the interoceptive effects of a commonly used vehicle.

Disclosures: K.K. Chadman: None. **A. Varghese:** None. **S. Sayakkara:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.02/Web Only

Topic: G.08. Other Psychiatric Disorders

Title: Variation in theta beta ratio and cognitive performance in subjects with ADHD symptoms with or without depression symptoms

Authors: K. N. TUZ-CASTELLANOS[1], W. V. HERRERA-MORALES[2], J. REYES-LÓPEZ[4], *L. NUÑEZ-JARAMILLO[3];
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Abstract: ADHD is a neurodevelopmental disorder presenting with hyperactivity, impulsivity and inattention. It is common for ADHD patients to present comorbidities, such as depression. Both ADHD and depression have been associated with changes in brain activity, as well as with cognitive impairment. However, despite the frequent presence of comorbidities, there are few studies addressing the effect of the presence of both conditions on brain electrical activity and cognitive performance. Additionally, while many children with ADHD transit into adults with ADHD, there are fewer studies addressing ADHD and its comorbidities in this age group. Herein we performed qEEG analysis and neuropsychological tests in young adults with symptoms of ADHD, either with or without symptoms of depression, and compared them with controls. We found that subjects with ADHD symptoms presented higher impulsivity scores than controls, but lower than subjects with symptoms of both ADHD and depression did. We also found higher theta/beta ratio at Cz and O2 in subjects with symptoms of both ADHD and depression than in the other two groups. Our results indicate that the presence of depression symptoms in subjects with ADHD leads to higher impulsivity and lower processing capacity when compared with

subjects presenting only ADHD symptoms, highlighting the importance of screening for comorbidities in ADHD patients in order to attain a correct diagnosis and treatment selection

Disclosures: K.N. Tuz-Castellanos: None. **W.V. Herrera-Morales:** None. **J. Reyes-López:** None. **L. Nuñez-Jaramillo:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.03/PP21

Topic: H.01. Attention

Support: NIH RO3AA019798

Title: Alcohol-related attitudes, behaviors, and cognitions in female college freshman: Event-related potentials and frontal midline theta

Authors: *R. GRAHAM[1], J. T. CORBETT[2], N. A. CEBALLOS[2];
[1]Psychology, Texas State Univ. San Marcos, San Marcos, TX; [2]Psychology, Texas State Univ., San Marcos, TX

Abstract: Adjustment to college depends on how students' pre-college norms/behaviors align with the college environment. Females, in particular, may experience difficulty adjusting to the college drinking culture because drinking to intoxication has historically been considered more appropriate for men. The purpose of this pilot was to examine changes in alcohol consumption and related attitudes and cognitions in female freshmen at a Hispanic-serving college in Southwest Texas. Seventy females were recruited in the first semester of freshman year and completed a battery of measures (e.g., demographics, alcohol expectancies/attitudes and consumption) and a 3-stimulus oddball paradigm using images of alcoholic and nonalcoholic beverages. Participants returned after 6 months to repeat these measurements, yielding a final sample of 40. Analyses examined changes in attitudes and drinking patterns over time as a function of baseline drinking patterns. EEG was also examined via traditional averaging and the extraction of frontal midline theta (FMT) via wavelet analyses. At recruitment, 17 participants self-identified as regular drinkers, which remained constant over the study, as did alcohol consumption. Both groups were more likely to endorse the benefits of alcohol for tension reduction at Time 2 and less likely to believe that alcohol provides liquid courage and harms self-perceptions. ERP data revealed a frontal negativity (N2), while FMT data showed a prominent theta peak during the timeframe of the N2. Analyses focused on the N2 and FMT coinciding with the onset/peak/offset of the N2 (100-300 ms). For N2 amplitude, a time (T1 vs. T2) x stimulus (alcohol vs. non-alcohol) x group (regular vs. nonregular drinker) was observed, such that N2 amplitude was enhanced at T2, but more for alcohol images in the regular drinkers and more for nonalcohol images in nonregular drinkers. FMT was sensitive to drinking status, such that regular drinkers had enhanced FMT to alcohol images at T1 and T2, regardless of

target status. In the non-regular drinking group, FMT to alcohol images was only larger when they were targets. Together, these results suggest that the drinking patterns established prior to entry into college have important implications for how female students respond, physiologically and behaviorally, to the presence of alcohol and alcohol-related messages in the collegiate setting.

Disclosures: R. Graham: None. **J.T. Corbett:** None. **N.A. Ceballos:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.04/PP22

Topic: G.08. Other Psychiatric Disorders

Title: Symbiotic Ally: An overview of ayahuasca research in mental health and how new technology can bridge gaps

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[1]Temple Univ., Philadelphia, PA; [2]Independent Researcher, Denver, CO; [3]Equideum Hlth., Pittsburgh, PA

Abstract: Ayahuasca is an ancient plant medicine from South America utilized for its psychoactive effects that ethnopharmacologist Dennis McKenna referred to as, “a symbiotic ally of the human species.” Extracted from the bark of the Banisteriopsis caapi vine, the main psychoactive component in the plant is N,N-dimethyltryptamine commonly referred to as DMT. Interest and demand for the ritualistic use of ayahuasca has expanded to the United States and globally in facilities with qualified facilitators and staff who administer it for spiritual and therapeutic purposes. Ayahuasca has potential therapeutic value for mental health issues such as major depressive disorder, post-traumatic stress, and anxiety. Research on the therapeutic value of ayahuasca has been limited due to a variety of factors including novelty of focus as a therapeutic drug, respect towards and limited scrutiny of its use in spiritual ceremonies, illegal status in some jurisdictions, stigma towards use of certain psychoactive compounds for mental health therapy, and lack of formal controlled studies with appropriate follow-up and validated outcome metrics. This overview aims to systematically analyze research reviews and systematic reviews on the therapeutic aspects of ayahuasca for mental health. A literature search on the topic identified 136 reviews and systematic reviews with the intention of identifying trends and gaps in existing research. Given the relatively high number of reviews to original research on this topic (approximately 1 in 4 publications is a review), we anticipate significant overlap in the source material. Research on this topic is extremely limited which causes gaps in understanding. The most common limitations include small study size and lack of a control group. The ritualistic nature of ayahuasca use makes benefits difficult to quantify, and the illegal nature of the substance deters participants from sharing their results publicly. These issues prevent existing research pools from growing. After careful consideration of research limitations, we make

recommendations on how to address these gaps, including new technological approaches to data privacy. An enhanced, privacy-focused research effort may allow more thorough examination of the therapeutic effects of the drug and potentially point to opportunities and challenges with respect to its increasing use.

Disclosures: C. Tengowski: None. **E. Beerbower:** None. **S. Manion:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.05/PP23

Topic: G.08. Other Psychiatric Disorders

Support: Sino-Danish Center for Research and Education
A.P. Møller Fonden
Jascha Fonden
Fru C. Hermansens Fonden
Torben & Alice Frimodts Fonden

Title: Exploring ultrastructural alterations in Slitrk5$^{-/-}$Mouse: Insight into OCD pathogenesis

Authors: *O. B. SVENDSEN[1,2], J. MIDTGAARD[3], N. XU[4], F. S. LEE[5], J. R. NYENGAARD[1]; [1]Dept. of Clin. Med., Aarhus Univ., Aarhus N, Denmark; [2]Sino-Danish Ctr., Beijing, China; [3]Dept of Neurosci., Univ. of Copenhagen, Copenhagen 2200-N, Denmark; [4]Inst. of Neurosci., Chinese Acad. of Sci., Shanghai, China; [5]Departments of Psychiatry and Pharmacol., Weill Med. Col. of Cornell Univ., New York, NY

Abstract: Up to 3% of the world's population suffers from obsessive-compulsive disorder (OCD), which has led to high costs in treatment and loss of work. Several hypotheses have been proposed to explain the development of OCD, but our current understanding is still limited, and today's treatments are only partially effective. We are investigating the potential role of the neurotransmembrane protein, Slitrk5, which may be one of the missing pieces in our understanding of developing OCD. Recent studies have associated a deficient Slitrk5 with OCD. In our exploratory study, we utilized ultrastructural analysis with Serial-Block Face Scanning Electron Microscope (SBF-SEM) to identify and visualize ultrastructural alterations at nanometer resolution of the Slitrk5$^{-/-}$ mouse. The Slitrk5$^{-/-}$ is an OCD mouse model, where the Slitrk5 protein has been constitutively knocked out. We prepared 80 µm thick sections of tissue from the dorsal medial area of the striatum (DMS) of the Slitrk5$^{-/-}$ (n= 5, male) and wild type mice, C57BL/6 (n = 3, male) for the SBF-SEM. The outcome of the SBF-SEM was a series of images (Block of ~1000 40 nm thin sections) covering an area of approximately 30x30x30 µm in the DMS. Each block was rendered, visualized, and analyzed to uncover structural alterations of myelinated axons. The ultrastructural analysis revealed deformations and degenerations of myelinated axons in the tissue of the Slitrk5$^{-/-}$ mouse. The preliminary result suggests that

experimental deletion of the Slitrk5 protein in mice leads to the development of abnormal myelin membrane extensions, myelin redundancy, and degenerations of myelinated axons within fiber bundles of the DMS. These findings highlight the previously unknown crucial role of the Slitrk5, as a structurally important synaptic-associated protein that has a crucial regulatory effect on myelinated axons, and that a deficiency of this protein leads to degenerative processes, at least in the DMS. Overall, these data support the hypothesis that Slitrk5 plays an essential role in the development of OCD-like phenotypes. We are currently conducting additional structural studies on the Slitrk5$^{-/-}$ mouse to further validate our findings. These include mesoscale analysis to 3D visualize the Cortico-Striatal Circuitry by retrograde labeling combined with whole-brain tissue clearing technique to map the entire long-range connectivity of the Cortico-Striatal Circuit. Additionally, we are performing macroscale structural analysis of the dorsal medial striatum, using multiplexing methods to examine ~30 different structural proteins to reveal molecular and cellular structural alterations within the DMS of the Slitrk5$^{-/-}$ mouse.

Disclosures: O.B. Svendsen: None. **J. Midtgaard:** None. **N. Xu:** None. **F.S. Lee:** None. **J.R. Nyengaard:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.06/PP24

Topic: G.08. Other Psychiatric Disorders

Support: NS112390
3R01NS112390-02S1

Title: Synaptojanin-1 gene mutations produce sex-specific changes in cocaine reward through alterations in dopamine system function

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Abstract: The *synaptojanin-1 (SYNJ1)* gene is known to be important for dopamine-related disorders. Recent evidence has demonstrated that Synj1 deficient mice (*Synj1*$^{+/-}$) have impairments in dopaminergic synaptic vesicular recycling. Though these impairments in the dopamine system have been observed, little is known about how *Synj1* deficits affect the mesolimbic system, or reward processing. To examine the role of the *Synj1* gene in motivated behavior, we subjected male and female *Synj1*$^{+/-}$ and *Synj1*$^{+/+}$ mice to a battery of behavioral tests. These tests included a sucrose preference test, operant conditioning, and progressive ratio, as well as a cocaine conditioned place preference paradigm.

Overall, we observe that *Synj1*$^{+/-}$ mice exhibit a normal behavioral profile compared to controls, with normal hedonic responses and motivated behavior for sucrose. However, male *Synj1* $^{+/-}$ demonstrated an attenuated conditioned place preference for cocaine. To further investigate the mechanisms supporting the attenuated response to cocaine, we recorded levels of striatal DA in response to cocaine and levels of the dopamine transporter (DAT) in the midbrain and striatum. We observed that *Synj1* $^{+/-}$ male mice take longer to reach peak DA release to cocaine and failed to show cocaine-induced increases in midbrain or striatal DAT.
These findings provide new insights demonstrating that *SYNJ1* deficiencies result in abnormal DA system function in males. Additionally, our results also demonstrate a *SYNJ*-related mechanistic explanation for the strong existence of sex differences, among substance abuse and other dopamine-related disorders.

Disclosures: J.I. Mejaes: None. **J. Saenz:** None. **C. O'Brien:** None. **C. Pizzano:** None. **P. Pan:** None. **D.J. Barker:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

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Topic: G.08. Other Psychiatric Disorders

Support: Temple University Mid Atlantic Neuroscience Diversity Scholars Program
NIH BP ENDURE
NIH K08MH080239

Title: Brain Measures of Shared and Unique Variance between Internalizing and Externalizing Psychopathology

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Abstract: Introduction. P300 component amplitude from event-related potentials (ERPs) has been widely observed to be reduced in association with externalizing (EXT; substance use and antisocial behavior) and Internalizing behaviors (INT; depression and to a degree for anxiety), as well as thought disorders such as schizophrenia. The shared variance between INT and EXT behaviors, referred to as a general psychopathology factor p (p-factor), has been shown can mediate P3 amplitude reduction (P3AR) associated with internalizing and externalizing (Bernat et al., 2020). Recent work, from our group and others, has indicated that ERPs can be understood as a mixture of time-frequency components delta and theta (Bernat et al., 2011; Bernat et al, 2015, Harper et al., 2014). The present project aims to assess the role of theta in the observed P3 amplitude reduction related to the p-factor. Methods: The present project utilizes the same data as previously published for the time-domain P3 (Bernat et al., 2020), which contained 125

participants (70 females, 55 males; M = 20.01 years, SD = 3.77 years) who completed a visual oddball paradigm. Time-frequency theta activity was extracted using time-frequency principal components analysis (tfPCA; Buzzell et al., 2022) for target stimuli to assess the shared and unique variance between theta and the previously reported P3 in relation to the p-factor. Results. Pearson's correlations indicated that both target P3AR (r = -.195) and target theta for (r = -.237) were related to p-factor. Multiple regression was used to assess shared and unique variance by comparing models with P3 alone, and with theta. R2 change between the models indicated theta added significant incremental prediction for p-factor relative to P3 alone (R-squared change: .037, p < .03). The model with both indicated that theta shared variance with P3, but did maintain significant unique variance in relation to p-factor (t-2.52, p<.034), while P3 did not maintain significant unique variance relative to theta (t = -1.53, p < .129). Discussion. The present project implicates theta in this process, suggesting that *p*-factor involves mechanisms related to both P3 and theta. Medial-frontal theta has been strongly implicated in attention, salience, and cognitive control, suggesting that these functions may be measurably implicated in elevations of general psychopathology.

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Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.08/PP26

Topic: G.08. Other Psychiatric Disorders

Title: Development of novel selective morpholine trace amine-associated receptor 1 partial agonists with promising preclinical effects related to neuropsychiatric disorders and well tolerated in healthy volunteers

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[2]pRED, [3]Ricb, [4]Neurosciences and Rare Dis., [5]Pharmaceut. Sci., [6]Therapeut. Modalities, [1]F. Hoffmann-La Roche, Basel, Switzerland

Abstract: Trace amine-associated receptor 1 (TAAR1) is a novel target for antipsychotic and potentially also mood-stabilizing and anti-addictive drugs with high potential for differentiation, exploiting a fundamentally new mechanism of action based on the modulation of dopaminergic, serotonergic, and glutamatergic neurotransmission. TAAR1 agonists from a previous amino oxazolines series have been broadly studied preclinically (e.g. RO5263397, RO5256390, RO5203648) and in clinic (e.g. RO5263397). Unfortunately, its clinical development had to be halted as RO5263397 was mainly metabolized in humans by N-glucuronidation. High frequency of UGT2B10 splice site mutations in individuals of African origin resulted in poor

metabolization of RO5263397 and high plasma exposure. Through a medicinal chemistry program we identified potent and selective TAAR1 ligands from a new chemical series, the morpholines, and optimized their physicochemical and pharmacokinetic properties in rat, mouse, Cynomolgus monkey and human. We developed two selective partial agonists of TAAR1 (RO6889450 and RO6799477) and tested them extensively in nonclinical models predictive of antipsychotic, stress-response modulating and anti-addictive properties. We demonstrated that both compounds significantly reduce the hyperlocomotion induced by the NMDA receptor antagonists PCP (3.2 mg/kg, i.p) and by the dopamine transporter inhibitor cocaïne (15 mg/kg, i.p) in a dose dependent manner. Both compounds also showed a potentiation of the effect of olanzapine on PCP-induced hyperlocomotion in mice and a partial reversal of cocaïne-induced facilitation in intracranial self-stimulation threshold in rats. Anxiolytic-like properties of RO6889450 and RO6799477 were observed in the stress-induced hyperthermia test in mice with results comparable to chlordiazepoxide. Both compounds presented a neural activation profile measured by phMRI differing from first and second-generation antipsychotics. RO6889450 and RO6799477 showed a favorable preclinical safety profile, findings observed in rats and monkeys were clinically monitorable and/or reversible. Both molecules have been investigated in Phase I single ascending dose (SAD) and multiple ascending dose (MAD) studies conducted in healthy volunteers to evaluate the safety, tolerability, PK, and pharmacodynamics. Based on its unique behavioral profile TAAR1 activation could represent a novel therapeutic option for neuropsychiatric disorders, including schizophrenia and addiction.

Disclosures: **C. Fournier:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **D. Buchy:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **S. Mohr:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **A. Pähler:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **R. Norcross:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **P. Pflieger:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **S. Sewing:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **B. Kuennecke:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **S. Holiga:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **I. Gerlach:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche. **M.C. Hoener:** A. Employment/Salary (full or part-time):; F.Hoffmann La Roche.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.09/Web Only

Topic: G.08. Other Psychiatric Disorders

Title: Neural correlates of trait impulsivity among healthy individuals

Authors: *H. BAK[1,2], H.-Y. JUNG[2], H. KIM[2], K. LEE[2], S.-H. LEE[2];
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Abstract: Impulsivity is defined as a tendency to prompt, unplanned reactions to internal or external stimuli regardless of negative outcomes of these reactions. Although impulsivity is more likely to be present in individuals with some specific psychiatric disorders, it can be shown in any individuals with or without mental health conditions. By discovering neural correlates responsible for impulsivity, therefore, it can help to understand the severity of psychiatric symptoms, personality characteristics, and the degree of social adaptation. Herein, this study aimed to find out the gray matter substrates of trait impulsivity in healthy individuals. We also investigated the connection between impulsivity and psychological symptomatology, such as depression and anxiety, low resilience, and quality of life. A total of 75 right-handed healthy individuals were enrolled. Trait impulsivity was assessed using Barratt Impulsiveness Scale (BIS). At baseline, The Beck Anxiety Inventory (BAI), Beck depression Inventory-II, World Health Organization Quality of Life (WHOQOL-BREF) and the Connor-Davidson Resilience Scale (CD-RISC) were assessed. All participants underwent T1-weighted MRI scan. Freesurfer (version 7.1.1) general linear regression was applied to reconstruct a cortical surface model for calculating the mean cortical thickness (CT) and the local gyrification index (LGI) for performing whole-brain vertex-wise correlation analysis. Partial correlation analyses were carried out to examine the relationship between BIS scores and CT or LGI in each brain region. Exploratory correlation analysis was performed to examine relationships between significant brain regions and other measurements. In healthy individuals, total BIS scores were significantly negatively correlated with the mean CT of the left lateral occipital cortex (OC). Also, there was a significant negative correlation between total BIS scores and LGI of the left pars opercularis, the fold of the inferior frontal gyrus. In the left lateral OC, mean CT was negatively correlated with BAI scores and positively correlated with WHOQOL-BREF scores. The LGI values of the interior frontal gyrus showed a negative correlation with BAI scores. Our findings indicated that mean CT of the left lateral OC and LGI of the inferior frontal gyrus may contribute to trait impulsivity. The regions, therefore, could be neural basis for psychological symptom severity, resilience, and quality of life in general population.

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Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.10/PP27

Topic: G.08. Other Psychiatric Disorders

Support: CONACYT for the PhD scholarship no. 931445

Title: Evaluation of Post-COVID-19 Neurological Syndrome, its implications for social cognition and the presence of affective disorders in the general population.

Authors: E. ACOSTA-MARÍ[1], T. CIBRIAN-LLANDERAL[2], R. TRIANA-DEL RIO[4], Y. CAMPOS-USCANGA[3], H. ACOSTA-MESA[3], R. CASTILLO-LOPEZ[3];
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Abstract: Background: Most people who develop COVID-19 fully recover, but current evidence suggests approximately 10-20% of people experience a variety of mid and long-term effects after they recover from their initial illness. The most common symptoms associated with post COVID-19 condition include fatigue, breathlessness and cognitive dysfunction (for example, confusion, forgetfulness, or a lack of mental focus or clarity). Post COVID-19 condition can affect a person's ability to perform daily activities such as work or household chores. Some viruses have a certain affinity for neuronal tissue, such as SARS-CoV-2, they have the capacity to alter the complex organization of neuronal circuits through different pathways, with clinical manifestations of variable intensity and duration, predominantly neurological, which has been described as Post-COVID-19 Neurological Syndrome.**Objective:** To determine the levels of symptoms of stress, anxiety, depression, and scores on tests involving empathy in the general Post-COVID-19 population and compare them with the scores obtained in the general Pre-COVID-19 population. We collected the data of 86 general Post-COVID-19 population and compared to 126 general Pre-COVID-19 population. The Perceived Stress Scale (Gonzalez, Landero, 2007), the Beck Anxiety Inventory and Beck Depression Inventory (Villegas, 2004), the Davis Interpersonal Reactivity Index (Davis, 1980) and the Reading the Mind test were used for the psycho-affective evaluation (Baron-Cohen, 2001). **Results:** They were evaluated 126 Pre-COVID-19 participants and 86 Post-COVID-19 participants. In the analysis of the psycho-affective evaluation, the median test was used to compare two groups, which reported statistically significant differences in the evaluation of the perceived stress scale (P = 0.01), in the Beck Anxiety Inventory (P = 0.01), in the Beck Depression Inventory (P = 0.01). In the RME test, the mean was 23.67 (SD: 3.75) in Pre-COVID-19 while in the Post-COVID-19 group it was 21.51 (SD: 3.62) (P = 0.01). The interpersonal reactivity index of Davis showed statistically significant differences in the evaluation of the subscales perspective-taking scale (PT) (P=0.01), fantasy scale (FS) (P=0.01), empathic concern scale (EC) (P=0.01), personal distres scale (PD) (P=0.01)

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Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.11/PP28

Topic: G.08. Other Psychiatric Disorders

Support: CONACYT-727272

Title: Development of binge eating behavior in Female Wistar Kyoto rats; a better model with construct and appearance validity

Authors: D. S. RODRÍGUEZ-RANGEL, *C. LOPEZ-RUBALCAVA;
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Abstract: Binge eating disorder (BED) is the more prevalent eating disorder worldwide, mainly affects women, and presents elevated comorbidity with other psychiatric disorders such as anxiety. The main symptoms of BED are binge eating episodes, defined as consuming large amounts of food in a short time, and the absence of any compensatory behavior to prevent weight gain. Binge eating episodes are usually characterized by eating in lack of hunger, feeling uncomfortably full, eating much more rapidly than usual, and the presence of discomfort feelings after the episode. In the present work, we analyze the potential of the Wistar Kyoto (WKY) rat strain as a binge-eating (BE) model capable of developing an anxiety-like behavior associated with abnormal eating patterns. We compared its performance against the Sprague Dawley strain (SD) since it is the most used strain to study BE behavior. Thus, we implemented an intermittent palatable food access model without food deprivation on 7-week-old female WKY and SD rats. We used sucrose syrup (30%) and shortening as palatable foods. We assessed the development of BE behavior, the effect of the estrous cycle on it, the presence of anxiety-like behavior, and the serum corticosterone levels with and without stress. Even though the development of sucrose BE was similar between strains, WKY more easily developed the shortening BE than SD, required less time to establish the BE behavior, and exhibited an eating pattern similar to a binge episode during the palatable food access session. The parameters we evaluated in the BE behavior were unaffected by the estrous cycle in any strains. In the plus maze test, only WKY presented an anxiety-like behavior associated with the sucrose and shortening BE; however, the results in the modified marble test did not show a difference between strains but a difference between diets, in which only the sucrose groups expressed increased anxiety-like behavior. Finally, WKY showed a higher sensitivity to stress than SD. Sucrose BE also seems to affect both strains' hypothalamic pituitary adrenal axis; it facilitated response to stress exposure in SD and was blunted in WKY. In conclusion, WKY offers a better construct and appearance validity to the binge eating model than SD.

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Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

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Program #/Poster #: PSTR229.12/QQ1

Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant P50DA037844

Title: Sex differences in sensation/novelty seeking phenotypes in a large cohort of young adult heterogeneous stock rats

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Abstract: High levels of sensation/novelty seeking (SNS) is associated with elevated risk for maladaptive behaviors such as substance abuse and gambling addiction, especially in adolescents and young adults. In humans, men have generally been reported to have higher levels of SNS than women, although this difference may at least partially reflect culturally transmitted social norms. However, past studies in rodents that examined sex-related differences in SNS phenotypes using various behavioral paradigms have yielded mixed results. Few studies have examined sex differences in SNS in rats using very large samples. The present study examined sex differences in SNS phenotypes in a large cohort (1,254 males and 1,253 females) of young adult genetically diverse heterogeneous stock (HS) rats using two tasks in different behavioral paradigms designed to measure partially overlapping but different aspects of SNS. In the first task, the locomotor response to novelty test, rats were placed in a novel locomotor chamber for 18 min, and their locomotor activity was recorded by an infrared motion sensor-system. In the second task, the light reinforcement (operant sensation seeking) test, which measures voluntary operant responding for a purely sensory reinforcer (light), animals were placed in a dark operant chamber with poke holes in the left and right walls for 18 min per session. In the initial habituation phase (6 sessions), rats' snout-poke responses into either hole had no consequences. During the subsequent light reinforcement phase (6 sessions), snout-pokes into one of the holes, designated "active", produced a 5-sec light onset according to a variable-interval 1 min schedule of reinforcement, while pokes into the other "inactive" hole had no consequences. The results of the locomotor response to novelty test showed that female HS rats reacted to the novel chamber to a greater degree than males, in agreement with a number of other studies in rats. In the light reinforcement test, during the initial habituation phase, females made more pokes into both holes in the dark chamber than males, again suggesting that females had greater response to novelty than males. When the response-contingent light stimulus was introduced in the following phase, females made more active responses for the light stimulus than males. However, females also made more inactive responses than males, and no significant sex difference was found in the relative preference for the light reinforcer. Thus, our results indicate that, while female rats have greater reactivity to novel stimuli than males, the sexes may not differ in some other aspects of SNS such as their propensity to seek purely sensory stimulation.

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Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

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Program #/Poster #: PSTR229.13/QQ2

Topic: G.08. Other Psychiatric Disorders

Support: Bruce/Jones Graduate Fellowship in Addiction Biology
NIMH Grant R00MH121355

Title: Examining the role of parvalbumin interneurons in alcohol intake in the maternal immune activation model

Authors: *J. L. PALMER[1], D. SAN MIGUEL[2], J. J. DONEGAN[3];
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Abstract: Schizophrenia is a debilitating psychiatric disorder with symptoms ranging from paranoia and delusions to social withdrawal and cognitive dysfunction. People with schizophrenia are also at an increased risk of developing an alcohol use disorder (AUD). Although the co-occurrence of these disorders is frequent, the underlying neurobiological mechanisms remain understudied, making treatment a challenge. One brain region that has been implicated in both schizophrenia and AUD is the anterior hippocampus (ventral hippocampus (vHipp) in rodent), which plays a role in motivated behaviors and emotional regulation. Schizophrenia patients and rodent models with schizophrenia-like deficits show hippocampal hyperexcitability, which is thought to be caused by a loss of inhibitory interneuron function. Specifically, parvalbumin (PV) interneurons, which regulate pyramidal cell firing, show dysregulation. Recent work has also demonstrated that chemogenetic manipulation of vHipp pyramidal cells alters alcohol intake. Because changes in pyramidal cell firing accompany changes in both schizophrenia-like behaviors and alcohol consumption, we sought to examine the role of vHipp PV interneurons in these contexts. To achieve this aim, we used a maternal immune activation (MIA) model where pregnant mouse dams were injected with 10mg/kg of Poly(I:C) on gestational day 12.5. In adult offspring, an excitatory DREADD was expressed in vHipp PV interneurons. Four weeks later, mice were injected with Compound 21 (1mg/kg) before behavioral testing to determine whether vHipp PV interneuron activation alters anxiety-like, social, sensorimotor gating, and alcohol-drinking behaviors. Thus far, our preliminary data suggest attenuations in prepulse inhibition of startle in MIA mice that can be restored with PV interneuron activation. These experiments could identify a potential therapeutic target for alcohol use disorder associated with schizophrenia.

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Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

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Program #/Poster #: PSTR229.14/QQ3

Topic: G.08. Other Psychiatric Disorders

Support: R01AA028218

Title: Sex-related differences in binge-like eating and pituitary adenylate cyclase-activating polypeptide in the paraventricular nucleus of the thalamus

Authors: *B. A. CARPENTER, G. R. CURTIS, B. E. PIRINO, J. R. BARSON;
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Abstract: Binge eating disorder is the most common eating disorder in the United States, characterized by overconsumption of typically palatable food in a short time period, and is more often seen in women than in men. To study possible mechanisms behind this sex-related difference, we focused on the paraventricular nucleus of the thalamus (PVT) and the neuropeptide, pituitary adenylate cyclase-activating polypeptide (PACAP), both of which have known roles in eating behavior and neither of which has yet specifically been tied with binge-like eating. Using C57BL/6J mice, we first developed a binge eating paradigm, where we gave *ad libitum* fed mice limited access to Milk Chocolate Ensure Plus® (binge group; n = 12/sex) for 2 hours/day, 4 days/week, for 6 weeks. The control group had access to water and chow only (control group; n = 12 females, 8 males). We found that the binge group had significantly greater caloric consumption during the period of Ensure access compared to the control group, and that they escalated their Ensure intake over the course of the experiment. Moreover, the binge group females consumed significantly more than their male counterparts. With quantitative real-time (qRT-) PCR after the 6 weeks of binge-like eating, we found that control males had lower baseline levels of PACAP mRNA in the PVT than control females, and that, while a history of binge-like eating lead to increased levels of PACAP mRNA in males but not females, levels of PACAP mRNA in both sexes were reduced immediately prior to daily access to Ensure, suggesting that a decrease in PVT PACAP may disinhibit binge-like eating. To determine if PACAP in the PVT could act to inhibit binge-like eating, we trained PACAP-Cre transgenic mice to binge eat using the same limited access Ensure paradigm and injected them in the PVT with either a Cre-dependent excitatory DREADD (n = 6 females, 8 males) or control virus (n = 5 females, 7 males). During the 7th week of binge eating, we injected them within-subject with clozapine n-oxide (5 mg/kg IP) or saline vehicle, and we found that increased activity of PACAP^{+} cells in the PVT significantly and specifically decreased Ensure consumption, and that this effect was driven by male and not female mice. These results show that, like humans, female mice engage in binge-like eating more than male mice, and that this may be driven in part by inherent differences in the PACAP system in the PVT. Specifically, the endogenously higher baseline levels of PACAP in the PVT of female mice may contribute to their resilience to negative feedback signaling that would normally inhibit or reduce binge-like eating, allowing for more binge-like eating in the female population.

Disclosures: B.A. Carpenter: None. **G.R. Curtis:** None. **B.E. Pirino:** None. **J.R. Barson:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.15/QQ4

Topic: G.08. Other Psychiatric Disorders

Support: National Institutes of Health grant NS115631

Title: Longitudinal psycho-physio clustering of chronic pain states

Authors: *R. B. LERICHE[1], A. BEHA[2], J. LIN[2], A. SHAUGHNESSY[2], P. SHIRVALKAR[2], *R. LERICHE[2];
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Abstract: Chronic pain is a debilitating condition affecting 1 in 5 of Americans. Previously, tracking pain precisely and longitudinally has not been feasible. With advances in digital reporting and smart watch tracking, we report daily longitudinal pain fluctuations from five patients (3 females, 0.7-2.75 years, 1141-2153 pain reports) via the numeric rating scale (NRS), visual analog scale (VAS), and McGill Pain Questionnaire (MPQ), as well as, sleep, heart rate, and steps, respectively. Psychometric variation in pain reporting motivated our use of clustering algorithms to identify pain subspaces from the NRS, VAS, and MPQ (2-4 pain clusters per patient). As these patients were undergoing deep brain stimulation for chronic pain, with simultaneous sense capabilities, these pain subspaces may improve neural pain biomarker identification than any metric alone. Future directions include seeing if sleep, heart rate, and/or activity subsets exist within these pain subspaces, and if not, how this physiology may serve as a pain proxy. This work builds towards a personalized medicine approach for chronic pain with unprecedented volume and duration of pain reporting from individual patients.

Disclosures: R.B. Leriche: None. **A. Beha:** None. **J. Lin:** None. **A. Shaughnessy:** None. **P. Shirvalkar:** None. **R. Leriche:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

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Program #/Poster #: PSTR229.16/QQ5

Topic: G.08. Other Psychiatric Disorders

Support: NIDA Grant R00DA045749

Title: Differentiating Responders and Non-Responders to Repetitive Transcranial Magnetic Stimulation for Tobacco Use Disorder

Authors: *M. APOSTOL, T. JORDAN, G. HAASE, L. KIM, G. LIU, N. PETERSEN; UCLA, Los Angeles, CA

Abstract: Cigarette smoking is an immense public health challenge and a leading cause of disease and death worldwide. Non-invasive neuromodulation, such as repetitive transcranial magnetic stimulation (rTMS), may help people quit smoking by altering neural activity. In line with others (e.g., Li et al., 2013), our group previously found evidence that single-session rTMS to the left dorsolateral prefrontal cortex (dlPFC), but not a control target (v5), led to a significant reduction in cigarette craving symptoms (Shiffman-Jarvik Withdrawal Scale craving subscale; SJWS-C). The current study was designed to identify the variables associated with this response. Participants (N = 48) were adults ages 18 - 45 who met DSM-5 criteria for Tobacco Use Disorder. Single-session rTMS (10 Hz, 100% of motor threshold, 5s on / 10s off, 3000 pulses, 15 minutes) treatments were performed targeting left dlPFC (experimental target) or v5 (control target) in a randomized, crossover, placebo-controlled, single-blind experiment. Participants completed the SJWS-C before and after each rTMS treatment. Using only dlPFC data, responders were defined as participants with SJWS-C scores that were reduced after rTMS to dlPFC. We predicted that responders and nonresponders would have significantly different nicotine dependence levels, number of cigarettes smoked daily, depression/anxiety symptoms, and ages (all nondirectional). For the primary hypotheses, mixed-effects linear models were computed with responder/nonresponder status as a fixed effect, participants as random effects, and n = 21 nonresponders and n = 27 responders. A difference in cigarettes smoked per day was trending towards significance, $F(1,46) = 3.30$, $p = .08$, Cohen's $d = 0.54$ (moderate effect size), but no significant differences between responders and nonresponders were detected, all ps > .05. Then, confidence intervals and effect sizes were computed for exploratory variables. Pre-rTMS craving in responders (M = 5.30; 95% CI: 4.75 - 5.84) was higher than nonresponders (M = 3.68; 95% CI: 2.82 - 4.53), Cohen's d = 1.06 (large effect size). Similarly, expired CO was higher in responders (M = 7.04; 95% CI: 5.18 - 8.90) than nonresponders (M = 4.36; 95% CI: 3.21 - 5.61), Cohen's d = 0.69 (moderate effect size). Although none of the hypothesized statistically significant differences were observed in responders versus nonresponders, the results indicated that responders to rTMS displayed higher cigarette use, craving, and expired CO. This study was a step towards understanding how individual differences relate to neuromodulation efficacy. The study design and hypotheses were preregistered at https://osf.io/m58rs.

Disclosures: M. Apostol: None. **T. Jordan:** None. **G. Haase:** None. **L. Kim:** None. **G. Liu:** None. **N. Petersen:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.17/QQ6

Topic: H.01. Attention

Support: UVA Brain Institute
UVA Neurosurgery

Title: Stimulus-preceding Negativity during Focused Attention to Painful Laser Stimulation

Authors: I. BHANGUI, *D. WANG, W. CARTER, R. RAMESH, S. MOOSA, W. ELIAS, C.-C. LIU;
Univ. of Virgina, Charlottesville, VA

Abstract: Painful stimulation evokes physiological and behavioral responses for reducing immediate injury and avoiding future harm through the experience of pain and pain-related brain mechanisms of learning and memory. While neurophysiology studies have demonstrated a number of scalp EEG responses related to the experience of pain and anticipation of pain, little is known about whether these scalp EEG responses are modulated by gender and psychological factors such as pain catastrophizing. In the present study, we used a Nd:YAP laser stimulator (wavelength 1.34μm, beam diameter 5mm, pulse duration 4ms) for nociceptive specific painful stimulation, and measured the stimulus-preceding negativity (SPN) - a slow anticipatory attention-related cortical potential during a focused attention task. Painful laser stimulations were delivered in triplet fashion (i.e. S1, S2, S3 with a fixed inter-stimulus interval of 1.5s or 2s) with constant stimulus intensity (40-50/100 pain intensity adjusted at the baseline), and a random inter-triplet interval between 10-30s. Subjects were instructed to attend to the stimulation and rate each stimulus within each triplet stimulation set. Fourteen subjects (23.9 ± 10.9 yrs, 8 female) were enrolled to the study. Stimulus related EEG recordings (1000Hz sampling rate, 0.1-25Hz bandpass) were extracted into -1 to 5s epochs for SPN and evoked potential analyses. Our preliminary results showed that the SPN for S2 and S3 stimuli within triplet stimulation positively correlated with the amplitudes of laser evoked potential (i.e. negative-positive deflections at 200-400ms post-stimulation (LEP_N2P2); $r = -0.62$, $p = 0.02$, and $r = -0.77$, $p = 0.001$ for S2 and S3, respectively). A positive correlation was found between the SPN and pain intensity in male subjects ($p < 0.05$). Furthermore, pain catastrophizing recorded from the female subjects in the present study showed a significant positive correlation with the SPN enhancement from S2 to S3. These results support the notion that the brain mechanism of anticipatory attention plays a differential role in both the experience of pain and pain-related learning and memory for female versus male subjects.

Disclosures: I. Bhangui: None. **D. Wang:** None. **W. Carter:** None. **R. Ramesh:** None. **S. Moosa:** None. **W. Elias:** None. **C. Liu:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.18/QQ7

Topic: H.01. Attention

Support: UVA Brain Institute
UVA Neurosurgery

Title: Pain-related Cortical Excitatory and Inhibitory Responses in High Pain Catastrophizers

Authors: S. YE, D. WANG, W. CARTER, S. MOOSA, W. J. ELIAS, *C.-C. J. LIU;
Neurosurg., Univ. of Virginia Sch. of Med., Charlottesville, VA

Abstract: Pain catastrophizing is a cognitive and affective appraisal that involves the tendency to attend to or magnify the threat value of pain and feelings of helplessness to cope with pain. Studies have indicated that high pain catastrophizers have difficulty shifting their attention away from painful stimuli, suggesting that brain mechanisms of attention to painful stimuli play a differential role between high- versus low-pain catastrophizers. Cutaneous laser stimulation is an established technique suitable for studying pain-related brain mechanisms of attention. The most obvious scalp electroencephalogram (EEG) responses to painful laser stimulations are a negative-positive biphasic deflection (LEP_N2-P2, 220-350ms), and the amplitudes of LEP_N2-P2 responses has been demonstrated to represent complex cortical activities reflecting both the nociceptive transmission to the cerebral cortex and modulated by attention related cerebral information processing underlying the experience of pain. Furthermore, EEG responses may also depend on the cortical excitability and inhibitory processes that allows individuals to appropriately adapt to changes in the environment which can be assessed by repetitive stimulations (i.e. application of stimuli in close succession). In the present study, we used a Nd:YAP laser (wavelength 1.34μm, beam diameter 5mm, pulse duration 4ms) for painful stimulation in a focused attention task to test the hypothesis that painful-related cortical excitatory and inhibitory responses vary as a function of pain catastrophizing in health controls. Painful laser stimulations were delivered in triplet fashion (i.e. S1, S2, S3 with a fixed inter-stimulus interval of 1.5s or 2s) with a constant stimulus intensity (40-50/100 pain intensity adjusted at the baseline), and a random inter-triplet interval between 10-30s. Subjects were instructed to attend to the stimulation and rate each stimulus within each triplet stimulation set. Thirty-seven subjects (21.3 ± 4.2 yrs, 21 female) were enrolled to the study. Our preliminary results showed a positive significant correlation between pain magnification scores and the amplitude of LEP_N2P2 evoked by the first stimulus (S1) ($r = 0.4$, $p = 0.1$), indicating greater cortical excitability in high pain catastrophizers. Furthermore, subjects with higher a pain magnification scores showed greater inhibitory responses evoked by the test stimuli (i.e., S2 or S3) within the triplet stimulation trains (S2: $r = -0.43$, $p = 0.007$ and S3: $r = -0.34$, $p = 0.04$). These findings suggest pain-related cortical excitatory and inhibitory responses vary as a function of pain catastrophizing in health controls.

Disclosures: S. Ye: None. **D. Wang:** None. **W. Carter:** None. **S. Moosa:** None. **W.J. Elias:** None. **C.J. Liu:** None.

Poster

PSTR229. Basic and Clinical Neuroscience in Psychiatry

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR229.19/QQ8

Topic: G.08. Other Psychiatric Disorders

Support: Regis University - Center for Scholarship & Research Engagement

Title: Investigating the impacts of activity-based anorexia and recovery on anxiety behavior and stress-induced activation of brainstem glucagon-like peptide 1 and forebrain neurons in adolescent female rats

Authors: *M. BRANNON, M. UYEMURA, M. GOLDSCHMIDT, A. LARSON, M. LOPEZ, K. AUGER, J. W. MANISCALCO;
Neurosci., Regis Univ., Denver, CO

Abstract: Anorexia nervosa (AN) is characterized by body weight loss resulting from food restriction and increased physical activity in conjunction with body dysmorphia and fear of weight gain. AN has the highest mortality rate among all psychiatric diseases and although therapeutic treatments exist, relapse rate is high, ~20-30% of those suffering remain chronically ill, and >10% die from the disorder. This highlights the need for better preventative measures and therapeutic interventions, which rely on an improved understanding of the neurobiological mechanisms underlying AN. Rodent studies indicate that overnight fasting reduces anxiety – a key factor in the development and maintenance of AN – in part by reducing stress-induced activation of specific neural circuits, including brainstem glucagon-like peptide 1 neurons and their downstream targets in the paraventricular hypothalamus (PVN) and bed nucleus of the stria terminalis (BNST). It is possible that prolonged periods of food restriction, such as those used in animal models of AN, may alter anxiety via changes in these same neural circuits. Considering this, we exposed adolescent female rats to activity-based anorexia (ABA), in which rats are food restricted and allowed voluntary access to exercise. Rats in the ABA paradigm show precipitous body weight loss, hyperactivity, and other characteristics of AN. Once ABA rats lost 20-25% of baseline body weight, they underwent one of two treatments: 1) exposure to a 30-minute restraint stress followed by sacrifice via transcardial perfusion, or 2) a body weight recovery phase via removal of exercise wheels and restoration of *ad libitum* food. After regaining body weight to the level of controls, rats in cohort 2 were tested for anxiety-like behavior on the elevated plus maze (EPMZ) and open-field test (OFT) and later sacrificed via transcardial perfusion following 30-minute restraint stress. Immunohistochemical localization of cFos in the PVN and BNST, and colocalization of cFos and GLP-1 neurons in the hindbrain, was quantified to assess neural activation following stress exposure. Interestingly, we found no differences in anxiety-like behavior in the EPMZ (entries into and time spent in open/closed arms) or OFT (time/entries in perimeter vs. center of field) between body weight recovered ABA rats and controls ($p > .05$). Preliminary results show altered GLP-1 and forebrain neural responses to restraint stress in ABA rats, despite the lack of overt changes in anxiety behavior. Our findings suggest that a single, acute exposure to the ABA paradigm may not be sufficient to elicit prolonged changes in anxiety behavior but may be sufficient to alter neural stress responses.

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Poster

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Topic: G.08. Other Psychiatric Disorders

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Hjarnfonden PS2020-0049
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Title: Transcript expression profile of Substance P, NPY, CCK and their receptors in five brain regions in major depressive disorder with a focus on locus coeruleus.

Authors: *S. BARDE[1], J. AGUILA[1,2], W. ZHONG[3], A. SOLARZ[1], I. MEI[2], J. PRUD'HOMME[4,5], M. PALKOVITS[6], G. TURECKI[4,5], J. MULDER[1], M. UHLEN[3], C. NAGY[4,5], N. MECHAWAR[4,5], E. HEDLUND[1,2], T. G. HOKFELT[1];
[1]Karolinska Institutet, Stockholm, Sweden; [2]Stockholm Univ., Stockholm, Sweden; [3]Sci. for Life Lab., Stockholm, Sweden; [4]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [5]Mc Gill Univ., Montreal, QC, Canada; [6]Neuromorphological and Neuroendocrine Res. Laboratory, Dept. of Anatomy, Histology and Emb, Semmelweis Univ. and the Hungarian Acad. of Sci., Budapest, Hungary

Abstract: Major depressive disorder (MDD) is a predominant contributor to disability worldwide with approximately 800,000 suicide deaths per year and a substantial burden to patients, families and society. Medications pose certain drawbacks, including delayed therapeutic response, inefficiency in about 2/3 of patients and often side effects. Several preclinical studies suggest that neuropeptides participate in stress response and are modulated by stress. Thus, these peptide systems are potentially important candidates in depression and deserve further exploration. Substance P (SP)/tachykinin (TAC), neuropeptide Y (NPY), cholecystokinin (CCK) and their multiple receptors are widely expressed and distributed in the rodent and human brain, as shown in a large number of studies using various methods. In the present study, we focus on three peptide families, namely, SP/TAC, NPY and CCK and their respective receptors.Transcript expression was quantified for these three neuropeptide families using qPCR in five regions of the human brain from controls and depressed suicide subjects (DSS): dorsolateral prefrontal cortex

(DLPFC), anterior cingulate cortex, dorsal raphe nucleus (DRN), locus coeruleus (LC) and medullary raphe nucleus. In addition, using laser capture microdissection, individual neurons from human LC of 20 control subjects were isolated, pooled and subjected to Smart-seq2 RNA sequencing. Raw and dCT values reveal differences in the expression levels of the above markers with the predominant levels for several *TAC* and *TACR* transcripts in the DRN and LC, and for *NPY*, *NPYR1*, *CCK* and *CCKBR* transcripts in the PFC regions. Significant sex differences for controls were recorded in the DLPFC controls (*TAC1, TACR1 and NPY* higher in male controls) and LC (*NPY* higher in female controls). Elevated expression levels in the DLPFC of DSS were observed for SP, TAC and TAC3 in males, SP in males and NPYR1 in both sexes. In the LC, mRNA levels in DSS were increased for all TAC family transcripts in females, *SP*, *TACR1* and *TACR3* in males, *NPY* in both sexes, and *NPYR1* in males. Using the Smart-seq2 RNA sequencing, we observed significant levels of *GAL*, *NPY*, *TAC1*, *CCK*, and *TACR1* in subpopulations of LC neurons, as well as of many other peptide (e.g. *CARTPT*) and receptor (e.g. *ADCYAP1R1*) transcripts.Taken together these findings show parallel but differential changes in the transcript levels of two neuropeptide families in depressed suicide subjects. Our results may assist in the search for development of novel treatment strategies based on targeting the receptors of these three neuropeptides.

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Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR230.01/QQ10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R00DA043573
NIDA R21DA054929
NIGMS P20GM103434

Title: Intravenous fentanyl self-administration in genetically diverse mice

Authors: *A. KASTIGAR, M. LEONARDO, B. GOURLEY, J. LIPOVICH, A. WALDEN, Z. MCCONNELL, J. HUFFMAN, S. BRUNTY, P. E. DICKSON;
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Abstract: Fentanyl overdose is the leading cause of death for Americans aged 18 to 45. This striking statistic underscores the urgent need to discover and characterize the genetic drivers of fentanyl addiction. Although addiction and addiction-like behaviors are highly heritable in

humans and mice, the genetic underpinnings of fentanyl taking and seeking are poorly understood. Identifying these mechanisms will facilitate treatment in humans and will reduce the morbidity and mortality that is caused by fentanyl addiction. To this end, we quantified intravenous fentanyl self-administration in male and female mice (N = 175) from ten genetically diverse strains (C57BL/6J, DBA/2J, A/J, NOD/ShiLtJ, PWK/PhJ, CC002/Unc, CC005/TauUnc, CC007/Unc, CC019/TauUnc, CC051/TauUnc). These strains are members or founders of the Collaborative Cross (CC) and BXD recombinant inbred mouse panels. We used these strains because recombinant inbred mouse panels, due to their genetic diversity and when used in the context of a systems genetics approach, enable discovery and dissection of heritable mechanisms underlying complex traits such as addiction. We used intravenous drug self-administration because it enables exquisite decomposition of the multifaceted addiction construct into its component parts. To fully capture the range of behaviors that together drive fentanyl taking and seeking, we quantified classical pharmacological phenotypes (e.g., dose-response) and addiction-like behaviors (e.g., escalation of drug taking, resistance to extinction, vulnerability to relapse). From this experiment, we reached several conclusions. First, addiction-like fentanyl taking and seeking are highly heritable in mice. Second, the behaviors that collectively drive fentanyl taking and seeking are genetically dissociable. Third, the variation in intravenous fentanyl self-administration across the genetically diverse mouse strains used in this study is far outside the range observed in the commonly used C57BL/6J strain. Collectively, these observations underscore that genetic diversity is necessary to capture the phenotypic diversity that, at its extremes, represents vulnerability and resistance to addiction-like fentanyl taking and seeking. Moreover, the high heritability of the measured intravenous fentanyl self-administration behaviors reveals that recombinant inbred mouse panels can be used to discover and dissect the genetic underpinning of the distinct phenotypes that, together, drive fentanyl addiction.

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Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR230.02/QQ11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R00DA043573
NIDA R21DA054929
NIGMS P20GM103434

Title: Self-administration of social reward and IV cocaine in genetically diverse mice

Authors: *M. LEONARDO, A. KASTIGAR, A. WALDEN, B. GOURLEY, J. LIPOVICH, P. E. DICKSON;
Marshall Univ., Huntington, WV

Abstract: Social interaction is rewarding in humans and rodents, and this “social reward” phenotype varies across individuals. Variability in social reward may, at least in part, be heritable, and these genetic underpinnings may overlap with those driving drug use. These hypotheses have been largely unexplored. Identifying and dissecting shared genetic mechanisms driving social reward and drug reward may provide insight into addiction across rewards. In the present study, we quantified social reward in male and female mice from nine genetically diverse mouse strains (C57BL/6J, DBA/2J, A/J, NOD/ShiLtJ, PWK/PhJ, CC002/Unc, CC005/TauUnc, CC019/TauUnc, CC051/TauUnc). These strains are members or founders of the Collaborative Cross (CC) and BXD recombinant inbred mouse panels. We used these strains because recombinant inbred mouse panels, when used in the context of a systems genetics approach, enable discovery and dissection of heritable mechanisms underlying complex traits. To quantify social reward, we used an operant self-administration paradigm: mice could depress a lever to raise a guillotine door allowing brief interaction with another mouse of the same sex. In the first experiment, non-catheterized mice were tested for 28 daily two-hour sessions to quantify social reward; a rewarded group and control group were used. In a second experiment, we implanted a jugular catheter prior to social reward testing and mice could choose to self-administer either a social reward or an IV infusion of cocaine. From these experiments, we reached the following conclusions. First, exposure to a conspecific reinforced lever pressing in mice. Second, the magnitude of social reward varied across strains. Finally, mice exhibited a significant preference for social reward relative to cocaine. Collectively, these data indicate that social reward is a heritable phenotype in the mouse that can be quantified using an operant conditioning paradigm.

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Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR230.03/QQ12

Topic: G.09. Drugs of Abuse and Addiction

Title: Whole body vibration prevents neuronal and behavioral effects of chronic morphine withdrawal in a rat model

Authors: *A. AMENDOLARA, N. HILL, A. HILL, T. PURCELL, T. ALGER, G. JONES, C. SMALL, S. STEFFENSEN, D. SANT, K. BILLS;
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Abstract: Previous research suggests that applied mechanical stimulation (Mstim), like osteopathic or chiropractic manual manipulation, modulates substrates which activate the mesolimbic dopaminergic (DA) pathway between the ventral tegmental area (VTA) and nucleus accumbens (NAc). While this treatment has been explored in rats with alcohol dependency, it has not previously been studied in a context of opioid withdrawal. We aim to determine whether MStim treats the effects of morphine withdrawal as manifested behaviorally, electrophysiologically and electrochemically. Twenty-four male Wistar rats (n=12 with MStim treatment) received MStim (80 Hz; 15 min) applied via vibration plate 2 times per day for 2 weeks immediately following morphine administration (1mg/kg; IP). The effects of Mstim were evaluated using single-unit electrophysiological recordings of VTA GABA neurons in withdrawal, elevated plus maze and ultrasonic vocalizations (USV). Following chronic morphine exposure, a reinstatement dose in naïve versus MStim rats caused alterations to firing of VTA GABA neurons (117.5% baseline versus 32.7% baseline; 2.5mg/kg; $p<0.0001$) and to dopamine release in the nucleus accumbens (20 min: EtOH vs EtOH + MStim, $F_{(3,24)} = 3.4964$, $p=0.0310$; 80 min: $F_{(3,23)} = 9.0058$, EtOH vs EtOH +MStim, $p=0.0004$, EtOH and naïve, $p<0.0001$). Additionally, rats in withdrawal from morphine that underwent MStim treatment spent significantly more time ($p < 0.05$) in open areas of the Elevated Plus maze as compared to naive rats. Treated rats also showed increased pain tolerance (decreased hyperalgesia, $p < 0.05$) and generally exhibited higher USV call frequencies than naive rats ($p < 0.05$). This study represents a mechanistic look at the effects of targeted mechanoreceptor activation in the context of opioid exposure. It represents preliminary evidence that there is a mechanistic basis for future studies in humans. These data, when taken as a whole, provide a mechanistic rationale for future human studies to explore physical medicine modalities, including manipulative therapeutics and acupuncture as treatment options.

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Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Epigenomic investigation of TET1's cell-type and sex-specific role in cocaine addiction

Authors: *Y. LI, H. XU, J. M. CHITAMAN, N. WADDELL, G. KAPLAN, K. ABREU, C. REMEDIES, J. FENG;
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Abstract: DNA methylation is an essential epigenetic pathway profoundly influencing brain plasticity and function. TET1 is a methyl-cytosine dioxygenase facilitating the cytosine oxidation and methylation turnover in the brain which is dysregulated in response to cocaine usage. In our study, upon conditional knock-out of *Tet1* (*Tet1*cKO) in mouse *Drd1*-expressing medium spiny neurons (D1-MSN), we observed sex-dependent effects in cocaine addiction-related behaviors. In addition, these behavioral alterations were associated with diminished or reversed genome-wide DNA methylation changes between sexes. Further investigation unveiled that both the Tet1-dependent and sex-specific CG/non-CG differential methylation regions (DMRs) were often aggregated in megabase hotspot regions (DHRs). Employing chromosome conformational capture sequencing(Hi-C), we discovered that a portion of DHRs exhibited higher-order genome organization changes. Together, the integrated multi-omic analyses demonstrate TET1's effects are selectively enriched in synaptic function-related genes. Therefore, our study illuminates an intricate interplay between TET1 and addiction behaviors in a cell-type and sex-dependent manner.

Disclosures: **Y. Li:** None. **H. Xu:** None. **J.M. Chitaman:** None. **N. Waddell:** None. **G. Kaplan:** None. **K. Abreu:** None. **C. Remedies:** None. **J. Feng:** None.

Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR230.05/QQ14

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00045758

Title: Cocaine extinction day 1 exposure upregulates sex-specific transcripts in rat dorsal hippocampus pyramidal neurons

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Abstract: Abstinence success is particularly complex in women as psychological and biological responses to drugs of abuse differ in women compared to men. Current RNA-Seq analysis of gene expression in sex differences remains sparse, lacks depth, and has not been linked to specific high-risk behavioral outputs, e.g. cocaine-seeking. Furthermore, the understanding of transcripts that contribute to relapse susceptibility and/or resilience remains unknown. Thus, understanding circuit and molecular signatures that drive increased cocaine seeking among females is critical to the development of effective SUD therapies. Extinction day 1 (ED1) marks the initiation of abstinence where the expected drug is not available, and is a particularly stressful time point where drug cravings may increase. Since cravings during the initiation of abstinence in humans and rodents predicts later relapse in both species, ED1 may be a critical time point for

targeting treatment in addiction. Herein, we used whole-transcriptome sequencing (RNA-Seq) analysis to identify sex-specific gene expression patterns elicited by exposure to the cocaine self-administration context on ED1 that correlate with cocaine seeking behavior. Fresh-frozen whole dorsal hippocampus from male and female Sprague-Dawley rats were sacrificed as naïve, in 24hr withdrawal from cocaine (WD1), or immediately following ED1 testing. Gene Set Enrichment Analysis of 14,605 transcripts indicates that few transcripts and pathways were differentially regulated between males and females in the dorsal hippocampus under naïve, withdrawal, or ED1 conditions. However, we identified 22 transcripts in females and 149 transcripts in males that had 2.0-fold change differences on ED1 compared to naïve or withdrawal day 1 rats. Notably, of these transcripts, 5 in females significantly correlated to the expression of ED1 cocaine-seeking with a R2 &lt 0.70. Therefore, we used multiplexed RNAscope *in situ* hybridization to localize the cell-specific expression of five genes Erg1, Lrch4, Baiap3, Lrrcl4, Kcnt1; those that significantly correlated to cocaine-seeking behavior on ED1. We found that these cocaine-seeking predicting transcripts co-localized with Fos+ immunohistochemistry reactivity selectively in dorsal hippocampus pyramidal neurons of female rats on extinction day 1. We propose the localization of activity-dependent transcripts that respond to a critical time point in addiction that predicts later relapse may comprehensively identify key factors that inform circuit recruitment during cocaine-seeking in early abstinence.

Disclosures: **S. Kelsen:** None. **J. Zhao:** None. **M.M. Berry:** None. **A.S. Kohtz:** None.

Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

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Program #/Poster #: PSTR230.06/QQ16

Topic: G.09. Drugs of Abuse and Addiction

Title: The relationship between major psychiatric disorders, substance use behaviors, and longevity: A multivariable Mendelian randomization and multi-omics study

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Abstract: Observational studies suggest major psychiatric disorders, including bipolar disorder (BD), schizophrenia (SCZ) and major depressive disorder (MDD) reduce longevity; however, frequently co-occurring alcohol consumption and smoking have also been shown to impact longevity, making it difficult to disentangle which among the co-morbid disorders is driving this reduction. Here we deploy Mendelian randomization (MR) and transcriptomic imputation to parse the associations these disorders have to longevity. This study sourced existing summary-level genome-wide association study (GWAS) data from European-ancestry cohorts on psychiatric disorders, substance use behaviors, cardiometabolic diseases, multivariate longevity,

and epigenetic aging acceleration (EAA).MR analysis shows MDD, weekly alcohol consumption, and smoking negatively associate with longevity in a single-variable model; while only smoking negatively associates in a multivariable one. MR also shows a corresponding positive association between smoking and PhenoAge-measured EAA, and that these smoking associations are cardiometabolic disease-independent.Transcriptome-wide association studies on smoking across several tissue types identified 118 novel gene-smoking associations not captured by the original GWAS. Colocalization analysis revealed several molecular features which share a causal variant with both lifetime smoking index placement and longevity, including PRMT6 transcripts and those for five genes in locus 17q21.31. Broadly, novel and colocalized genes were involved in cellular proliferation, learning, and neurodevelopment. Drug-target MR analysis identified genes upstream of smoking behavior whose protein products are amenable to drug targeting and involved in reward processing and nicotine metabolism. Using genomic methods that enable the simultaneous assessment of major psychiatric disorders and substance use behaviors, we provide evidence that smoking—but not drinking, MDD, BD, nor SCZ—has a negative independent association with longevity that is recapitulated in the transcriptome. Our findings highlight the importance of developing smoking cessation therapies for the longevity of psychiatric populations, and identify prime drug-target candidates for such future translational research.

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Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

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Program #/Poster #: PSTR230.07/QQ17

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant DA026861

Title: Recent cocaine-use pattern and problem severity differ by corticotropin-releasing hormone receptor 1 (CRHR1) genotype

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Abstract: Background: Stress-exposure plays a critical and complex role in substance use disorders and comorbid negative affective states (e.g., depression, anxiety). Corticotropin-releasing hormone (CRH) signaling at receptor type 1 (CRHR1) mediates stress-reactivity and impacts drug-taking behaviors in animal models. CRHR1 genetic variation may underlie differences in cocaine-use phenotypes, but the role of common CRHR1 single nucleotide polymorphisms (SNPs) in cocaine-related behaviors remains unclear. Methods: The present

clinical study assessed relationships between two CRHR1 intronic SNPs, rs242924 and rs173365, and various aspects of cocaine use among recent cocaine users. As these SNPs were in linkage disequilibrium, results are reported for rs242924. Based on allelic frequencies, participants were grouped into A/A homozygotes or C-allele carriers (A/C or C/C). Results: Compared to CRHR1 rs242924 C-allele carriers (n=22), A/A homozygotes (n=40) exhibited significantly ($p<.05$) greater binge cocaine use and cocaine problem severity. A significantly higher proportion of A/A homozygotes reported having sought treatment for cocaine use, and this group scored significantly higher on measures of impulsivity and depressive symptoms than C-allele carriers. Conclusion: CRHR1 genetic variation (rs242924 A/A) is associated with increased binge cocaine use, problem severity and associated negative affective states. These findings merit further attention to elucidate mechanisms of and treatments for cocaine use disorder involving CRHR1.

Disclosures: A.T. Mascarin: None. **M. Burmeister:** None. **M.K. Greenwald:** None.

Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

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Program #/Poster #: PSTR230.08/Web Only

Topic: G.09. Drugs of Abuse and Addiction

Support: Tobacco-Related Disease Research Program Project Grant [22RT-0103, T31IP1427]
NIDA T32 training grant (T32DA050558)

Title: Potential protein coding activity in human CHRNA6 3'UTR

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Abstract: One of every three high school students in the United States have been exposed to nicotine through tobacco products during 2021. The inherent plasticity of this vulnerable developmental period results in heightened susceptibility for nicotine addiction and consequent drug dependency. Clinical and preclinical studies demonstrate nicotine exposure during adolescence, even at low doses, disrupts normal brain development and enhances the rewarding properties of other drugs (i.e., "gateway hypothesis"). Nicotine activates nicotinic acetylcholine receptors (nAChRs) which mediate neurotransmitter release and may be altered with early exposure to nicotine. In particular, α;6-containing nAChRs (α;6*nAChRs) have been implicated in nicotine-induced locomotion, dopamine release, and nicotine self-administration. Human studies have identified a single nucleotide polymorphism (SNP), a single base pair variation, within an untranslated region of the α;6*nAChR subunit gene, *CHRNA6*, associated with nicotine and other substance use. To study this specific SNP (C to G) and its role in nicotine

exposure, our lab engineered two rat lines replacing the entire rat 3'-untranslated region (UTR) with the human 3'-UTR containing the SNP of interest ($\alpha6^{CC}$ and $\alpha6^{GG}$). We have shown the *CHRNA6* 3'UTR SNP enhances nicotine related behaviors in a sex and genotype dependent manner *in vivo*. In addition to the SNP, each rat line contains two small open reading frames (smORFs) within the *CHRNA6* 3'-UTR with potential for translation into microproteins (4 total). The microproteins would include a putative 35 amino acid (AA) truncated smORF microprotein for $\alpha6^{GG}$, a 45 AA smORF microprotein for $\alpha6^{CC}$, and two 72 AA smORF microprotein with a non-synonymous radical substitution from $\alpha6^{CC}$ and $\alpha6^{GG}$ (Threonine (T) to Arginine (R) respectively). We used HEK293T cells transfected with each putative microprotein designed with an ALFA-tag to evaluate expression via Western blot. We show stable expression of the 45 AA microprotein for $\alpha6^{CC}$, but not $\alpha6^{GG}$. These results indicate novel protein coding activity in the 3'UTR of *CHRNA6 in vitro.* Our findings provide a potential mechanism by which the *CHRNA6* 3'-UTR SNP regulates these nicotine seeking behaviors.

Disclosures: E.M. Castro: None. **T.F. Martinez:** None. **S. Lotfipour:** None.

Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

Location: WCC Halls A-C

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Program #/Poster #: PSTR230.09/QQ18

Topic: G.09. Drugs of Abuse and Addiction

Support: Penn State internal funds

Title: The human dopamine transporter gene (SLC6A3) contains a hyper-variable number of tandem repeats

Authors: *D. J. VANDENBERGH[1], A. T. APLSEY[2], M. A. VERBIEST[3], W. J. STONE[1], E. R. DOMICO[1], M. ANISIMOVA[3];
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Abstract: The Dopamine Transporter plays a central role in regulating dopamine signaling, but genetic analyses of SLC6A3 have generated conflicting results with dopamine-related traits. This lack of consistency might result from an inability to test relevant loci in the gene. We used haplotype-phased, long-read genomic sequence data and Tandem Repeat Annotation Library (TRAL) to identify 5 Tandem Repeats that vary in copy number (VNTR) in SLC6A3 and characterized 3 that have not been studied. Copy number and sequence similarity of each repeat unit of the five VNTRs is presented. One VNTR in intron 8 is hyper-variable (hyVNTR) with a range of 3.4-133.4 repeat copies and 46 alleles in the 64 chromosomes analyzed (93% heterozygosity). The consensus sequence of the repeat unit is 38 bp with 82% G+C content. The repeat is predicted to form G-quadruplexes (G4s, G-tetrads) in silico, which was confirmed by

circular dichroism spectroscopy. Multiple putative PRDM9 binding sites (recruiter of recombination promoting proteins) are present in the repeat, and it is in low linkage disequilibrium (LD) with flanking genetic markers (r2=0.016 and 0.001), suggesting it is a hotspot for recombination. Studies of other sites in SLC6A3 cannot estimate genetic effects of this hyVNTR due to lack of LD.

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Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

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Program #/Poster #: PSTR230.10/QQ19

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA030505
NIH Grant DA048085

Title: Limbic Pallidum Deep Brain Stimulation for the Treatment of Severe Alcohol Use Disorder

Authors: *N. E. MILLER, O. STUDNICKI, N. ARNOLD, K. MOUSSAWI;
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Abstract: Alcohol Use Disorder (AUD) is a serious healthcare and societal burden with an estimated cost of $249 billion dollars per year. Current AUD treatments have limited efficacy, and relapse rates have not changed over the past 50 years. The mortality rate in individuals with severe AUD and advanced alcohol-related liver disease (ALD) is greater than 50% in 5 years but is reversible with abstinence from alcohol use. In those high-risk patients, we propose to the test the safety, feasibility, and preliminary efficacy of Deep Brain Stimulation (DBS) of the limbic pallidum, a critical node in the brain's reward circuit, for the treatment of AUD. The study population includes participants between the ages of 21 and 75 years with severe and refractory AUD and advanced ALD (stage 3 liver fibrosis or compensated cirrhosis). After informed consent, participants undergo baseline evaluations including clinical, neuropsychological, and AUD assessments (blood tests, questionnaires, alcohol Timeline Follow Back). In addition, neurocognitive tasks are performed to assess cognitive and behavioral domains associated with AUD (e.g., impulsivity, risk-taking, reward processing dysfunction). The DBS system (Sensight directional leads and Percept neurostimulator) is then implanted over two stages. The DBS system is turned on 3-4 weeks after surgery and titrated based on side effects and craving reports. Participants are followed closely (biweekly, then monthly) to assess adverse events and alcohol use. In one implanted subject, follow-up evaluations after DBS activation show reduced alcohol use as indicated by increased percent days abstinent and reduced drinks per drinking day (based on Alcohol Timeline Follow Back), alcohol-related cue reactivity (based on Cue Reactivity

Task), cravings (based on Alcohol Craving Questionnaire scores), drinking compulsivity (based on Obsessive Compulsive Drinking Scale scores), and alcohol dependence severity (based on Alcohol Dependence Scale scores). As this study is ongoing, more data are expected in the coming weeks/months. Overall, results from one study participant so far show reduced alcohol use and improved behavioral measures associated with severe AUD with limbic pallidum DBS.

Disclosures: N.E. Miller: None. **O. Studnicki:** None. **N. Arnold:** None. **K. Moussawi:** None.

Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

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Program #/Poster #: PSTR230.11/QQ20

Topic: G.09. Drugs of Abuse and Addiction

Support: NCCIH Grant R01AT010627
NIDA Grant R01DA048301
NIDA grant T32DA053558

Title: Sex differences in drug cue-reactivity and its reappraisal in heroin and cocaine use disorders -- an fMRI study

Authors: *Y. HUANG[1], A. O. CECELI[1], G. KRONBERG[1], S. G. KING[2], A. BRACKETT[1], G. N. HOBERMAN[1], N. E. MCCLAIN[1], N. ALIA-KLEIN[3], R. Z. GOLDSTEIN[3];
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Abstract: In drug addiction, women tend to present with a higher risk for relapse, greater severity of drug abuse, and an earlier age onset of drug use. Previous fMRI studies have shown sex differences in cocaine use disorder (CUD), with men exhibiting higher drug-related, and women exhibiting more stress-related, corticostriatal reactivity. Strikingly, our recent findings have revealed higher opioid/stimulant mortality rates in men than women. However, given inconsistent sex-specific findings in preclinical studies, and a paucity of clinical investigations of sex differences in drug, including heroin use disorder (HUD), our understanding of the sex differences in the neural processes underlying drug cue-reactivity and its potential regulation is incomplete. Here, 51 men (age = 42.38 ± 9.88) with HUD and 28 women [16 with HUD and 12 with CUD; age = 39.50 ± 10.02] underwent a novel cue reactivity fMRI task where they were instructed to passively view heroin or cocaine (congruent with drug of choice), food, and neutral cues, reappraise heroin or cocaine cues, and savor food cues. We hypothesized sex differences in drug cue-related corticostratal reactivity, including in the prefrontal cortex (PFC). Given absence of prior study into sex specific effects, exploratory analyses examined emotion regulation strategies (reappraisal and savoring). Although subjective pre-, post-, and post- minus pre-task drug craving as well as cue-induced drug craving, valence, and arousal ratings revealed no significant sex differences [W=468.5, p=.02>(0.05/6)], preliminary region of interest analyses,

targeting subgenual and rostral anterior cingulate cortex, showed that passive drug vs. neutral cue viewing was associated with hyperactivations in these two regions in women vs. men. Whole brain analyses further showed hyperactivations in the medial and dorsomedial PFC during drug vs. food cue viewing and hypoactivation in the frontal eye fields when reappraising (vs. passively viewing) drug cues in women vs. men (all above mentioned significant results are $Z>3.1$, corrected to $p<0.05$). These results were not driven by differences between women with HUD vs. CUD. There were no sex differences in savoring (vs. passively viewing) food cues, nor in the contrast directly comparing the two emotion regulation strategies. Our findings suggest enhanced drug cue-reactivity and impaired reappraisal-related corticostriatal engagement in women vs. men with drug addiction, underscoring the importance of considering sex differences in future explorations of craving and other main symptomatology in drug addiction.

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Poster

PSTR230. Addiction: Genetics, Translational, and Clinical Studies

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: JSPS Grant-in-Aid for Challenging Exploratory Research (19K22511)
JSPS Research Fellowship for Young Scientists (21J23266)
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Institute of Seizon and Life Sciences
Kyoto University Foundation
Nakatani Foundation

Title: Neurobehavioral Characteristics of the Impulse Control Disorder Kleptomania

Authors: *Y. ASAOKA[1], M. WON[3], T. MORITA[4], E. ISHIKAWA[4], Y. GOTO[2];
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Abstract: Kleptomania an impulse control disorder that is characterized by compulsive and impulsive stealing for the sake of act itself but not motivated by the sense of needs. The disorder was already described in early 19the century, but has barely been targeted for neuropsychological investigations, and its mechanisms have remained largely unclear to date. In this study, we investigated cognitive and affective deficits and associated neural activities, as well as physiological characteristics, such as peripheral monoamine and hormone concentrations and epigenetic changes, in patients with kleptomania. Administration of a psychological task such as Jumping-to-Conclusion test combined with recordings of prefrontal cortical (PFC)

activity using functional near-infrared spectroscopy (fNIRS) unveiled deficits in probability estimation and decreased PFC activity in kleptomania patients. Moreover, kleptomania patients exhibited distinct gazing patterns and PFC responses to situational cues associated with their symptoms. Kleptomania patients reported heighted negative affects, such as stress, depression, and anxiety, compared to healthy subjects. In addition, although kleptomania patients also rated themselves more aggressive than healthy subjects, implicit assessments with a point-subtraction aggression test revealed comparable level of aggression between patients and healthy subjects, suggesting altered self-recognition. Consistent with these behavioral observations, testosterone and cortisol blood concentrations were lower and higher, respectively, than those of healthy subjects. Further biochemical assays in blood samples obtained from kleptomania patients revealed that increased ratio of dopamine to its metabolites and decreased ratio of norepinephrine to its metabolites. Moreover, genome-wide DNA methylation analysis found alterations of methylation status on the gene encoding membrane trafficking and associated with other psychiatric disorders, such as schizophrenia, autism spectrum disorder, and drug addiction. Collectively, these multiple lines of evidence suggest that many, but not all neurobehavioral characteristics overlap with those reported in drug addiction, and therefore kleptomania may be considered as behavioral addiction.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.01/QQ22

Topic: G.09. Drugs of Abuse and Addiction

Support: DA038042
MD007579

Title: Beta-adrenergic receptor blockade persistently disrupts retrieval of fentanyl-associated memory

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Abstract: Retrieval of drug-associated memories triggers craving and relapse in substance users, hindering treatment and cessation of use. Disrupting retrieval of drug-associated memories could significantly reduce or eliminate seeking and relapse rates in addicts. Previous work from our lab has shown that systemic β-adrenergic receptor blockade persistently impairs retrieval of cocaine-associated memories (Otis et al., 2011), an effect that could be isolated to the prelimbic medial prefrontal cortex (mPFC; Otis et al., 2013) and dorsal hippocampus (Otis et al., 2014). These findings suggest a retrieval network critical for the maintenance of substance abuse, but whether

this serves as a common network for retrieval following any drug of abuse is unknown. Here we assessed whether retrieval of opioid-associated memory using the potent synthetic opioid, fentanyl, could be disrupted by β-adrenergic receptor blockade. We established a fentanyl conditioned place preference (CPP) following conditioning over 8 days in which rats (N=16) associated fentanyl with one of two distinct chambers. At test, rats had free access between chambers and time in each was recorded. We found that propranolol (10 mg/kg, i.p.), a β-adrenergic receptor antagonist, administered prior to the first retrieval test persistently impaired fentanyl CPP compared to saline ($p<0.005$). Next, we determined whether the prelimbic mPFC was a common node in the retrieval network for drug-associated memories. Following conditioning, rats were bilaterally infused with propranolol (10 ug/ul at a volume of 0.3 ul per side) or saline into the prelimbic mPFC. These investigations are underway to determine the specific brain regions common to addiction-retrieval pathways across drug classes. Overall, these findings suggest a widespread therapeutic use for propranolol in the treatment of addiction through prevention of drug-associated memory retrieval to reduce cue-elicited drug seeking and relapse.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Topic: G.09. Drugs of Abuse and Addiction

Support: MBRS-SCORE-1SC2DA047809
NIGMS-RISE-R25GM061838
NeuroID
NIGMS-1R16GM149491-01

Title: Increased BDNF expression in the hippocampus of male and female rats in the extinction of morphine place preference

Authors: *W. V. RICHIEZ MATEO[1,2], M. E. LLORET TORRES[3], C. M. TORRES RODRIGUEZ[4], P. BONILLA RULLAN[3], R. AYALA[3], L. HERNANDEZ ROMAN[4];
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Abstract: Drug-related overdoses in the United States have steadily increased over the past decades;however, opioids account for 74.8% of these deaths (CDC, 2022). Addiction is recognized as acognitive disorder of chronic drug-seeking relapse; caused by aberrant learning patterns thatinduce neuroplasticity damage in the circuits of the mesocorticolimbic dopaminergic system.Understanding the role of brain plasticity in addiction is essential for mitigating the

currentpublic health crisis. Previously, we showed that three distinctive behavioral phenotypes wereobserved after morphine conditioning and forced-extinction training: the sham-extinction,extinction, and extinction resistant groups. In addition, the neuroplasticity transcript bdnf wasupregulated within the ventral striatum/nucleus accumbens (VS/Nac) of males that extinguishedmorphine-conditioned place preference (CPP) after extinction training. Using the same methods,we found that although BDNF protein expression showed no significant difference in theVS/Nac, both males and females showed an upregulation within the hippocampus (HIP). Incontrast, BDNF expression in the amygdala (AMY) was increased in both extinction andextinction-resistant groups, only in males. Withdrawal-related symptoms (rearings, grooming),and exploratory-based anxiety (side changes) were also assessed during morphine conditioningand extinction test days. Results showed similar conditioning patterns between male and femalerats; however, forty (40) percent of female rats were able to extinguish their morphine CPP, ascompared to fifty (50) percent in males. Furthermore, females show a higher likeability toextinguish their morphine CPP without extinction training. On the other hand, withdrawal-relatedsymptoms were significantly reduced in males that received extinction training, compared tosham-extinction animals. However, female withdrawal symptoms were unaffected by training,although they showed significantly less withdrawal symptoms than males in their baseline trials.Results demonstrate that although increased BDNF expression in the AMY might be responsiblefor contextual learning during extinction training, the increased BDNF expression in the HPCplays a crucial role in the successful extinction of opioid seeking behavior.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.03/QQ24

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant NIAAA 7R01AA024526

Title: The effect of cannabidiol (CBD) on behavioral deficits of comorbid alcohol use disorder and post-traumatic stress disorder

Authors: *B. MCGUFFIN[1,2], B. SCHWARTZ[2], L. WILLS[2], J. GASS[2];
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Abstract: Alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD) are debilitating conditions that often co-occur, with prevalence rates reaching almost 79%. A major concern of this comorbidity is the tendency for one disorder to exacerbate the other. Symptoms related to PTSD are a significant risk factor for the development of AUD, and alcohol abuse worsens PTSD symptoms. This cycle, along with a lack of effective pharmacological treatments,

leads to behavioral and physiological deficits. Arousal symptoms are a fundamental aspect of PTSD that are characterized by exaggerated physical and emotional reactions, leading to maladaptive fear learning and overgeneralization. Additionally, remission for comorbid AUD and PTSD is difficult to attain due to intensified symptomology and a lack of FDA-approved medications. In recent years, cannabidiol (CBD), a non-psychoactive compound found in cannabis, has been a focus of study due to its therapeutic potential and lack of side effects. Researchers have demonstrated the anxiolytic effects of CBD in both humans and animals, showing its promise as a novel therapeutic agent for disorders such as PTSD/AUD. The purpose of this study is to investigate the hypothesis that CBD will reduce fear-related behaviors in a rat model of comorbid AUD and PTSD. Male Wistar rats underwent a 2h restraint stress (RS) procedure followed by 2-weeks of chronic intermittent ethanol exposure (CIE). To investigate changes in future stress sensitivity, rats were exposed to a standard contextual fear conditioning paradigm, used to train the animals to associate environmental and auditory cues (environment appearance and tone) with an aversive stimulus (mild foot-shock). 30 minutes prior to each conditioning session, rats received an intraperitoneal injection of CBD (20mg/kg) or 0.9% Saline. The amount of time rats remained still (freezing) in the absence of the foot-shock during the tone represents fear-related behavior. Our current results indicate rats with a history of stress and alcohol exposure displayed significantly higher freezing behaviors compared to control animals and this effect was attenuated with CBD treatment. This suggests that when CBD is administered during fear learning, it can prevent heightened stress sensitivity associated with AUD/PTSD and enhance extinction learning. Taken together, the current results show promise for CBD to reduce enhanced fear-related behaviors associated with comorbid AUD and PTSD.

Disclosures: B. McGuffin: None. **B. Schwartz:** None. **L. Wills:** None. **J. Gass:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR231.04/QQ25

Topic: G.09. Drugs of Abuse and Addiction

Support: CVU: 1102522

Title: Intermittent model of binge-intake behavior in mice

Authors: *S. ORTEGA-TINOCO[1], W. A. ZEPEDA RUIZ[1], A. MONDRAGÓN-GARCÍA[1], J. E. RAMÍREZ-SÁNCHEZ[2], J. GARDUÑO TORRES[2], S. L. HERNÁNDEZ-LÓPEZ[2]; [1]UNAM, Ciudad de México, Mexico; [2]UNAM, CDMX, Mexico

Abstract: Intermittent model of binge-intake behavior in miceAccording to the OMS, it has been reported that in all regions of the Americas, 62% of adults are overweight or obese (OMS, 2018). Overweight and obesity are mainly triggered by eating disorders such as binge-eating, which consists of an excessive consumption of high-calorie foods such as sugar and fats in a short

period of time, approximately 2h (Corwin, 2006; Corwin et al., 2016). Most rodent overconsumption models utilize dietary restriction and stress (Avena et al., 2008; Anversa et al., 2020). On the other hand, the model proposed by Corwin always provides standard food and water, to induce binge eating, a limited access to a palatable food is given in a 2h period. This model has not been tested in mice, and it would be of the utmost importance to verify that the intermittent access model functions in the same way in rats. Therefore, the objective of this work was to standardize the intermittent model in mice proposed by Corwin.Binge-type intake was evaluated using Corwin's intermittent model. The 24 subjects were separated as follows: control (n=12) and binge-intake (n=12).Twenty-eight 1-month-old male C57BL/6 mice weighing between 19 and 22 g were used. All experiments were carried out in accordance with the official Mexican standard of technical specifications for the production, care, and use of laboratory animals (NOM-062-ZOO-1999) and in accordance with the provisions of the Internal Committee for the Care and Use of Laboratory Animals (CICUAL) of the National Autonomous University of Mexico. The subjects were kept in individual cages with controlled environmental conditions: light-dark cycle 12-12 h, temperature 21 ± 2 ºC and humidity 70 ± 10%. The subjects were randomly assigned, and their initial weight was controlled. For 28 days all subjects had *ad libitum* access to standard food and water. The experimental group with binge-intake had access to palatable food 12 days (Monday, Wednesday, and Friday, for 4 weeks). We measured water consumption, standard foods, palatable foods, and weight. For the statistical analysis we used the Mann Whitney and Kruskal Wallis U trials. Meaningful differences were observed when $p < 0.05$.Our results indicate that a combination of high fat and sugar can be used as a palatable food in Corwin's over-eating model. We were able to observe that the groups with binge-intake had a high consumption of palatable food. Interestingly, we did not observe statistically significant differences in weight between subjects.

Disclosures: **S. Ortega-Tinoco:** None. **W.A. Zepeda Ruiz:** None. **A. Mondragón-García:** None. **J.E. Ramírez-Sánchez:** None. **J. Garduño Torres:** None. **S.L. Hernández-López:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

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German Research Foundation Grant SCHL1969/5-1
German Research Foundation Grant EN906/8-1

Title: Regaining control over alcohol intake: Goal-directed behavior and hippocampal activity predict the real-life implementation of drinking intentions in alcohol use disorder

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Abstract: The transition from recreational alcohol use to alcohol use disorder (AUD) has been conceptualized as a shift from goal-directed towards habitual behavior, but previous research has not demonstrated categorical differences between individuals with and without AUD regarding model-based control - a reinforcement-learning-based formalization of goal-directed behavior. While previous studies have shown associations between alcohol consumption (AC) measures and the degree of model-based behavior, they relied on long-term retrospective self-reports. In this study, we investigated whether control over alcohol intake in day-to-day life could be predicted by experimentally assessed goal-directed behavior and its neural correlates. Sixty-seven human participants (20 women; mean 35.2 ± SD 9.4 years old; 2 - 7 12-months AUD criteria) completed long-term smartphone-based ecological momentary assessment of their AC (assessed every 2 days) and their intention to reduce AC (assessed every 8 days) over a 1-year period. At the start of the assessment period, participants performed a two-step sequential decision-making task during functional magnetic resonance imaging. This allowed to derive the degree of model-based behavior, ω, via Bayesian computational modeling as well as neural correlates of model-based reward prediction errors (RPEs) in regions previously associated with model-based learning, i. e. ventral striatum, ventromedial prefrontal cortex, and hippocampus. Using linear mixed-effects regression, we found that the association of the weekly intention to reduce AC (predictor; 'drink less than usual' vs. 'drink no more than usual / no intention') and daily AC (outcome) was moderated by the degree of model-based behavior in the two-step task (moderator 1; $\beta = -0.27$, $t_{11776} = -2.77$, $p = .006$) as well as by model-based RPE-correlated blood oxygenation level dependent signal in bilateral hippocampus (moderator 2; $\beta = -0.19$, $t_{11776} = -2.06$, $p = .040$). Specifically, participants with higher levels of model-based behavior and stronger hippocampal encoding of model-based RPEs were comparatively more successful at reducing their AC in weeks in which they intended to do so. Our results demonstrate that goal-directed behavior and its hippocampal correlates prospectively predict how well individuals with AUD are able to intentionally modify their AC over the course of one year. In conclusion, experimentally assessed goal-directed behavior directly relates to intentional real-life modification of AC, offering itself as a target for prevention and treatment of escalated substance use.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.06/QQ27

Topic: G.09. Drugs of Abuse and Addiction

Support: Swiss National Science Foundation 3100030_189188 / 1

Title: The impact of persistent accumbal dopamine transients on the preference between natural and "drug-like" rewards

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Abstract: Drug-choice at the expense of natural reward has been suggested to be a hallmark of addiction. It has been hypothesised that a bias towards drug rewards might be due to the pharmacological power of the drugs on the dopamine (DA) system, which results in an overvaluation of the reward. Indeed, unlike natural rewards, drugs cause an increase in DA concentration in the Nucleus Accumbens (NAc), even when the reward delivery is well predicted by a cue. However, these influential theories have not been experimentally tested. Here we recorded DA transients using a genetically encoded DA sensor in mice during an operant choice task between a natural and an artificial "drug-like" reward (optogenetic dopamine neuron self-stimulation oDASS or intravenous cocaine). We observed that the individual preferences correlated with the amplitude of transients at the predictive cue: DA transients are stronger for the cue predicting the preferred reward. Moreover, after revaluation of the oDASS reward by associating it with a punishment risk, the change in preference is reflected in a change of DA transient amplitude at the cue. Unexpectedly, we found that most mice do not prefer artificial rewards, although the DA transients at reward delivery disappear for natural rewards while staying high for cocaine and oDASS. Based on these findings, we propose a relaxed implementation of temporal difference learning, where the effect of the DA on the system is modelled to decrease as a function of the total error accumulated. *Our data are in line with a model where DA transients at the cue correlate with choice behaviour in the context of natural and artificial rewards. They also show that persistence of DA may not necessarily lead to an overvaluation of the artificial reward in our operant choice task.*

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA052460
P50 DA006634
P50 AA026117
Peter F. McManus Charitable Trust

Title: Diurnal variation in effects of cholinergic interneuron modulation on Pavlovian conditioned responding

Authors: *I. GALLINGER[1], T. A. STOWE[3], L. L. SEXTON[1], M. J. FERRIS[2];
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Abstract: Substance use disorder (SUD) is a chronic mental disorder involving pathological changes in learning and motivation. Only a fraction of drug users develop SUD, and understanding determinants of this variation is critical to improving treatment outcomes. In animals, higher rates of sign tracking (ST) behavior in the Pavlovian Conditioned Approach (PCA) task are associated with increased drug-taking and represent a preclinical model of individual differences conferring vulnerability to SUD. Rates of ST peak in the dark, or active, portion of the 24-hour light cycle in male rats. Induction of this behavior is known to be dependent on phasic release of dopamine (DA) in the nucleus accumbens core (NAcc), a regional neurotransmitter system implicated in SUD. Our group has demonstrated greater relative phasic DA firing during the dark cycle, an effect which is mediated by a reduction in cholinergic interneuron (CIN) inputs. These data are consistent with clinical evidence for diurnal rhythms in substance use behavior. In the present study, we further explore these findings by manipulating NAcc DA release and CIN firing in vivo.
To test the hypothesis that altering accumbal acetylcholine (ACh) release would elicit changes in a DA-dependent behavioral output, we used designer drugs exclusively activated by designer receptors (DREADDs) to alter CIN firing across times of day. We surgically infused Cre-dependent DREADDs into the NAcc of male Long-Evans rats expressing Cre-recombinase under control of the cholinergic acetyltransferase (ChAT) promoter. Rats were assigned to run during their light cycle and receive an inhibitory DREADD (N=4) or run during their dark cycle and receive an excitatory DREADD (N=4). All 8 rats were habituated to an operant chamber and trained to receive sugar pellets from a food hopper. Next, they received an injection of clozapine n-oxide dihydrochloride (CNO) 30 minutes before completing a PCA test daily for 10 days, and for the remainder of the sessions they received a saline injection. Consistent with prior work, the rate of ST increased in the dark, but not light, cycle when the DREADDs were not activated. DREADD activation via 1 mg/kg CNO blocked ST in the dark cycle. Additional studies are

using optogenetic stimulation to examine the effects of evoked phasic-like DA release on acquisition of ST behavior.
These data support the hypothesis that ACh release from NAcc CINs suppresses phasic to tonic ration of DA signaling by increasing tonic dopamine. This reduces the induction of ST. These findings further our understanding of the mechanisms underlying diurnal variation in reward-seeking behavior.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.08/RR1

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R00045758

Title: Sex differences in hippocampal β-adrenergic receptor subtypes driving retrieval, retention, and consolidation of cocaine-associated memories.

Authors: *M. BERRY[1], C. COCKRELL[2], S. KELSEN[1], A. KOHTZ[1];
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Abstract: The exposure to environmental context and drug associated cues maintains and induces drug seeking behavior. Hippocampal β-adrenergic receptors (β-ARs) have a long-standing historical implication in driving processes associated with contextual memory retrieval, retention, and consolidation that extend to contextual drug memories. However, sex differences in the role of β-adrenergic receptors in drug memory remains unknown. It has previously been shown that there is a selective role for β2-ARs in the retrieval and retention of contextual drug memories in males. Additionally, there are substantial sex differences that exist in the Adrenergic system. We hypothesized that there are sex differences in selective recruitment of β-ARs during memory retrieval, consolidation, and retention. We implanted cannula into the dorsal CA1 region of the hippocampus to intracranially administer β-AR antagonists and used cocaine conditioned place preference (CPP) in adult male and female Sprague-Dawley rats. CPP was conducted using a two compartment chamber automatic door box. During conditioning, rats were isolated to either the black or white side and administered 10mg/kg, IP cocaine. On test day, time spent on the cocaine-paired side (preference) was assessed. We used the antagonists ICI 118,551 (β1) Betaxolol (β2), and/or Propranolol (β1 and β2) administered to the hippocampus prior to testing in CPP to test if retrieval of cocaine-associated memories was driven by β1- or β2-ARs. Rats were then retested 2 weeks later to determine the effects of β-AR antagonist intervention on memory retention. In a separate group of rats, β-AR antagonists were administered prior to each conditioning session, to determine their effects on drug memory consolidation. Our results showed that administration of either β1, β2, or combined β1 and β2-ARs before the initial CPP

testing reduced the expression of a CPP compared to vehicle administration. These effects contrast with prior reports in males showing that β1, but not β2, ARs drive retrieval and retention of cocaine CPP. Our results indicate novel relevance for sex-specific involvement of β-AR receptors in driving cocaine memory retrieval, retention, and consolidation. These results will inform sex-specific pharmacological therapies for reducing drug relapse.

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Poster

PSTR231. Addiction: Learning, Memory and Development

Location: WCC Halls A-C

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Program #/Poster #: PSTR231.09/RR2

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Research Center of Excellence (P50)
T32

Title: Circadian Sex Differences in 5 Choice Behavior of Heterozygous Rats

Authors: *H. K. DOLLISH, Dollish, C. MCCLUNG, M. TORREGROSSA;
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Abstract: Adolescence is characterized by profound neurodevelopmental and behavioral changes. Among these changes, significant alterations in circadian rhythms, including a shift towards an evening chronotype, are observed. These circadian disruptions pose challenges as they often clash with social and academic demands of teens, exacerbating academic, social, and behavioral difficulties. Furthermore, this period is associated with increased risk-taking behavior and impulsivity, which heightens the susceptibility to drug experimentation. Investigating changes in these adolescent circadian rhythms can serve as an early indicator for identifying teens at higher risk of drug initiation. Early intervention is crucial in reducing the likelihood of developing substance use disorder (SUD). Thus, understanding the mechanisms and factors contributing to these behaviors is of utmost importance. Sex differences in adolescent research are understudied and are desperately needed to inform subsequent experiments and intervention strategies effectively. In this study, we utilized male and female heterozygous rats to examine the impact of circadian rhythms on 5-choice behavior outcomes (premature response, accuracy, and omission rates) which to measure impulsivity. By investigating the intricate relationship between circadian rhythms, sex differences, and impulsivity using an animal model, this research contributes to our understanding of the underlying mechanisms and factors influencing adolescent behavior. The findings have the potential to inform targeted interventions and guide future studies, ultimately improving our ability to address challenges associated with adolescent substance use and related behavioral issues. Our results show correlations between circadian rhythms and 5-choice task behaviors are present with sex differences. During the 5-choice task, choice accuracy is affected heavily by the phase of the activity rhythm. Male rats, on the other

hand, show a marked difference in premature response rate that is correlated with amplitude, period, phase, and non-parametric circadian metrics such as intradaily stability and average activity peak during the active phase. Overall, circadian measures in males correlate more widely with impulsive behaviors compared to females, but females have stronger correlations between circadian rhythmicity and 5-choice performance.

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Poster

PSTR231. Addiction: Learning, Memory and Development

Location: WCC Halls A-C

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Program #/Poster #: PSTR231.10/RR3

Topic: G.09. Drugs of Abuse and Addiction

Title: Persistence of Behavioral Sensitization After a Single Methamphetamine Injection in Mice

Authors: *A. S. RAUHUT[1], K. MEHTA[2], A. MENGEL[1], N. FEDORCZAK[2], T. KURUP[2], G. RAUHUT[3];
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Abstract: The Pavlovian excitatory conditioning account of behavioral sensitization suggests that the sensitized response reflects both associative (i.e., classical conditioning) and non-associative processes. That is, the sensitized response reflects the summation of the classically conditioned response (i.e., CR) with the pharmacological, unconditioned response (i.e., UR). This experiment, using an animal model of drug addiction (i.e., behavioral sensitization), evaluated the Pavlovian excitatory conditioning account by examining the temporal persistence of conditioned hyperactivity and sensitization after a single methamphetamine injection in male, Swiss Webster mice (N = 80). Following 6 weeks of acclimation, mice received either a single injection (intraperitoneal, i.p.) of physiological saline (vehicle) or methamphetamine (2.0 mg/kg) prior to a 30-minute locomotor activity session (Conditioning Day). Following the conditioning day, tests for conditioned hyperactivity (CR Test) and behavioral sensitization (Methamphetamine Challenge Test) occurred after a delay of 2 and 3 days (Immediate), 6 and 7 days (Short), 14 and 15 days (Moderate), or 27 and 28 days (Long), respectively. An injection of physiological saline or methamphetamine (1.0 mg/kg) occurred on the CR Test and Methamphetamine Challenge Test, respectively. Distance traveled and vertical counts served as the dependent measures of locomotor activity. The 2.0 mg/kg methamphetamine dose produced robust, acute locomotor activity on the conditioning day in all groups. In addition, both dependent measures of locomotor activity revealed that conditioned hyperactivity was detected at all time points, and the robustness did not decline over time. In contrast, behavioral sensitization was robust early (Immediate- and Short-Delay time points) and only detected by the vertical count measure but weakened over time and was not evident later (i.e., Moderate-Delay). Collectively, these results suggest that the 1) the pharmacological UR diminishes over time whereas the CR does not, and 2) the conditioned and pharmacological components of behavioral

sensitization are dissociable following a single methamphetamine injection. These observations speak against a simple Pavlovian excitatory conditioning account.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

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VA Merit BLRD I01 BX005367-01A2

Title: Behavioral and Neuroinflammatory Sex Differences in Comorbid Posttraumatic Stress Disorder and Alcohol Use Disorder

Authors: *B. SCHWARTZ[1,2], B. MCGUFFIN[2], L. J. WILLS[3], J. T. GASS[3];
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Abstract: Post-traumatic stress disorder (PTSD) is a debilitating disorder with a prevalence rate of approximately 5%. Of those diagnosed with PTSD, 30%-59% also suffer from alcohol use disorder (AUD). Currently, there are limited effective treatment options for those suffering from comorbid PTSD/AUD. Previous research has suggested that biological sex differentially impacts PTSD comorbid with AUD, however, the underlying mechanisms are enigmatic. The goal of this study was to better understand sex-dependent mechanisms associated with comorbid PTSD/AUD by using a rodent model to analyze specific behavioral tasks and changes in neuronal function. Chronic inflammation has been implicated in PTSD and AUD respectively, with differences between sexes being observed. Females tend to express elevated levels of inflammation in both disorders compared to males in brain regions such as the medial prefrontal cortex (mPFC). Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that is commonly used as a biomarker for psychiatric diseases such as PTSD and AUUD, since it has been shown to be elevated in these patient populations. To further examine these sex differences, a comorbid PTSD/AUD rat model was implemented in adult male and female Wistar rats (n=24) by exposing the rat to 2h restraint stress (RS) followed by 2 weeks of chronic intermittent ethanol vapor (CIE). Upon completion of CIE, a fear conditioning (FC) task was conducted to assess future stress sensitivity. The animals were then euthanized, and the brain tissue harvested. Subsequently, 2 subregions of the mPFC, prlimbic cortex (PrL) and the infralimbic cortex (IfL), were dissected from the brain tissue to analyze TNF-α protein expression using an enzyme-linked immunosorbent assay (ELISA). The PrL and IfL were chosen as regions of interest because of their role in learning, memory, and The FC data revealed that RS+CIE females froze

significantly more than the female controls and all males during extinction recall. During context renewal, the RS+CIE females froze significantly more than all the males, but less than the the female controls. Additionally, we found that all females had increased levels of TNF-α protein expression compared to males without a history of RS+CIE. The current study suggests that RS+CIE females demonstrate increases in fear after extinction as well as increases in TNF-α protein expression within the PrL. This research provides further knowledge on the possible cause of the sex differences in comorbid PTSD/AUD that could aid in future discoveries of treatment options that are sex-dependent for those diagnosed with comorbid PTSD/AUD.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.12/RR5

Topic: G.09. Drugs of Abuse and Addiction

Support: RISE NIH 5R25GM060665-22

Title: Investigating the molecular mechanisms underlying HIV-1 Tat/Opiate Interactions in the dorsal striatum of female rodent brains.

Authors: *T. RODRIGUEZ[1,2], A. N. QRAREYA[3], J. GOMEZ[1], F. ABDUL WALI[1], M. AGUILAR[1], N. MUNOZ[1], S. DILAWARI[1], J. PARIS[3,4], P. SERRANO[1,2];
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Abstract: The human immunodeficiency virus 1 (HIV-1) protein, transactivator of transcription (Tat) is a potent mediator in the progression of HIV-1 associated neurocognitive disorders (HAND). HIV-1 Tat promotes neuronal cell dysfunction and death by disrupting intracellular calcium (Ca^{2+}) homeostasis via the N-methyl-D-aspartate receptor (NMDAr). The neurotoxic effects of HIV-1 Tat on HAND are further exacerbated by opiate drug abuse. Women who are diagnosed with HAND and abuse opiates, may display worsening effects of HAND due to the known interactions of estradiol (E_2) potentiating drug-driven reward behaviors. Our goal is to investigate the molecular mechanisms underlying the complex interaction between HIV-1 Tat and opiate drug abuse to mitigate the effects of HAND in the female brain. We hypothesize that HIV-1 Tat will enhance NMDAr activity resulting in the dysregulation of Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit composition and trafficking in the dorsal striatum (DS), which can be exacerbated following acute morphine. The dysregulation of NMDAr activity and AMPAr subunit composition promotes synaptodendritic damage that correlates with cognitive decline observed in HAND patients. We performed a western blot analysis to quantify NMDA and AMPA receptor subunit expression in the DS of adult female

HIV-1 Tat -/+ Transgenic ovariectomized (ovx) mice, following an unbiased morphine conditioned place preference (CPP) task. We found a significant difference between Tat (-) and Tat (+) mice in CPP, whereas Tat (+) mice demonstrate a higher preference for the morphine-conditioned chamber compared to the control Tat (-) mice. In the DS, we show a significant decrease in the GluA1 subunit, which promotes AMPAr retention on the post-synaptic membrane. This is accompanied by the downregulation of the GluA1 regulatory residue, serine 845, a phosphorylation site essential for stabilizing and maintaining GluA1-containing AMPARs at post-synaptic membrane. We also show a significant decrease in GluA2, the Ca^{2+} impermeable subunit important for anchoring synaptic AMPAr and limiting Ca^{2+} influx. Interestingly, we observed the downregulation of the NR1 subunit expression in the DS, an obligatory subunit required for the assembly of functional NMDARs. The downregulation of the NR1 subunit is correlated with the excessive activation of the NMDAr. These results highlight the effect of HIV-1 Tat /*acute* morphine interactions on NMDAr activity, which may result in NMDAr-mediated excessive Ca^{2+} influx, initiating the dysregulation of the AMPAr GluA1/2 subunit expression pattern in the DS and exacerbating synaptodendritic damage.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.13/RR6

Topic: G.09. Drugs of Abuse and Addiction

Title: Human models of craving: instrumental behavior and self reports of craving

Authors: *N. RUIZ, D. ECKARDT, L. BRIAND, M. E. WIMMER, V. P. MURTY; Temple Univ., Philadelphia, PA

Abstract: Incubation of craving, a prominent animal model of substance use, is a phenomenon where craving for a reward intensifies over extended periods of abstinence. In these models, craving is expressed by instrumental behavior to reward cues after different periods of abstinence. Homologous studies in humans have also shown escalated craving after delays, but have mainly relied on subjective self-reports. Thus, in humans, there is a disconnect between the subjective feelings of cravings and instrumental actions to seek those incentives. To resolve this cross-species gap, we developed a novel human paradigm to characterize the sense of craving for reward into an instrumental behavior more akin to the rodent models. Across two studies, participants (study 1: n = 103, study 2: n = 164) completed a task where they would make an instrumental button press to food items, previously rated on liking, craving and time since last consumption. The amount of button presses indicated how much they desired to later describe an

experience with that food. We found a significant relationship where subjective craving predicted button presses, above and beyond liking (study 1: $p < 0.001$, study 2: $p < 0.001$). Further, the time since last consumption had an inverted U-shaped relationship with subjective craving (study 1: $p = 0.03$, study 2: $p = 0.03$), mirroring incubation data in humans. Critically, both of these effects replicated in an independent sample. This study provides the first step in establishing a translational link between animal and human models of craving.

Disclosures: **N. ruiz:** None. **D. Eckardt:** None. **L. Briand:** None. **M.E. Wimmer:** None. **V.P. Murty:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR231.14/RR7

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R21(CEBRA) DA048633 (NCS)
NIDA Grant R21 DA050821 (NCS)
Overland Foundation (NCS)

Title: Drug-induced dopamine signaling changes the transmitter identity of prefrontal neurons which in turn generates cognitive deficits.

Authors: *M. PRATELLI[1,3], *M. PRATELLI[4], N. C. SPITZER[2,3];
[1]Neurobio. Section 0357, Div. of Biol. Sci., The Univ. of California, San Diego, SAN DIEGO, CA; [2]Neurobio. Section 0357, Div. of Biol. Sci., The Univ. of California, San Diego, La Jolla, CA; [3]Kavli Inst. for Brain and Mind, Univ. of California San Diego, la Jolla, CA; [4]UC San Diego, san diego, CA

Abstract: Repeated exposure to drugs of abuse can affect the function of the medial prefrontal cortex and induce cognitive deficits. Phencyclidine (PCP) and methamphetamine (METH), despite having different molecular targets in the brain, are well known to cause long-lasting cognitive impairments and have both been used to mimic deficits observed in schizophrenia. Because studies have focused on the effect of a single drug, it is unclear whether PCP and METH cause these deficits via a common mechanism of action. We previously found that repeated exposure to either drug impairs memory by causing glutamatergic neurons in the prelimbic subregion of the prefrontal cortex (PL) to gain a GABAergic phenotype and decrease their expression of the vesicular glutamate transporter. Using a tamoxifen-inducible system to obtain time-specific genetic labeling of neurons expressing GABAergic markers, we found that PCP and METH change the transmitter identity of the same neurons in the PL. This discovery prompted an investigation of the underlying mechanism of action. Like other addictive substances, PCP and METH acutely promote the phasic firing of dopaminergic neurons in the ventral tegmental area (VTA), increasing dopamine levels in the striatum and prefrontal cortex.

We found that chemogenetic silencing of VTA dopaminergic neurons during PCP- or METH-treatment prevented the change in PL neuron's transmitter identity, indicating that drug-induced dopamine signaling is needed for the switch in transmitter to occur. Furthermore, repeatedly inducing phasic firing of VTA dopaminergic neurons via optogenetic stimulation was alone sufficient to induce PL glutamatergic neurons to change transmitter identity, raising the possibility that other drugs can have a similar effect. The results reveal that a switch in the transmitter identity of PL neurons is a shared mechanism by which exposure to different drugs can generate cognitive deficits.

Disclosures: **M. Pratelli:** None. **M. Pratelli:** None. **N.C. Spitzer:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.15/RR8

Topic: G.09. Drugs of Abuse and Addiction

Support: Center for Integrated Biomedical and Bioengineering Research
NIGMS GM11313 Grant

Title: The role of the corticostriatal pathway in learning with interoceptive nicotine stimulus

Authors: *T. W. ALLEN, J. HENSLEY, A. KALINOWSKI, M. E. DEANE, E. O'KEEFE, E. KELLER, H. MANNING, S. CHARNTIKOV;
Psychology, Univ. of New Hampshire, Durham, NH

Abstract: Tobacco use, primarily in the form of smoking, is the foremost preventable cause of death, responsible for approximately 8 million fatalities each year. This is more than deaths from alcohol and all illegal substances combined. Nicotine, the main addictive component in tobacco, alters neural circuits in ways that could potentially stimulate addictive behaviors and impact cognitive functions. Although there is a wealth of information about pharmacology and neurobiology associated with the reinforcing effects of nicotine, the neurobiology underlying learning with nicotine stimulus is virtually unexplored. Thus, this study assessed the role of the corticostriatal pathway associative learning with self-administered interoceptive nicotine stimulus. To this end, we used a chemogenetic approach to express Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) on the neurons projecting from the prelimbic cortex to posterior dorsomedial Caudate Putamen (p-dmCPu). Rats then learned to self-administer nicotine in conjunction with access to sucrose, an approach that results in the rapid development of learned nicotine-evoked goal-tracking response. After the establishment of a stable nicotine-evoked goal-tracking response, we assessed the role of corticostriatal projections in the expression of this learning by inhibiting these projections on a test day and then assessing the magnitude of a nicotine-evoked goal-tracking response. Based on our previous studies, we hypothesized that the suppression of projections from the prelimbic cortex to the p-

dmCPu would decrease the expression of associative learning with nicotine stimulus. As expected, our findings reveal that inhibiting the corticostriatal projections to the p-dmCPu inhibits the nicotine-induced goal-tracking behavior. These results are consistent with previous literature on the role of the corticostriatal pathway in associative learning with nicotine stimulus. Our research underscores the significance of the corticostriatal pathway in nicotine-associated learning, illuminating a potential neurobiological target for understanding the mechanism of nicotine use and dependency.

Disclosures: T.W. Allen: None. **J. Hensley:** None. **A. Kalinowski:** None. **M.E. Deane:** None. **E. O'Keefe:** None. **E. Keller:** None. **H. Manning:** None. **S. Charntikov:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.16/RR9

Topic: G.09. Drugs of Abuse and Addiction

Support: HBI Pioneer Postdoc Award

Title: Circuits and mechanisms underlying the vulnerability to cocaine-seeking relapse behavior.

Authors: *Y. ESCOBEDO LOZOYA[1], N. SHUKR[2], S. M. DYMECKI[1];
[2]Genet., [1]Harvard Med. Sch., Boston, MA

Abstract: Substance use disorders (SUDs) are chronic and relapsing conditions impairing approximately twenty million young people in the United States annually. Affective disorders are comorbid with SUDs, suggesting synergy between these public health epidemics. Therapeutically useful 5HT agonists exert complex off-target effects due to their broad action across serotonin neurons that serve different, even opposing, functions. Here, we build on our recent discovery of a specialized subtype of brain serotonin-producing neuron, *r2Hoxa2-Pet1*, that controls the strength or durability of cocaine-reward memory and cocaine-seeking behavior. These neurons appear to have the potential to communicate with and regulate downstream neural circuits in the brain by deploying serotonin and glutamate. This raises the possibility of signaling across different time scales and even valence, with serotonin acting more slowly and with positive or negative valence depending on the nature of the receptor expressed by the downstream neurons and glutamate acting more quickly and positively. In addition to possible dual signaling means, these *r2Hoxa2-Pet1* neurons display a unique axon terminal arborization that ensheaths targeted neuron cell bodies with numerous boutons, forming a specialized structure first termed pericellular basket by S. Ramon y Cajal. *r2Hoxa2-Pet1* pericellular baskets are found in the medial prefrontal cortex, the hippocampus (HPC), and most densely in the lateral septum intermediate (LSi), which is known to contribute to reward reinforcement through interconnections with the lateral hypothalamus and the mesolimbic dopamine system. LSi activity is causally implicated in the retrieval of relapse-inducing cocaine memories and selective

neurons within the LSi gate mobility contextually. We hypothesize that LSi neurons basketed by *r2Hoxa2-Pet1* boutons modulate the persistence of memory traces, i.e., their neural representation, called an engram, related to cocaine use. In this way, *r2Hoxa2-Pet1* neurons may bias relapse vulnerability. Here we deploy our arsenal of molecular genetics tools in mouse, scRNAseq, HCR, and histological and electrophysiological profiling of cells in the LSi to reveal the identity and connectivity of basketed cells in LSi, suggesting possible mechanisms by which *r2Hoxa2-Pet1* pericellular baskets engage their LSi target neurons and the output circuits that are recruited. Our work revealing these limbic neural pathways, cell types, and signaling mechanisms may provide insight into similar human brain pathways underlying vulnerability for drug-use relapse and thus might inform leads for the prevention and treatment of SUDs.

Disclosures: **Y. Escobedo Lozoya:** None. **N. Shukr:** None. **S.M. Dymecki:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

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Topic: G.09. Drugs of Abuse and Addiction

Support: CONACYT 1222825
PAPIIT DGAPA UNAM IA202120
PAPIIT DGAPA UNAM IA201622

Title: Cerebellar and behavioral alterations induced by chronic self administration of opioids in male Wistar rats

Authors: *M. SERRANO[1], J. RASGADO-TOLEDO[2], C. CARRANZA-AGUILAR[2], D. ANGELES-VALDEZ[2,3], E. GARZA-VILLARREAL[2];
[2]Inst. de Neurobiología, [1]Inst. de Neurobiología, UNAM, Queretaro, Mexico; [3]Dept. of Biomed. Sci. of Cells and Systems, Cognitive Neurosci. Ctr., Univ. of Groningen, Groningen, Netherlands

Abstract: The cerebellum is considered an integrative system that supports cognitive and emotional functions. Opioid use disorder is due to activation of μ, δ, and κ opioid receptors. The μ opioid receptor is distributed in the cerebellum. Chronic opioid use affects the structure of the cerebellum and its relationship to behavior. Chronic morphine self-administration produces changes in cerebellar volume and affects learning and spatial memory during acquisition and recall. These changes are a consequence of the consumption of morphine.Eleven male Wistar rats at P35 were used, jugular vein cannulation was performed (six control and five morphine consumption) for self-administration in automated cages (Med-Associates model ENV-018V). The model was carried out in a fixed ratio scheme (FR1) for an acquisition phase and progressive ratio 9-4 (PR9-4) for a maintenance phase with morphine (0.01 or 0.1 mg/kg/infusion) orsaline (0.9%) for the control group. During FR1, motor function rehabilitation with a rotarod was implemented for five days, followed by behavioral tests: open field and elevated plus maze.

During the maintenance phase, behavioral tests were performed; novel object recognition, open field, elevated plus maze, and spatial and procedural Morris maze. A FLASH 3D structural magnetic resonance sequence was performed before starting FR1 (P57), during FR1 (P70), and at the end of PR9-4 (P105) with the following parameters: TR = 30.76 ms, TE = 5 ms, angle of rotation = 10°, slice thickness = 25.6 mm, FOV = 28.2 x 19 x 25 .6 mm, isometric voxel = 160 µm.A decrease in latency over time was observed in the acquisition and recall phase in spatial memory, procedural memory, and learning tasks. The results obtained in spatial MWM were p = 0.003 on day three and p= 0.0008 on day four in relation to the first day. In addition, the morphine-treated group showed anxious behaviors in open field tests with results of P = 0.001 and elevated plus-maze. MRI results revealed local volume structural changes, decreased cerebellar fissure, and increased superior cerebellar peduncle and ventral spinocerebellar tract.These findings suggest that chronic opioid use may have detrimental effects on cerebellar structure and behavior, highlighting the complex role of the cerebellum after morphine exposure beyond its traditional motor functions.

Disclosures: M. Serrano: None. **J. Rasgado-Toledo:** None. **C. Carranza-Aguilar:** None. **D. Angeles-Valdez:** None. **E. Garza-Villarreal:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.18/RR11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA047981

Title: A role for Gilz in plasticity underlying cocaine-associated behaviors

Authors: *J. ROUNDS[1], C. CHINN[3], J. CHILDS[4], E. A. KRAMÁR[2], M. A. WOOD[5]; [2]Neurobio. and Behavior and Ctr. for the Neurobio. of Learning & Memory, [1]Univ. of California, Irvine, Irvine, CA; [3]Neurobio. and Behavior, UC Irvine, Irvine, CA; [4]Neurobio. and Behavior, [5]Neurobiol & Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Repeated exposure to drugs of abuse can alter the structure and function of synaptic connections, giving rise to drug-associated memories that can persistently influence behavior. These memories and their corresponding behaviors can be differentially recruited by environmental stress. However, there remains a paucity of knowledge in the mechanistic interactions between stressful stimuli and drug exposure underlying long-term behavioral adaptations. Here, we investigated the role of a stress-responsive candidate gene (glucocorticoid-induced leucine zipper, *Gilz*) in long term cocaine-associated memory processes within the nucleus accumbens (NAc) and ventral tegmental area (VTA) of mice. RT-qPCR revealed sex-specific *Gilz* expression patterns in both the NAc and VTA, where adult male and female mice exhibit differential expression of mRNA splice variants that code for distinct GILZ protein

isoforms. These GILZ isoforms regulate transcription as well as the integration of key signaling cascades involved in plasticity, and their differential expression suggests possible sex differences in the reward-related actions of GILZ. To determine whether GILZ expression is necessary for the formation of cocaine-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. While these findings are limited to spatiotemporally specific, rather than global, knockdown, we found that male hemizygous $Gilz^{KO}$ mice exhibit impairments in cue-induced reinstatement of lever pressing in cocaine self-administration. In addition, we found that *Gilz*-1 is a necessary and sufficient component of NAc 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.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.19/RR12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R25 DA020537
NIDA Grant 1UG3DA048507-01A1

Title: Is medial orbitofrontal cortex (mOFC) repetitive transcranial magnetic stimulation (rTMS) placement best for treating addiction and preserving working memory in nicotine-dependent populations? A literature review and RCT in South Carolina patients presenting for smoking cessation

Authors: *T. D. DUBIN, X. LI;
Psychiatry and Behavioral Sci., Med. Univ. of South Carolina, at the MUSC Hlth. Inst. of Psychiatry, Charleston, SC

Abstract: High-frequency repetitive transcranial magnetic stimulation (HF-rTMS) over the dorsal lateral prefrontal cortex (DLPFC) is the generally accepted neurostimulation protocol for smoking cessation treatment, although medial orbitofrontal cortex (mOFC) placement may better aid in preserving working memory. This exploratory study assessed DLPFC versus mOFC rTMS placement in patients with Tobacco Use Disorder (TUD) to reveal possible memory harms, as tested using the N-back working memory task. The Medical University of South Carolina conducted a double-blind, sham-controlled, randomized clinical trial of participants (n=18, 9

female) aged 49.8 [9.7] (mean [SD]) nearby Charleston who enrolled for rTMS smoking cessation treatment (15 sessions over 3 weeks). rTMS was sham or active MRI-guided to the DLPFC (10 Hz, 3000 pulses each session) for facilitation protocol or to the mOFC (1 Hz, 900 pulses each session) for inhibition. N-back studies occurred prior to rTMS treatment #1, #6, #11, #15, and 1 month after rTMS #15. 16 participants began treatment: 9 received DLPFC rTMS; 7 received mOFC rTMS. 12 participants (7 DLPFC vs. 5 mOFC) were analyzed. Mixed model results showed significantly different correct trials between 0-back (5.29±0.31), 1-back (3.27±0.31), and 2-back (2.21±0.31), ($p < 0.01$). A trend difference existed between DLPFC treatment (3.24±0.24) and mOFC treatment (3.93±0.25), ($p=0.054$), in stimulating working-memory accuracy. Though the DLPFC controls over working memory, the mOFC mediates task retrieval, outcome-specific information, and goal-oriented action. This could account for the trend difference favoring mOFC placement; more research must be done to affirm this is the best placement protocol.

Disclosures: **T.D. Dubin:** None. **X. Li:** A. Employment/Salary (full or part-time):; Medical University of South Carolina. 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.; Medical University of South Carolina. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medical University of South Carolina.

Poster

PSTR231. Addiction: Learning, Memory and Development

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIGMS Grant SC3GM130467
NIGMS Grant R16GM145552

Title: Juvenile exposure to psychological stress or ketamine, but not their combination, decreases hippocampal AKT-mTOR signaling in adult C57BL/6 male mice.

Authors: *M. RODRIGUEZ, I. GARCIA-CARACHURE, J. REYES, O. LIRA, S. D. IÑIGUEZ;
Dept. of Psychology, The Univ. of Texas At El Paso, El Paso, TX

Abstract: Ketamine is administered to manage major depression in adolescent patients. However, the long-term effects of juvenile ketamine exposure on memory-related tasks have not been thoroughly assessed. Thus, we examined whether exposure to ketamine, psychological stress, or both, results in long-lasting alterations in spatial memory in C57BL/6 male mice. Furthermore, we evaluated how ketamine and/or psychological-stress history influenced hippocampal protein-kinase b-mammalian target of rapamycin (AKT-mTOR)-related signaling.

Specifically, male postnatal day (PD)-35 mice underwent vicarious defeat stress (VDS), a form of psychological stress that reduces sociability, with or without ketamine exposure (20 mg/kg/day; PD35-44). In adulthood (PD70) mice were assessed for spatial memory performance on a water-maze task or euthanized for hippocampal tissue collection. We found that juvenile pre-exposure to ketamine or VDS, individually, increased the latency (sec) to locate the escape platform in adulthood. However, juvenile history of concomitant ketamine-and-VDS prevented memory impairment. Furthermore, individual ketamine or VDS pre-exposure, unlike their combined history, decreased hippocampal AKT-mTOR-signaling. Collectively, our preclinical model displays how ketamine treatment, for the management of adolescent psychological stress-induced sequelae, does not impair spatial-memory later in life. However, juvenile recreational ketamine misuse, like psychological stress history, results in long-term spatial memory deficits, along with decreases of hippocampal AKT-mTOR signaling.

Disclosures: M. Rodriguez: None. **I. Garcia-Carachure:** None. **J. Reyes:** None. **O. Lira:** None. **S.D. Iñiguez:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

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Topic: G.09. Drugs of Abuse and Addiction

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College of Liberal Arts, CSULB
Associated Students Inc., CSULB
CSUPERB

Title: Liraglutide, a glucagon-like peptide-1 receptor agonist, does not affect the expression of methamphetamine preference in adolescent male and female rats

Authors: *T. P. NGUYEN, A. LIN, S. P. VARGAS, E. N. SIU, S. P. MOORE, A. R. ZAVALA;
Psychology, California State University, Long Beach, Long Beach, CA

Abstract: Methamphetamine (METH) use is especially damaging in adolescence as early initiation leads to poorer treatment outcomes. Currently, there are no FDA-approved treatments for stimulant use disorder. Preclinical studies have shown that Liraglutide, an FDA-approved drug that activates glucagon-like peptide-1 (GLP-1) receptors, lessens the rewarding effects of cocaine, heroin, and oxycodone. However, the role of activating GLP-1 receptors on METH reward has not been examined, nor has it been examined in adolescent rats. Thus, the present study investigated whether stimulating GLP-1 receptors with Liraglutide decreases METH-seeking behavior in adolescent rats using the conditioned place preference (CPP) method, a

validated animal model of drug reward. Male and female Sprague-Dawley rats underwent a 10-day CPP procedure. During day 1, baseline, rats were assessed for initial side preference in a two-chamber-sided box. Conditioning days took place over the next 8 days, during which rats had alternating drug and saline treatment sessions. Rats were administered METH (0.0, 0.3, or 1.0 mg/kg) or saline on their respective days and immediately confined to one side of the CPP box for 30 min. On day 10, testing, the rats were pretreated with Liraglutide (0.1 mg/kg) or saline 60 min prior to being placed in the CPP apparatus with free access to both sides for 15 min. A preference score was computed by taking the time spent in the drug-paired side during testing minus the time spent in the same compartment during baseline. Additionally, changes in the time spent in the METH-paired side between baseline and testing sessions for each group were examined. Overall, male rats exhibited a robust preference for the METH-paired side during testing, which was not attenuated by pretreatment with Liraglutide. Female rats did not strongly prefer the METH-paired side relative to controls. However, within-group preferences for the METH-paired side were evident between baseline and testing. Nonetheless, similar to the male rats, Liraglutide failed to affect the preferences of female rats. Additional studies examining higher doses of Liraglutide are needed to determine if adolescent rats are less sensitive to the effects of Liraglutide and examine the effects of Liraglutide on the acquisition of METH-induced CPP. When considered together, developmental differences in the effects of Liraglutide are evident, given the lack of an effect of Liraglutide on METH-seeking behavior in adolescent male and female rats.

Disclosures: **T.P. Nguyen:** None. **A. Lin:** None. **S.P. Vargas:** None. **E.N. Siu:** None. **S.P. Moore:** None. **A.R. Zavala:** None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: SC3GM130467
R16GM145552
R25DA050687
R25MH055929

Title: Increases in Hippocampal AKT-mTOR Signaling in Adult Female C57BL/6 mice with Juvenile History of Ketamine exposure

Authors: *A. THEMANN, O. LIRA, I. GARCIA–CARACHURE, P. A. FOGEL, P. VARGAS, S. D. IÑIGUEZ;
The Univ. of Texas at El Paso, El Paso, TX

Abstract: Major Depressive Disorder (MDD) is more frequently diagnosed in women when compared to men - a sex-difference that emerges during adolescence. Consequently, teenage girls are more likely to be prescribed with antidepressant medications, including ketamine (KET), for the treatment of MDD. Yet, whether early-life exposure to KET yields lasting side effects remains understudied. To address this issue, we evaluated if juvenile exposure to vicarious defeat stress (VDS) and/or KET would alter spatial memory performance and hippocampal protein-kinase B-mammalian target of rapamycin (AKT-mTOR) signaling in adulthood. Specifically, postnatal day (PD)-35 C57BL/6 female mice underwent 10 days of VDS, with or without KET (20 mg/kg) administration immediately after each stress session. Once mice reached adulthood (PD70) separate groups were assessed for spatial memory performance in the Morris water maze (MWM) or were euthanized for whole hippocampus tissue dissection. No lasting changes in spatial memory performance were noted in adult female mice with juvenile VDS and/or KET pre-exposure. However, independent of stress history, adult female mice pretreated with KET displayed increased hippocampal AKT-mTOR protein expression when compared to controls. To delineate the role of this signaling pathway on spatial memory performance under normosensitive conditions, a separate group of stress-naïve adult females were administered with rapamycin (30 mg/kg/i.p.), an inhibitor of mTOR, prior to MWM testing. Here, we found that rapamycin-treated adult females displayed impaired spatial memory performance, per increases in time to locate the escape platform on the MWM. Together, our findings demonstrate that juvenile KET exposure mediates enduring increases of hippocampal AKT-mTOR signaling while not influencing spatial memory performance - highlighting that early-life KET exposure alters AKT-mTOR signaling in later life.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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NIH Center for Compulsive Behaviors
NIDA Scientific Director's Fellowship for Diversity in Research
NIDA-UMBC Undergraduate Research Internship Program

Title: Development of multipleXed Population Selection and Enrichment single nucleus RNA sequencing to characterize transcriptional programs of neuronal ensembles and other rare populations

Authors: *K. WOODS[1], R. MADANGOPAL[1], R. PALAGANAS[1], O. R. DRAKE[1], D. PHAM[1], M. B. BRENNER[1], T. L. MARTIN[1], M. STEINBERG[2], J. MARTIN[2], C.

CHARENDOFF[2], W. AUSTIN[2], J. CHOI[1], M. MERRIMAN[1], S. J. WEBER[1], V. A. LENNON[1], E. VAN LEER[1], L. E. KOMER[1], B. T. HOPE[1], K. E. SAVELL[1];
[1]NIH, NIDA IRP, Baltimore, MD; [2]BD Biosci., San Jose, CA

Abstract: Relapse is an ongoing clinical problem, and there are currently no effective treatments to reduce the risk of relapse to psychostimulants like cocaine. Environmental stimuli previously associated with drug-taking can precipitate relapse long after cessation of drug use. These maladaptive cue-drug associations are hypothesized to be encoded within specific patterns of strongly activated neurons (neuronal ensembles) that can be identified by Fos. Our lab and others have shown causal roles for Fos-expressing neuronal ensembles in reward-seeking behaviors and identified unique molecular and functional alterations within them. However, due to methodological limitations, previous studies pooled samples of Fos-negative and Fos-positive neuron from different animals, could not characterize cell-type diversity of ensembles or identify molecular alterations within specific ensemble cell-types. To address this gap, we developed a new Multiple**X**ed **Po**pulation **S**election and **E**nrichment single nucleus RNA-**seq**uencing (XPoSE-seq) pipeline to determine cell-type composition of rare ensemble populations and define their transcriptional profiles following learned behaviors.
XPoSE-seq pairs an antibody-based nucleus multiplexing strategy with fluorescence-activated nucleus sorting to allow for user-defined ratios of desired cell populations from individual subjects as input to snRNA-seq. We used male and female Fos-based transgenic rats to label behaviorally active neurons following novel context exploration (1 h). We observed maximal Fos-driven mRFP expression occurs 3 hours after the start of the behavior. After sequencing, we found robust multiplexing oligo detection and were able to successfully demultiplex to assign sample identity to nuclei. Analysis revealed distinct clusters corresponding to known excitatory and inhibitory cell types in the mPFC that further subcluster into expected layer and interneuron sub-types. We found that novel context-active neurons are overrepresented in select cell types, and that these sub-types have distinct transcriptional signatures after novel context exploration. Using the XPoSE-seq pipeline, ongoing analysis is aimed at characterizing cell-type and ensemble-specific transcriptional signatures involved in cocaine-relapse ensembles. In future studies, we will employ CRISPR-based transcriptional modulators to assess causal roles for identified cocaine memory-specific transcriptional fingerprints in persistent cocaine relapse during abstinence.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

Title: Ribosomal protein S6 upregulation in rat medial prefrontal cortex to nucleus accumbens synapses following cue-induced cocaine seeking

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Abstract: Learned associations between discrete cues (or contexts) and drug effects are thought to be encoded by sparsely distributed patterns of neurons called neuronal ensembles. Cue-specific synaptic inputs (synaptic ensembles) on these ensemble neurons are thought to be altered during learning to mediate long-lasting memories. However, we do not have endogenous markers of activated synaptic ensembles to study them in the same way we use Fos as a marker of activated cell bodies. We developed a flow cytometry of synaptoneurosomes (FCS) procedure to examine synaptoneurosomes with single synapse resolution and identified S6 ribosomal protein as an endogenous marker of synaptic activity. Neural activity increased S6 in synaptoneurosomes 5-60 min following cocaine injections and/or non-drug-related novel context exposure. Here we assessed S6 protein expression in synaptoneurosomes from medial prefrontal cortex (mPFC) to nucleus accumbens (NAc) projections in cue-induced cocaine seeking.All rats were injected in mPFC with AAV expressing ChR2-eYFP to tag the specific mPFC terminals in NAc. We trained all rats for 10 days (2 x 3 h session a day) to lever press for cocaine infusions paired with a 3.5-s light cue. After 3-4 weeks in their home cages, rats were exposed for 30 min to either the cocaine-paired light cue (seeking test group) or kept in their home cages. We compared these with a naïve group that did not undergo training. Brains were obtained 60 min after cue exposure. We then assessed S6 protein expression after cocaine seeking used our flow cytometry of synaptoneurosomes (FCS) procedure. S6-positive synaptoneurosomes increased significantly after cocaine seeking, and this effect was specific to the mPFC to NAc pathway. Taking together our previous and current findings we postulate that S6 upregulation can be used as a marker of activated synapses not only for acute general activation but for specific drug-related learned associations, similar to how Fos expression has been used to identify nuclei in neuronal ensembles. Our future studies will focus on applying the S6-based FCS approach to study synaptic plasticity changes in protein expression in cocaine cue-related synaptic ensembles.

Disclosures: F. Rubio: None. **E. Ukpong:** None. **D.E. Olivares:** None. **Y. Gera:** None. **D. Pham:** None. **C. Dunn:** None. **R. Madangopal:** None. **B.T. Hope:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

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Topic: G.09. Drugs of Abuse and Addiction

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NIH Center for Compulsive Behaviors
NIDA Scientific Director's Fellowship for Diversity in Research
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NIDA-UMBC Undergraduate Research Internship Program

Title: Characterization of cocaine-relapse ensemble-specific cell types and transcriptional programs in prefrontal cortex using single nucleus RNA sequencing in Fos-mRFP transgenic rats

Authors: *K. SAVELL[1], R. MADANGOPAL[2], K. WOODS[3], P. SARAVANAN[1], D. THOMPSON[1], D. PHAM[4], O. R. DRAKE[5], M. B. BRENNER[1], B. T. HOPE[1];
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Abstract: Drug-associated memories can drive relapse long after the last instance of drug use. Associative memories, including cue-drug memories, are thought to be encoded within specific patterns of strongly activated neurons (neuronal ensembles) that can be identified using immediate early genes such as Fos. Our lab and others showed that Fos-expressing neuronal ensembles play a causal role in drug-seeking behaviors and identified ensemble-specific transcriptional alterations. However, these studies used pooled samples of Fos-positive and Fos-negative neurons, which contained multiple cell types, and used a targeted analysis of relatively few candidate genes. These results cannot inform which cell types are participating in the drug memory ensemble and cannot distinguish differential transcriptional responses within individual cell types. To address this issue, we combined ensemble-based transgenic tools with our recently developed Multiple**X**ed **Po**pulation **S**election and **E**nrichment single nucleus RNA-**seq**uencing pipeline (XPoSE-seq) to conduct an unbiased screen of ensemble-specific cell types and transcriptional changes during cocaine relapse.
We used male and female Fos-based transgenic rats to label cocaine relapse ensemble neurons in the medial prefrontal cortex (mPFC) following cocaine self-administration training. We trained rats to self-administer cocaine (FR1 reinforcement schedule, 0.75 mg/kg/inf. cocaine paired with a 3.5-s light cue) during twice daily 3 h sessions. Following training and 21 days of abstinence, we tested rats for cocaine seeking (30 min, extinction conditions) and collected brains 3 h after test (peak Fos-driven mRFP expression). We observed reliable cocaine self-administration during training and robust cue-induced cocaine seeking following abstinence. We applied the XPoSE-seq pipeline to identify which cell types comprise the cocaine relapse ensemble and characterize the cell-type specific transcriptional signatures of cocaine relapse. Our analysis revealed distinct clusters corresponding to known cell types in the mPFC (excitatory and inhibitory neurons) that further subcluster into expected layer and interneuron sub-types within mPFC. Using this unbiased approach, ongoing analysis is aimed at characterizing cell-type and ensemble-specific transcriptional signatures that contribute to drug-seeking behaviors. We will employ transcriptional modulators in future experiments to assess causal roles for these cocaine memory-specific genes in relapse.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH, NIDA IRP

Title: In vivo labeling and molecular characterization of cocaine memory-specific active neurons using the photo-convertible calcium integrator CaMPARI2

Authors: ***Y. GERA**[1], K. E. SAVELL[1], O. R. DRAKE[1], M. B. BRENNER[1], V. A. LENNON[1], D. PHAM[1], S. J. WEBER[1], L. E. KOMER[1], L. WANG[2], K. SCHAEFER[2], J. RUBIO[1], A. LEMIRE[2], E. R. SCHREITER[2], V. MENON[3], B. T. HOPE[1], R. MADANGOPAL[1];
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Abstract: Background: In abstinent drug users, cues previously associated with drug-taking can provoke drug craving and promote relapse long after the last instance of drug use. These maladaptive drug-cue associations are thought to be encoded by sparse patterns of neurons (ensembles) that are strongly activated during learning. However previous immediate early gene (IEG, eg. Fos) based ensemble labeling approaches lacked the temporal resolution needed to label active neurons during short behavioral events (e.g, lever press). To address this gap, we developed procedures to label active neurons *in vivo* with sub-second temporal specificity using the photo-convertible calcium-based activity marker CaMPARI2. We delivered ultraviolet photoconversion (PC) light into the infralimbic cortex (IL) during cocaine seeking to convert CaMPARI2 protein in active IL neurons from green to red fluorescent state and permanently label these cocaine-memory neurons.
Methods: We used male and female Sprague-Dawley rats in all experiments. We delivered AAVs into IL for CaMPARI2 expression, implanted optical fibers for PC light delivery and inserted a jugular catheter for cocaine self-administration. We trained rats to self-administer cocaine (FR1, 0.75 mg/kg/infusion + light cue) during twice daily trial-based cocaine self-administration sessions (30 trials/ 3 h session, 1 min lever access/trial). Following training and 21 abstinence days, we used CaMPARI2-photoconversion to permanently label IL cocaine-memory ensembles during a 1 min cocaine-seeking test. We collected brains either immediately after test (0-min group) or waited 10 minutes for experience-induced gene expression (10-min group). We isolated red (active) and green (inactive) CaMPARI2-labeled nuclei and performed single-nucleus RNA sequencing (snRNAseq) to identify unique molecular alterations (differentially expressed genes, DEGs) within IL cocaine-memory ensembles.

Results: We observed reliable cocaine self-administration during training and robust cue-induced cocaine seeking during the 1 min seeking test. CaMPARI2-snRNAseq revealed distinct clusters of glutamatergic and GABAergic IL neurons that subclustered into expected layer and subtypes. Further, IEGs were selectively induced in red 'active' neurons in the 10-min, but not 0-min group.
Discussion: We will identify unique DEGs within CaMPARI-labeled IL cocaine-memory ensembles and investigate DEG distribution across IL cell types. Molecular and cell-type characterization of drug-memory ensembles could help prevent relapse by selectively weakening persistent drug memories, without influencing other memories

Disclosures: Y. Gera: None. **K.E. Savell:** None. **O.R. Drake:** None. **M.B. Brenner:** None. **V.A. Lennon:** None. **D. Pham:** None. **S.J. Weber:** None. **L.E. Komer:** None. **L. Wang:** None. **K. Schaefer:** None. **J. Rubio:** None. **A. Lemire:** None. **E.R. Schreiter:** None. **V. Menon:** None. **B.T. Hope:** None. **R. Madangopal:** None.

Poster

PSTR231. Addiction: Learning, Memory and Development

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA042792

Title: Specificity of cocaine seeking and fear recall ensembles in the dorsal medial prefrontal cortex

Authors: S. LIU[1], X. LIU[2], Q.-S. LIU[3], ***C. OLSEN**[4];
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Abstract: Cocaine use disorder is a chronic and relapsing neuropsychiatric disorder characterized by a strong propensity for relapse upon re-exposure to a previously drug-associated environment. The dorsal medial prefrontal cortex (dmPFC) is a critical node in the mesocorticolimbic system related to cocaine seeking. There is evidence that learned associations between drug cues and drug seeking behavior are encoded by a specific ensemble of neurons sparsely scattered throughout the dmPFC. We hypothesized that inhibition of dmPFC cocaine seeking ensembles inhibits cocaine seeking memory retrieval, and these ensembles are not involved in fear conditioning memory retrieval, which is also mediated by the dmPFC. We tested this hypothesis by co-injection of viruses expressing TRE-Cre and a cre-dependent inhibitory PSAM-GlyR into the dmPFC of male and female cfos-tTA mice to enable "tagging" of ensemble neurons with an inhibitory chemogenetic receptor. Mice then underwent cocaine self-administration (0.5 mg/kg, 14 days) and fear conditioning. In Experiment 1, a dmPFC cocaine seeking ensemble was tagged, while in Experiment 2, a dmPFC ensemble for fear recall was

tagged. In both experiments, subsequent cocaine seeking and fear recall were tested during inhibition of the tagged dmPFC ensemble (0.3 mg/kg uPSEM792s, 30 min prior to sessions). In both sexes, inhibition of the cocaine seeking ensemble suppressed cocaine seeking, but not recall of fear memory, while inhibition of the fear ensemble reduced conditioned freezing but not cocaine seeking. These data demonstrate that cocaine and fear recall ensembles in the dmPFC are stable, but mutually exclusive from one another.

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Poster

PSTR231. Addiction: Learning, Memory and Development

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Program #/Poster #: PSTR231.28/SS1

Topic: G.09. Drugs of Abuse and Addiction

Support: P01DA047233
R01DA014133

Title: Capturing and profiling Arc neuronal ensembles encoding drug-context associations in the nucleus accumbens

Authors: *M. SALERY[1], *M. SALERY[3], A. GODINO[1], Y. XU[1], R. DURAND-DE CUTTOLI[1], A. R. LABANCA[1], L. M. HOLT[1], J. F. FULLARD[2], P. ROUSSOS[2], E. J. NESTLER[1];
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Abstract: Learned associations between the rewarding effects of a drug and the context in which it is experienced are decisive for precipitated drug-seeking and relapse. As learned associative memories have been proposed to be stored in sparse and highly discriminative populations of neurons, growing evidence suggests that drug-recruited neuronal ensembles could similarly encode addiction-related pathological memories. In this study, we explore the dynamics and molecular mechanisms of both the recruitment of these ensembles upon initial drug exposure and their contribution to the encoding, strengthening, and ultimately expression of drug-related memories. Additionally, we explore the intrinsic (phenotypical) or acquired (plastic) cellular properties that would favor the allocation of specific cells to these functional ensembles and predict their further reactivation. Capitalizing on the activity-dependent labeling of neuronal ensembles in *Arc*-CreER^{T2} mice (Denny et al., 2014), we captured cocaine-activated cells in the nucleus accumbens and permanently tagged them with fluorophores or channel-rhodopsin for further characterization, optogenetic manipulation, and single-nuclei sorting. We identified a subset of neurons activated at both early and late stages of drug exposure and show that the level of reactivation of the initial ensemble correlates with locomotor sensitization. Similarly, re-exposure to a cocaine-paired context in a conditioned place preference (CPP) paradigm was

associated with an increased reactivation of cocaine-recruited ensembles. The behavioral consequences of such reactivation were further assessed using optogenetics-mediated artificial reactivation. We found that the reactivation of ensembles recruited at early- versus late-stages of drug exposure had opposite effects on CPP expression. We then isolated tagged nuclei with FACS and performed single nucleus RNA sequencing to analyze their transcriptional signature. We show that D1- and D2-dopamine receptor-expressing subtypes of medium spiny neurons are differentially recruited through the encoding and expression of drug-context associations. Using activity-dependent transcriptional programs as a marker of recent activation, we isolated a cluster of reactivated cells within the initially activated ensemble and identified gene programs predictive of context-dependent reactivation. Together, such multiscale and ensemble-specific approaches represent a pivotal step towards a better understanding of the cellular and molecular processes involved in the encoding of pathological memories associated with drug addiction. Supported by NIDA

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Poster

PSTR232. Alcohol: Molecular Mechanisms

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Program #/Poster #: PSTR232.01/SS2

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIAAA 5R21AA026066
NIH/NIAAA 5R21AA028352

Title: Circuit & molecular encoding of memory-like states for alcohol tolerance in Drosophila

Authors: *N. R. KACHEWAR[1], C. LARNERD[3], A. LANGE[3], P. ADHIKARI[3], F. W. WOLF[2]; [1]Hlth. Sci. Res. Inst., [2]Dept. of Mol. and Cell Biol., Univ. of California, Merced, Merced, CA; [3]Quantitative and Systems Biol. Grad. Group, UC Merced, Merced, CA

Abstract: How initial neural plasticity induced by drugs of abuse lays the foundation for addiction is unclear. Ethanol tolerance is an initial form of neural and behavioral plasticity that is amenable to complete characterization. The intensity and pattern of ethanol exposure creates molecularly and anatomically distinct tolerance types. We discovered that binge-like short high ethanol dose (for rapid tolerance) and long low ethanol dose (for chronic tolerance) form intermediate and long-term memory-like states, respectively. The intermediate memory-like state is composed of labile and consolidated traces. Hence, initial ethanol exposure creates multiple distinct types of behavioral plasticity. Determining the molecular and circuit mechanisms for each trace, will let us better understand their contribution to alcohol use disorder. Our findings indicate distinct encoding mechanisms that interact with one another for each state. Both forms

of tolerance require the mushroom body learning and memory centers, but they require anatomically distinct regions. Moreover, the mushroom bodies are differentially required by tolerance type for acquisition, consolidation, and expression. A functional screen revealed new rapid tolerance-specific circuit components, including glutamatergic clock neurons and their postsynaptic partners that also regulate evening sleep. Genetic manipulation of long-term memory genes revealed that the trace - or engram - for chronic tolerance resides outside the mushroom bodies. Thus, rapid and chronic tolerance are encoded by different neural circuits that are also distinct from known learning and memory circuits.Chronic tolerance is encoded into the genome through reversible changes in histone acetylation. This mechanism occludes rapid tolerance development following a chronic exposure, indicating crosstalk between tolerance forms. The histone deacetylase Sirt1 is strongly downregulated by ethanol exposure: it promotes rapid tolerance but inhibits chronic tolerance. Sirt1 permits binge-like ethanol to regulate gene expression, including those for presynaptic function. Rapid and chronic tolerance changes in gene expression are markedly different, indicating different molecular mechanisms. We are poised to identify key molecular events that encode specific ethanol experiences into memory-like states in individual neurons.

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Poster

PSTR232. Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R25NS127776-01
NIH-NIGMS COBRE
PRST Catalyzer Research Grant

Title: A potential role of the gut microbiome in promoting age related alcohol tolerance in Drosophila

Authors: *P. PUJOLS, K. RIVERA, A. GHEZZI, I. RODRÍGUEZ-FERNÁDEZ;
Univ. of Puerto Rico, Rio Piedras, San Juan, PR

Abstract: Alcohol Use Disorder (AUD) is a medical condition characterized by a limited ability to stop or control alcohol use despite adverse social, occupational, or health consequences. In older adults, the habit of excessive alcohol consumption represents 20% between the ages 60 to 64 years and 11% over 65 years of age, according to the National Survey and Drug Use and Health. Aging can affect the response to alcohol in aspects of sensitivity and tolerance, which puts the elderly at greater risk. Thus, there is an urgent need to characterize the cellular and molecular effects of alcohol consumption in this aging population. Aging has twelve proposed

hallmarks but of particular interest for our project is the altered intercellular communication between the animal genome and the microbiome leading to microbial dysbiosis. The human being is home to around 38 trillion bacteria and this collective commensal, symbiotic, and pathogenic microorganisms is known as the microbiota, the majority of which is found in the intestine. Our study aims to understand the effects of alcohol on the bidirectional interaction of the gut microbiome and the brain in aspects of tolerance and sensitivity in young and old animals. We are using *Drosophila melanogaster* as a model organism as it has homology with humans in alcohol response, intestinal microbiome composition, and displays aging phenotypes. To measure age-related changes in alcohol behaviors, we exposed young (7 days) and old (45 days) Canton-S female flies to 50% ethanol vapor. We then calculated the time they take to sedate. For sensitivity assays, we measured resistance to alcohol in a first exposure compared to a control group that is exposed to water. While for tolerance we calculated the difference between resistance from a second exposure generated by a previous one. We then measure the changes in the gut microbiome after one or two exposures to alcohol. To do this we dissected the intestine of treated flies and plated the homogenate in selective media for *Lactobacilli*, *Acetobacteria,* and *Enterobacteria* and measure abundance in Colony Forming Units (CFUs). Our data shows that there is an increase in sensitivity and tolerance in old flies. In young and old flies, we find that after one exposure to alcohol, all CFUs increase and then decrease during the second exposure. Young flies treated with antibiotics have a decrease in tolerance. Future studies are focused on finding the molecular mechanism used by specific bacterial species to influence sensitivity and tolerance in flies. We hope to elucidate the underlying causes of neuroadaptation possibly caused by changes in the gut microbiome and to further understand these changes in the aging context.

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Poster

PSTR232. Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA022414-01

Title: Dunc13, RIM, and Rab3 mediated differential regulation of alcohol-related behavior in Drosophila

Authors: *G. SHRESTHA[1], G. W. ROMAN[2];
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Abstract: <*Dunc13* is an active zone protein homologous to the mammalian Munc13-1 that interacts with RIM and Rab3 as a heterotrimer. Alcohol binds to the C1 domain of

Dunc13/Munc13-1 and inhibits its ability to bind to Diacylglycerol. The genetic reduction of *Dunc13* activity in the *Dunc-13*^{P84200}/+ heterozygotes results in an increased self-administration and behavioral, physiological, and molecular resistance to the effects of alcohol, mimicking tolerance. The targeted reduction of *Dunc13* in the mushroom body neurons, through the expression of *Dunc13* RNAi, decreased alcohol sedation sensitivity ($p<0.03$). In contrast, the reduction in the ellipsoid body neurons led to increased alcohol sedation sensitivity ($p<0.009$). Hence, alcohol's inhibition of Dunc13 activity will likely lead to distinct brain region-specific outcomes. We further examined the role of *Dunc13* in regulating gene expression of presynaptic proteins 4 hours after alcohol exposure with RT-qPCR. In the heads of wild-type flies, we observed a significant decrease in *Dunc 13* and *synaptobrevin* expression and a significant increase in *RIM* and *Rab3* expression ($p<0.05$). The reduced levels of *Dunc13* mRNA in ethanol-treated wildtype flies closely approximated the levels found in the *Dunc-13*^{P84200}/+ heterozygotes. We examined the Loss-of-Righting phenotype of the *RIM*^{MB07541} insertional mutants, *RIM*^{EX73} null mutants, and *Rab3*^{rup} null mutants to determine if RIM and Rab3 also regulate alcohol sedation sensitivity. Both *RIM*^{MB07541} and *RIM*^{EX73} mutants have a dominant increase in alcohol sensitivity ($p<0.001$). Furthermore, reducing *RIM* expression in the mushroom bodies is sufficient to increase alcohol sedation sensitivity ($p<0.015$), and ectopic RIM expression in the mushroom bodies of *RIM*^{EX7} mutants resulted in the rescue of the mutant phenotype ($p<0.001$). Similarly, *Rab3*^{rup} mutants also displayed a dominant increase in alcohol sensitivity ($p<0.01$), suggesting both RIM and Rab3 activity affect alcohol resistance. The haploinsufficiency of *Dunc13*, *RIM*, and *Rab3* indicates alcohol sedation sensitivity is very responsive to the levels of these three proteins. Interestingly, the *RIM*^{MB07541}/+; *Dunc-13*^{P84200}/+ trans-heterozygotes have wild-type levels of alcohol sedation sensitivity ($p<0.02$), demonstrating an additive genetic interaction between *Dunc13* and *RIM* mutations.>

Disclosures: G. Shrestha: None. **G.W. Roman:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

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NIH Grant R01AA019536
NIH Grant R01DK110358

Title: Acute alcohol exposure synergizes with cholinergic neuron suppression to cause long-lasting sleep deficits in Drosophila

Authors: *M. CHVILICEK, I. TITOS, J. D. CHEN, A. R. RODAN, A. ROTHENFLUH; Univ. of Utah, Salt Lake City, UT

Abstract: Alcohol consumption causes short- and long-term sleep impairments that persist even during recovery from alcohol use disorder (AUD) when individuals are abstinent from drinking. Two significant elements of alcohol-induced sleep disruption include shortened nighttime sleep duration and increased sleep latency (the time it takes to fall asleep). These measures continue to be disturbed even after several weeks without alcohol. Sleep deficits are highly prevalent, affecting as many as 72% of AUD patients, and are one of the strongest predictors of relapse to drinking. Despite the severity of these problems and the high number of people facing them, the underlying biological mechanisms of alcohol-induced sleep deficits are poorly understood, making them difficult to treat in a targeted manner. To address this gap in knowledge, we take advantage of *Drosophila melanogaster*'s translatability for human sleep and alcohol responses to model alcohol-induced sleep disturbances. We utilized an alcohol exposure paradigm in which experimental flies become intoxicated due to inhalation of vaporized ethanol, eventually becoming sedated, while control flies receive water and experience no behavioral alterations. We performed these exposures in two different wild-type fly strains and then analyzed sleep using the *Drosophila* Activity Monitor system. We show that a single, sedating alcohol exposure causes strong loss of nighttime sleep and increases in sleep latency. Like in humans in a period of abstinence following heavy alcohol use, these effects last for days but eventually recover. We then altered the duration of the ethanol exposure and show that shorter, hyperactivating exposures failed to induce sleep deficits, even when repeated. This suggests that CNS-depressant ethanol action is required for long-lasting effects on sleep since hyperactivating exposures fail to depress the CNS. Then, we facilitated CNS depression by reducing or increasing neuronal activity throughout the brain or in subsets of neurons for specific neurotransmitters (acetylcholine, glutamate, and GABA). These manipulations synergized with ethanol exposure to induce behavioral incapacitation. However, of these various manipulations, sleep deficits only occurred when ethanol exposure occurred concurrently with reduced activity pan-neuronally or in cholinergic neurons. These data therefore suggest that ethanol-induced suppression of cholinergic neurons induces long-lasting loss of nighttime sleep and increases in sleep latency, which are conserved from *Drosophila* to humans.

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Poster

PSTR232. Alcohol: Molecular Mechanisms

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Support: Swedish Medical Research Council 2018-02814
Swedish Medical Research Council 2020-00559
Swedish Medical Research Council 2020-01346
Swedish Medical Research Council 2020-02105
LUA/ALF

Title: Ethanol intake in the rat is regulated by accumbal cholinergic signaling

Authors: *A. LOFTÉN[1,4], D. CADEDDU[2], L. ADERMARK[2], M. ERICSON[3], B. SÖDERPALM[3,4];
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Abstract: Alcohol use disorder is associated with serious medical consequences leading to preterm death. Although few in numbers, cholinergic interneurons have arisen as an important cell population within the nucleus accumbens that may exert a regulatory impact on local dopamine neurotransmission. In fact, a defect in cholinergic interneurons have been implied in psychiatric disorders such as alcohol addiction. The exact mechanisms through which endogenous cholinergic activity modulates dopamine release in response to ethanol administration and its role in development of addiction is not known. However, we have recently shown that the ethanol-induced increase in extracellular levels of accumbal dopamine is blocked by a combination of a muscarinic and a nicotinic antagonist given locally, implicating cholinergic signaling. Moreover, ablation of accumbal cholinergic interneurons attenuate the ethanol-induced increase of extracellular dopamine. Together these data suggest that activation of cholinergic interneurons, probably resulting in increased acetylcholine level, is involved in ethanol's dopamine releasing effect. The objective of this project was to investigate the functional role of accumbal cholinergic interneurons in ethanol intake in rat, to confirm the importance of the recent molecular findings. To this end, an intermittent ethanol consumption paradigm, know to induce high ethanol intake in outbreed rats, was used together with a toxin-based method to selectively ablate accumbal cholinergic interneurons. A selection of male rats presenting a high ethanol intake during an initial screening period, underwent stereotactic surgery to receive anti-choline acetyltransferase-saporin or a sham solution bilaterally into the nucleus accumbens. Rats treated with anti-choline acetyltransferase-saporin consumed significantly less ethanol than sham-treated controls. Further, the alcohol deprivation effect was abolished in toxin-treated animals whilst being present in sham-treated rats. The water intake and the weight gain were not affected by the depletion. In conclusion, these data support a functional role of accumbal cholinergic interneurons in ethanol´s rewarding effect, opening up for new potential pharmacological targets for treatments of alcohol use disorder.

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Poster

PSTR232. Alcohol: Molecular Mechanisms

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Program #/Poster #: PSTR232.06/SS7

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01AA027807-04
T32GM135751

Title: Influence of Sex and Age on Synaptic Gating in Nucleus Accumbens Medium Spiny Neurons

Authors: *T. LE[1], P. GIMENEZ GOMEZ[2], G. E. MARTIN[2];
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Abstract: **Purpose:** Binge alcohol drinking is a drinking pattern typically associated with adolescents and young adults. Bechara and Damasio postulated that alcohol exposure during adolescence weakens the frontocortial-reflective system, a system involved in decision-making and the ability to assess one's actions. Conversely, it strengthens the amygdala-impulsive system, which links pleasant or aversive stimuli to their emotional attributes. These two systems project onto the nucleus accumbens (NAc), a brain region that plays a pivotal role in the reward circuit and addictive behavior. We recently reported that these competing cognitive and emotional inputs primarily converge onto NAc medium spiny neurons (MSN's), where they are processed, integrated, and translated into behavior through a phenomenon known as "synaptic gating." We also found that synaptic gating, defined as the ability of the neural circuit to facilitate or suppress specific inputs (i.e., emotional or cognitive), is sensitive to binge alcohol drinking. However, little is known about how sexes and age affect synaptic gating. Such an understanding can help elucidate the biological propensity as to why girls and young women from the ages of 12-20 years old are more likely to drink more alcohol than 12-20 years old boys and young men.**Material and Methods:** To address this key biological question regarding sex and age differences, we expressed channelrhodopsin and chrimson in the PFC and BLA, respectively, of male and female C57BL/6 mice through stereotaxic surgery at four weeks old. Afterward, we performed NAc MSNs whole-cell recordings after independent light stimulation of BLA and PFC afferents at 6-, 8-, and 12-weeks old.**Results**: We found that, in males, the ability of cortical inputs to inhibit the transmission of information from the BLA region increases significantly in 8 weeks old mice compared to younger animals (i.e., 6-week-old). Interestingly, PFC inhibition of BLA transmission is strongest at 12 weeks old in females relative to 6- and 8-weeks old. Moreover, 20% ethanol negates the PFC inhibition of the BLA in both sexes.**Conclusion:** Our work suggests that the reciprocal control of emotional and cognitive information in the NAc is a flexible phenomenon that depends on age and sex.

Disclosures: **T. Le:** None. **P. Gimenez Gomez:** None. **G.E. Martin:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.07/SS8

Topic: C.01. Brain Wellness and Aging

Support: Beca de Doctorado Nacional ANID 21201061
Fondecyt 3210260
Fondecyt 1221080
NIH R01 AA025718

Title: Reduced level of glycine receptors in the aged nucleus accumbens is accompanied by a diminution in ethanol condition place preference

Authors: *A. GUZMÁN[1,2], L. ARMIJO-WEINGART[1], L. SAN MARTÍN[1], L. AGUAYO[1]; [1]Physiol., Univ. de Concepción, Concepción, Chile; [2]Basic Sci. and Morphology, Univ. Católica de la Santísima Concepción, Concepción, Chile

Abstract: The nucleus accumbens (nAc) is associated with addictive behaviors and undergoes significant changes with aging. It mainly comprises medium spiny neurons (MSNs), namely MSN-D1 or MSN-D2. MSNs express glycine receptors (GlyRs) that inhibit excitability and regulate dopamine release. Since GlyRs are allosterically modulated by ethanol (EtOH), changes in their expression may affect EtOH-associated behaviors. Therefore, we aimed to determine changes in GlyRs expression and neuronal activity in nAc MSNs during aging and subsequently study whether they influence EtOH conditioning. Using C57BL/6 mice (3, 6 and 12 months), we studied the dynamics of intracellular Ca^{2+} increase utilizing the expression of GCaMP6s in nAc slices in the presence of strychnine (STN) and EtOH. In BAC Drd1a-tdTomato mice, immunohistochemistry (IHC) and cell sorting-coupled RTqPCR assays were performed to obtain information on the differential expression of α1 and α2 GlyRs subunits in MSN-$D1^+$ and MSN-$D1^-$. We found that electrically generated Ca^{2+} transients, after adding 1 μM STN, progressively decreased with age ($p<0.05$), with a neuronal activation level of 276±83.7, 177±56.3 and 112±8.7% at 3, 6, and 12 months, respectively. Treatment with 20 mM EtOH caused a 19% increase at six months relative to 3 months, where the response was reduced (67±8.6). On the other hand, IHC for α subunits in MSN-$D1^+$ and MSN-$D1^-$ in the nAc showed a 32% decrease at 6 months in MSN-$D1^+$ ($p<0.01$) with no change in MSN-$D1^-$ as compared to the 3-month group. More specifically, the expression of α1 and α2 subunits showed that the α1 subunit of MSN-$D1^+$ was higher at two months than at 6 and 12 months of age ($p<0.05$), while no differences were found in MSN-$D1^-$. Similarly, the expression of the α2 subunit was not modified during aging. Finally, conditioned place preference studies showed that EtOH caused no conditioning at six months, which was reversed by inducing α1-subunit overexpression in a group of mice of the same age ($p<0.05$). These results show decreased expression and activation of GlyRs in aged nAc MSNs, with the α1 subunit in MSN-$D1^+$ being the most affected from 6 months onwards. Since α1 is one of the most ethanol-sensitive subunits, this would lead to dopaminergic dyshomeostasis and, consequently, to behavioral changes associated with the absence of conditioning. These data could help elucidate the neurobiological basis of EtOH-associated behavioral changes during aging, which could also be relevant in other behaviors related to decreased responsiveness to rewarding stimuli in aging individuals.

Disclosures: A. Guzmán: None. **L. Armijo-Weingart:** None. **L. San Martín:** None. **L. Aguayo:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.08/SS9

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in alcohol consumption are related to specific characteristics of the opioid system in mesolimbic structures

Authors: *L. M. MOLINA-MARTÍNEZ[1,2], Y. L. NAVA-CISNEROS[2], J. JUÁREZ[2]; [1]Biología Celular y Mol., CUCBA, Univ. de Guadalajara, Zapopan, Jalisco, Mexico; [2]Inst. de Neurociencias, CUCBA, Univ. de Guadalajara, Guadalajara, Jalisco, Mexico

Abstract: The participation of the endogenous opioid system in the reward and reinforcement mechanisms of alcohol intake, involves the β-endorphin release from the hypothalamic arcuate nucleus (ArN) and their binding to mu-opioid receptors (MOR) located in mesolimbic areas such as the Nucleus Accumbens (NAc), Amygdala (Amy) and Ventral Tegmental Area (VTA). Considering the evidence of sexually dimorphic characteristics in alcohol consumption and the opioid system, the aim of this work was to compare the number of positive β-endorphin neurons in the ArN and the MOR expression in the NAc, Amy and VTA in male and female Wistar rats with high (HC) and low levels of alcohol consumption (LC). The count of β-endorphin-immunopositive neurons in the ArN and the expression of MOR in the mesolimbic areas were performed by immunohistochemical and Western blot analysis, respectively. Our results showed that female rats consumed approximately 33% more alcohol compared to male rats. The high-drinking group of rats had a lower number of β-endorphin-positive neurons compared to the low-drinking group of rats regardless of sex. In female HC rats there was a high expression of MOR in both the NAc and VTA compared to female LC rats, while in males this only occurred in the Amy. Sex differences in alcohol consumption could be explained by the variability that exists in the activity of the endogenous opioid system between males and females.

Disclosures: L.M. Molina-Martínez: None. **Y.L. Nava-Cisneros:** None. **J. Juárez:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.09/SS10

Topic: G.09. Drugs of Abuse and Addiction

Support: NICHD, DIR

Title: Sex-specific regulation of ethanol intake and preference by PKA RIIα

Authors: *E. LONDON;
Eunice Kennedy Shriver Natl. Inst. of Child Hlth. an, Bethesda, MD

Abstract: SfN 2023 abstract title: **Sex-specific regulation of ethanol intake and preference by PKA RIIα**
Edra London,[1] Michelle Bloyd,[1] Erica Lesko,[1] Anna Vlachos,[1] Daniel Abebe,[1] Constantine A Stratakis,[2, 3] and Chris J McBain[11] Section on Cellular and Synaptic Physiology, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD); [2] Section on Endocrinology & Genetics, Program on Developmental Endocrinology & Genetics, *Eunice Kennedy Shriver* NICHD; [3]Human Genetics & Precision Medicine, IMBB, FORTH, Heraklion, Crete, & ELPEN Research Institute, Athens, Greece
Mice deficient for protein kinase A regulatory subunit IIα (PKA RIIαKO) have altered reward pathway-related behaviors including reduced intake of palatable foods and increased voluntary running. These behavioral phenotypes are mediated by altered PKA signaling in medial habenula (MHb) where RIIα is highly and specifically expressed. The MHb-interpeduncular nucleus (IPN) axis plays central roles in the regulation of mid-brain monoamines, value-based decision making, mood, addiction/withdrawal, and nociception. We hypothesized that ethanol drinking and preference would be impacted by the previously observed MHb defects in PKA localization and signaling in the RIIαKO mouse. We assayed ethanol sensitivity, ethanol intake and preference, and evaluated the impact of acute ethanol treatment on c-Fos expression in Hb, IPN and important downstream targets of MHb-IPN signaling. Sexual dimorphism is a documented phenomenon among some PKA-dysregulation-associated phenotypes. Interestingly, while RIIαKO females consumed significantly less 10% ethanol than same-sex WT littermates, male KO mice consumed more 10% ethanol than their WT counterparts. c-Fos staining revealed differences in ethanol-induced neuronal activity in Hb-IPN signaling between WT and KO mice. These data provide new insight into the role of habenular PKA signaling and the RIIα subunit in ethanol intake.

Disclosures: E. London: None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.10/SS11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant AA022534
NIAAA T32-AA014127

Title: Prenatal Alcohol Exposure (PAE) and Acute Stress Alter Limbic Levels of Corticotropin-Releasing Hormone (CRH) and Neuroimmune Factors in Mice

Authors: *J. R. ZIMMERLY, A. K. FERNANDEZ OROPEZA, M. S. SUN, S. NOOR, A. A. PASMAY, A. N. PRITHA, C. F. VALENZUELA, D. D. SAVAGE, E. D. MILLIGAN; Univ. of New Mexico Dept. of Neurosciences, Albuquerque, NM

Abstract: Prenatal alcohol exposure (PAE) results in a constellation of negative consequences clinically known as fetal alcohol spectrum disorders (FASD), which include altered stress responses and mood disorders. Limbic structures, including the hypothalamus and amygdala, influence the hypothalamic-pituitary-adrenal (HPA) response to stress that is initiated by the production of corticotropin-releasing hormone (CRH) in the hypothalamus. Stress-evoked HPA activation is associated with production of proinflammatory cytokines such as tumor necrosis factor α (TNFα) via activation of the toll-like receptor 4 (TLR4) cascade. Stress-evoked glucocorticoid (CORT) release into circulation exerts negative feedback via glucocorticoid receptors (GR) that inhibits further HPA activation and CORT release. PAE has been linked to adult brain GR dysregulation in response to stress. We hypothesized that PAE desensitizes the limbic GR response and simultaneously sensitizes the TLR4 pathway in response to acute stress. Pretreatment with the synthetic GR agonist dexamethasone (DEX) was administered to suppress stress-induced CORT levels. At 1.5 hr prior to 30-min of restraint stress or no stress, subcutaneous vehicle (DMSO; 1:100 in sterile phosphate buffered saline PBS, pH 7.4) or DEX (25 or 50 µg in DMSO:PBS) was given to 3-5 month old C57BL/6 mice, or offspring that underwent prenatal control exposure (saccharine; SAC), or PAE (10% EtOH) were given 25 ug DEX or VEH. Tail vein blood collection occurred immediately after stress. At 3 or 24 hr after stress, the hypothalamus and amygdala were collected. Messenger RNA (mRNA) expression levels of CRH, TLR4, and TNFα were assessed by RT-qPCR. Blood plasma CORT levels were assayed by enzyme-linked immunosorbent assay. All mice with stress exposure showed significantly elevated CORT levels without DEX pretreatment compared to controls while DEX blunted stress-induced CORT elevations. The PAE-stressed amygdala revealed blunted levels of TNFα at 3 hours, with elevations in TNFα in PAE animals emerging at 24 hrs. At 3 hr, the PAE-stressed hypothalamus revealed elevated TLR4 expression and strong trends in blunted CRH expression. At 24 hr, CRH returned to basal levels with TLR4 remaining elevated. These data support that the peripheral GR response is unaltered by PAE, while PAE dysregulates the neuroimmune stress-evoked corticolimbic response. Ongoing studies are examining mRNA levels of stress- and neuroimmune factors in the prefrontal cortex and anterior cingulate cortex.

Disclosures: J.R. Zimmerly: None. **A.K. Fernandez Oropeza:** None. **M.S. Sun:** None. **S. Noor:** None. **A.A. Pasmay:** None. **A.N. Pritha:** None. **C.F. Valenzuela:** None. **D.D. Savage:** None. **E.D. Milligan:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.11/SS12

Topic: G.09. Drugs of Abuse and Addiction

Support: F31AA030727
P50AA022537
R01AA027581

Title: Mechanisms of Ethanol Anxiolysis: Role of Global Ninein Deletion on Ethanol and Anxiety-like Behaviors

Authors: *E. R. GNATOWSKI[1], M. F. MILES[2];
[2]Virginia Commonwealth Univ., [1]Virginia Commonwealth Univ., Richmond, VA

Abstract: Purpose: Anxiety disorders serve as a predictor of developing Alcohol Use Disorder (AUD) with human subjects reporting stress and anxiety as drivers of ethanol consumption. The Miles laboratory previously identified Ninein (*Nin*) as a candidate gene underlying ethanol's acute anxiolytic-like properties in BXD recombinant inbred mice, using the light-dark box (LDB) transition model of anxiety. We have obtained global Ninein deletion mice and hypothesize deletion of *Nin* will decrease basal anxiety, increase ethanol anxiolysis, and increase ethanol consumption.
Methods: Mice wild-type (WT, $Nin^{+/+}$), heterozygous (HET, $Nin^{+/-}$), or homozygous (HOM, $Nin^{-/-}$) for *Nin* deletion underwent a behavioral battery to assess basal and withdrawal anxiety-like behavior, acute ethanol anxiolytic-like response, ethanol consumption and preference, and ethanol sensitivity. Anxiety-like behavior was measured using the light dark box transitional model (LDB). Increased anxiety-like behavior is measured by a decreased percent time spent in the light (%TIL) and a decreased percent distance traveled in the light (%DTL) of the LDB. Ethanol anxiolysis was examined using intraperitoneal (i.p.) injections of 1.8 g/kg of ethanol or saline (v/v) 5 minutes prior to testing in the LDB. Ethanol consumption was measured using the two-bottle choice intermittent access model (2BC-IEA) (24h access, 20% v/v ethanol, and water) on a M-W-F schedule for 5 weeks. Withdrawal anxiety was measured in LDB 24-hours following drinking. Loss of righting reflex (3.8 g/kg, i.p.) was assessed to examine ethanol sensitivity.
Results: There was no significant effect of sex or genotype on basal anxiety in the LDB. For ethanol anxiolysis studies, there was an overall effect of genotype on %TIL and a significant increase in both %TIL and %DTL in response to ethanol. 2BC-IEA studies show no changes in ethanol intake (g/kg), but HOM animals exhibit a significant decrease in ethanol preference (%) compared to WT. Female HOM mice showed a significant increase in anxiety-like behavior during acute withdrawal compared to female WT animals. No effect of withdrawal was seen in the male animals. Across all LDB tests, female animals exhibited a significant increase in locomotor activity than males.
Conclusion: Ninein may be a novel contributor to mechanisms underlying ethanol's anxiolytic properties and ethanol withdrawal-induced anxiety. Understanding the role of Ninein in these behaviors may contribute to future treatment of AUD. Future experiments will investigate selective *Nin* deletion in the central amygdala (CeA) on anxiety-like and ethanol-related behaviors.

Disclosures: E.R. Gnatowski: None. **M.F. Miles:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.12/SS13

Topic: F.04. Neuroimmunology

Support: NIH Grant K99 AA029454
NIH Grant P50 AA012870
NIH Grant P30 DA018343

Title: Prebiotics modulate central and peripheral effects of alcohol

Authors: *S. L. THOMPSON[1], N. PARK[1], J. R. TAYLOR[1,2,3];
[1]Psychiatry, [2]Neurosci., [3]Psychology, Yale Univ., New Haven, CT

Abstract: Alcohol (EtOH) alters reward circuitry in the brain, which promotes maladaptive behavior. EtOH also has vast peripheral effects including alteration of the gut microbiota, damage to the intestinal barrier, systemic inflammation, and liver damage. Recent work suggests that neuroinflammation may contribute to maladaptive behaviors associated with EtOH, and that the gut microbiota modulate behavioral and peripheral tissue damage effects of EtOH. The modulating role of the microbiota in central and peripheral EtOH effects is not well understood. Prebiotics are indigestible fiber products that 'feed' beneficial bacteria in the gut. Prebiotics protect the intestinal barrier and liver from EtOH, which suggests they may be good candidates to prevent peripheral EtOH-induced pathology. However, effects of prebiotics on EtOH consumption and EtOH-induced brain changes are unknown. Here, we provided male C57BL/6N mice with the prebiotic galacto-oligosaccharides (GOS; n = 15) or control (CTRL; n = 10) in the drinking water prior to and throughout 12 weeks of intermittent access to EtOH. GOS protected the intestinal lining, as measured by fecal albumin levels, but elevated EtOH consumption. To test whether consumption may be increased due to GOS-induced enhancement of EtOH metabolism, we provided naïve mice with GOS or CTRL (n = 11/group) in the drinking water preceding and throughout 8 wks of daily gavage of 2 g/kg EtOH. One hour following the first and final dose, blood EtOH concentration (BEC) was measured. GOS decreased BEC and spleen weight, a marker of peripheral inflammation. Next, we sought to determine whether prebiotics altered EtOH metabolism and immune signaling. We performed untargeted proteomics profiling of the spleen, liver, medial prefrontal cortex (mPFC), and nucleus accumbens (NAc) from a subset of these mice plus a no-EtOH control group (n = 6/group). Strikingly, one of the top proteins increased by GOS in liver was Aldh2, which metabolizes acetaldehyde, a toxic metabolite of EtOH. The top pathways rescued in the spleen were immune-related. Interestingly, levels of key differentially expressed immune proteins in NAc correlated with Aldh2 in liver or immune protein levels in spleen, suggesting that neuroimmune effects of EtOH in the NAc may be modulated by related pathways in the periphery that are orchestrated by the gut. Together, these findings suggest that GOS enhances EtOH metabolism in the liver, reducing peripheral inflammation in the spleen as well as downstream alterations in the mPFC and NAc that contribute to increased drinking. Future work will determine whether these effects are driven by the gut microbiota.

Disclosures: S.L. Thompson: None. **N. Park:** None. **J.R. Taylor:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.13/SS14

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA029924

Title: Role of the epigenetic enzyme EZH2 in compulsive alcohol intake

Authors: *E. BARBIER, E. DOMI, L. HOGLUND, L. XU, S. TOIVAINEN ELOFF, K. CHANTHONGDEE, M. HEILIG;
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Abstract: Alcohol use despite adverse consequences (i.e., compulsive use) is a core feature of AUD and can be modelled in rats using a contingent footshock-punished alcohol self-administration procedure. In previous works, we found that punishment-resistant alcohol self-administration was associated with increased activity of neurons expressing protein kinase C delta (PKCδ) in the central amygdala (CeA). Activity-dependent tagging, followed by chemogenetic inhibition of neurons activated during punishment-resistant self-administration, suppressed alcohol taking, suggesting a functional role of this neuronal population. In the present study, we aim to identify the molecular mechanisms that promote the activation of the CeA PKCδ neuronal ensemble during compulsive alcohol intake. Given the important role of epigenetic mechanisms in regulating alcohol-induced long-term neuroadaptation, we particularly focused on epigenetic enzymes. Using the NanoString® technology, we found an increased expression of the Enhancer of Zeste 2 (EZH2) in the CeA of punishment-resistant rats when compared to rats that decreased alcohol intake in presence of footshock. EZH2 is a histone 3 lysine 27 (H3K27) methyltransferase and is a catalytic component of the polycomb repressive complex 2 (PRC2). To determine the functional role of EZH2 in compulsive alcohol intake, we knocked down EZH2 in the CeA using a viral vector approach (AAV-5/2-hSyn1-chI[4xsh(rEzh2)]-mScarlet-I-WPRE-bGHp(A)). Ezh2 knockdown decreased resistance to punishment in “resistant” rats and had no effect on “sensitive” rats, indicating a functional role of this epigenetic enzyme in compulsive alcohol intake. In line with these findings, we also found that pharmacological inhibition of EZH2 using tazemetostat, a potent and selective Ezh2 inhibitor, decreased resistance to punishment in “compulsive” rats and showed no effect in “non-compulsive” rats. Together our findings highlight the contribution of epigenetic mechanisms and more particularly EZH2, in mediating compulsive alcohol intake.

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Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.14/SS15

Topic: G.09. Drugs of Abuse and Addiction

Support: 1 K01 AA028059-02

Title: Epigenetic changes in the mouse cerebellar cortex following chronic intermittent alcohol exposure

Authors: *P. ZAMUDIO-BULCOCK[1], J. J. WOODWARD[2];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]Neurosci., Med. Univ. Of South Carolina, Charleston, SC

Abstract: Alcohol intoxication impairs cerebellar-dependent tasks and individuals with alcohol use disorder (AUD) suffer from persistent alterations in cerebellar function. While the molecular mechanisms underlying the effects of acute alcohol exposure on cerebellar physiology have been extensively investigated, the effects of chronic alcohol exposure on the cerebellum are much less understood. Chemical modifications of the genome have emerged as important molecular mechanisms that contribute to the pathophysiology of AUD. Changes in acetylation or methylation in histone H3, frequently in H3K9, occur in substance abuse and have been implicated in AUD-related behaviors. In the nucleus accumbens, for example, repeated exposure to alcohol results in locomotor sensitization that is associated with increased acetylation of H3K9 while excessive binge-like ethanol intake and chronic intermittent vapor EtOH exposure (CIE) are associated with reductions in H3K9 methylation. Thus, we hypothesized that CIE induces changes in chromatin structure due to covalent modifications at H3K9 in the cerebellar cortex. To test this, we measured H3K9 methylation in C57BL/6J male CIE exposed mice, and controls. Each CIE cycle consisted of 4 days of 16 hrs of ethanol vapor followed by 8 hrs of withdrawal and resulted in escalated EtOH drinking. A separate set of naïve mice was used to test G9a-mediated H3K9-dimethylation in the cerebellar cortex using the G9a inhibitor UNC0642. Confocal images of Purkinje (PC) and granule (gC) cell layers in lobules V and VIII, were analyzed using Imaris software. First, we found that G9a inhibition resulted in a significant decrease in PC H3K9-dimethyl expression in lobules V and VIII (One-way ANOVA, ****p<0.0001, n=3 mice/condition, 73-117 cells). H3K9-dimethyl expression in gCs, was also significantly reduced after G9a inhibition (One-way ANOVA, ***p=0.0002, *p=0.34 n=3-7 mice/condition, 12-15 images). H3K9-dimethyl expression was measured in lobules V, VI and VIII in mice that underwent homecage EtOH consumption and those that were exposed to CIE. Mice exposed to EtOH, either by CIE or via voluntary consumption showed reduced expression of H3K9-dimethyl inside PC somas when compared to EtOH naïve aged-matched controls (One-way ANOVA, ****p<0.0001, n=3-7 mice/condition, 172-718 cells). In contrast, H3K9-dimethyl expression in gCs was reduced only in lobules VI and VIII after CIE with no changes in non-CIE EtOH drinking mice (One-way ANOVA, **p=0.0012, n=3-7 mice/condition, 39-103 images). These findings are among the first to describe epigenetic alterations in the cerebellar cortex following chronic exposure to alcohol.

Disclosures: P. Zamudio-Bulcock: None. **J.J. Woodward:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.15/SS16

Topic: G.04. Emotion

Support: K01DA054449
R01AA023183
R01AA027555
R01AA014351

Title: Recruitment of neuronal ensembles linked to compulsive ethanol seeking in rats with a history of negative reinforcement by ethanol

Authors: E. I. MEAMARI[1], N. RAJAEI[1], A. GREY[1], J. LOPEZ[1], R. BULLARD[1], A. H. THAN[2], G. E. WAGNER[1], O. O. KOZANIAN[1], N. O'CONNOR[3], N. SUTO[1], F. WEISS[1], *H. NEDELESCU[1];
[1]Neurosci., Scripps Res. Inst., La Jolla, CA; [2]Univ. of California Los Angeles, Los Angeles, CA; [3]MBF Biosci., Williston, VT

Abstract: Fos protein can be utilized to visualize activated neurons that mediate stimulus information and learned associations underlying drug seeking behavior. Here, we sought to examine whether the activation patterns of stimulus-reactive neurons are distinct in rats having learned to associate environmental stimuli with (1) positive reinforcement by ethanol in the nondependent state vs. (2) negative reinforcement (alleviation of withdrawal) by ethanol in rats with a dependence history. The experience of negative reinforcement or *withdrawal-related learning* (WDL) separates the learning taking place during casual alcohol use from the learning that occurs when alcohol is consumed during withdrawal. WDL-associated stimuli produce significant craving as reflected by several experimental measures of compulsive ethanol seeking compared to stimuli conditioned to ethanol in the non-dependent state. The WDL model is, therefore, ideal to investigate the neurobiological control specifically of compulsive ethanol seeking, and to identify how learned associations between ethanol and the environment are established in the brain. To accomplish this, we have developed a semi-automated brain-wide profiling method for imaging data to count Fos-expressing cells. We identified recruitment of neuronal ensembles that mediate ethanol seeking linked to WDL in seven out of eight brain regions analyzed, including the amygdala and paraventricular nucleus of the thalamus. Moreover, the findings revealed an overall highly differential pattern of neurocircuitry activation by ethanol-associated stimuli linked to negative (WDL) vs. positive reinforcement and suggest the formation of distinct engrams as a consequence of learning that occurs during repeated consumption of ethanol during withdrawal in the presence of contextual stimuli.

Disclosures: **E.I. Meamari:** None. **N. Rajaei:** None. **A. Grey:** None. **J. Lopez:** None. **R. Bullard:** None. **A.H. Than:** None. **G.E. Wagner:** None. **O.O. Kozanian:** None. **N. O'Connor:** None. **N. Suto:** None. **F. Weiss:** None. **H. Nedelescu:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

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Program #/Poster #: PSTR232.16/SS17

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA025120
NIH Grant P50-AA022534
NIH Grant AA015614

Title: Repeated High-intensity Binge Drinking and Alcohol Frontloading Leads to Alterations in Circadian Behavior and Circadian Corticostriatal Gene Expression in C57BL/6J Mice

Authors: *M. OROZCO[1], R. HUFFMAN[3], M. R. WESTENSKOW[2], D. N. LINSENBARDT[1]; [1]Neurosciences, [2]Cell Biol., Univ. of New Mexico, Albuquerque, NM; [3]Chem. Biology, Biochem. and Biophysics, Univ. of California Santa Cruz, Santa Cruz, CA

Abstract: There is increasing evidence that regular excessive alcohol consumption can lead to disrupted circadian rhythms, and that this disruption may in turn facilitate increases in drinking intensity. The purpose of the present study was to assess the impact repeated daily binge drinking on circadian home cage behavior and brain gene transcription. Young adult male and female C57BL/6J mice were provided with access to either alcohol or water for 2 hours a day for 6 weeks while home cage activity was continuously monitored 24 hours a day, 7 days a week. Mice were maintained on a 12:12 hour light-dark (LD) cycle for the initial 2 weeks, immediately after which a subset were sacrificed for RNA sequencing (RNAseq) of nucleus accumbens (Acb) and medial prefrontal cortex (mPFC) brain tissue. All remaining mice were switched to constant low light conditions for the following two weeks to assess endogenous circadian behavior (i.e. free-running period; aka 'tau') and were then returned to their original 12-hour cycle for the remainder of the experiment to assess re-acclimation to circadian cues. Repeated ethanol exposure led to high intensity binge drinking and alcohol frontloading that was associated with hyperactivity in females, hypoactivity in males, and alterations in endogenous behavioral rhythms. Three key circadian genes were found to be upregulated by repeated intensive alcohol drinking within both brain regions (Ncoa2, Gsk3b, and Rora), the magnitude of which varied by sex but that were consistent in direction. These findings indicate that repeated high intensity binge alcohol consumption and associated alcohol frontloading are associated with sex-specific and non-sex-specific alterations in circadian behavioral and neurobiology, which may be critical to the establishment of more permanently patterned alcohol drinking.

Disclosures: **M. Orozco:** None. **R. Huffman:** None. **M.R. Westenskow:** None. **D.N. Linsenbardt:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA AA025120-05S1
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NIH Loan Repayment Program, LRP-REACH

Title: Binge drinking during early adulthood disrupts gait, circadian rhythm, tauopathy and neuroinflammation in a preclinical mouse model of tauopathy (P301S) driven by brain region-specific transcriptional alterations

Authors: *N. M. MAPHIS, S. A. DAVID, D. FURLANO, D. N. LINSENBARDT; Neurosciences, Univ. of New Mexico, Albuquerque, NM

Abstract: Background: Excessive alcohol use has very recently emerged as a risk factor for the development and progression of Alzheimer's disease (AD), thought to be mediated by patterns of frequent heavy consumption, like binge drinking (BD). BD is defined as any pattern of alcohol consumption resulting in a blood alcohol concentration of 0.08 g/dL or higher within a 2-hour period. Despite the high rates of BD observed throughout early adulthood, it remains unknown how binge drinking leads to an increased risk of developing AD or how frequent alcohol use could drive pathological tau (pTau) accumulation. Given the troubling increases in frequency and intensity of BD, it is critical to uncover the neurobiological mechanisms that may underlie BD-induced AD risk. Since pTau is a pathological driver of AD, we sought to explore BD-induced AD risk by exposing the P301S tauopathy model to alcohol, using a well-validated voluntary BD model known as Drinking-in-the-dark (DID). **Methods:** 24 nTg (non-transgenic) and 24 P301S mice (equal sex) were exposed to a 21-day Drinking-In-The-Dark (DID) protocol with half receiving water (WAT) and the other half receiving 20% ethanol (EtOH). Average fluid intake, rate of fluid intake, and home cage activity were recorded daily. Following the conclusion of the experiment we measured blood ethanol concentration (BEC) and performed catwalk analysis to assess gait. Sagittal brain sections were processed for immunohistochemical markers for pTau and (AT8 & AT180) and microglia (iba1), while tissue punches of the hippocampus (HP) and brainstem (BSTM) from the opposite hemisphere were sequenced to identify differences in gene expression. **Results:** Both nTg and P301S mice consumed similar amounts of EtOH between D1 and D21, but both genotypes significantly increased the rate of

EtOH consumption over the 21-day exposure period. There were no significant difference in average fluid consumption (g/kg) or rate of consumption between genotype or when separated by sex. EtOH shortened circadian rhythm (period) in both nTg and P301S mice, but selectively exacerbated known gait deficits in the P301S. No significant differences were observed in pTau in the regions evaluated in P301S. However, two genes were identified in the HP and one in the BSTM that dissociated EtOH from water exposure in P301S. Finally, hub genes were identified from within alcohol-associated co-expression networks that represent potential treatment targets. **Conclusions:** These data support alcohol-induced increases in the rate of neurobehavioral decline associated with pTau and identify key brain areas and genes that may be involved.

Disclosures: **N.M. Maphis:** None. **S.A. David:** None. **D. Furlano:** None. **D.N. Linsenbardt:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.18/SS19

Topic: F.04. Neuroimmunology

Support: VA Merit Review Award, I01BX004712
NIMH R01 MH122954
NIGMS U54GM128729
Great Plains Veterans Research Foundation

Title: Inhibitory Effects of the Immunosuppressant Cyclosporine A on Binge Alcohol Drinking and Neuroinflammation in Two Conditional Neuronal Calcineurin Knockout Lines

Authors: *P. RONAN[1], T. P. BERESFORD[2];
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Abstract: We have found that thecalcineurin mediated immunosuppressants cyclosporine and tacrolimus inhibitbinge alcohol drinking in mice. Further, we have shown that this effect ismediated directly in brain, as intracerebroventricular administration alsosignificantly decreases drinking. As these immunosuppressants have severesystemic toxic effects, our goal is to determine proximal mechanisms by whichthese immunosuppressants are working in order to develop effective treatmentsfor alcohol use disorder (AUD) with fewer side effects. To this end, we areemploying genomic, molecular, transcriptomic, metabolomic, anatomic, andbehavioral approaches to explore the relationship between binge alcoholdrinking and calcineurin mediated immunosuppressants in signaling andneuroinflammatory suppression. Calcineurin is a somewhat ubiquitousphosphatase, involved in a wide range of signaling pathways - both in neuronsand glia. One major question is whether immunosuppressants are acting throughneuronal signaling pathways, regulating reward and stress/anxiety pathways, orin glia, mediating

neuroinflammatory effects. To address this, we have developed multiple transgenic models using afloxed calcineurin line (C57BL/6-Ppp3r1tm1Stl/J) crossed with various Credriver lines to knockout CN in various neuronal or glial populations. Here we report complete results for twoconditional neuronal CN knockouts; a “panneuronal” CN knockout line(CamKIIα-Cre) and a corticotropin releasing factor specific CN knockout line(CRH-Cre). Extensive experiments have determined that binge-like drinking inthese lines, both acute and chronic, is not affected by CN knockout.Furthermore, cyclosporine still had a robust inhibitory effect on alcoholdrinking in this population. We are furthering this work with other CN knockoutlines as well as using a focal knockout approach employing AAV-Cre vectors totarget specific populations of both neurons and glia. Together, these andmolecular data showing immunosuppressant effects on a range ofneuroinflammatory and stress signaling are converging to suggest thatimmunosuppressants are acting through glial mediated neuroinflammatorymechanisms to reduce binge-like alcohol consumption in mice.

Disclosures: P. Ronan: None. **T.P. Beresford:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR232.19/SS20

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P50AA017823
NIH Grant T32AA025606

Title: The potential role of glia in brain ethanol metabolism and the development of tolerance following adolescent intermittent ethanol exposure

Authors: *S. TRAPP, A. S. VORE, A. LUTZKE, P. MARSLAND, A. GANO, T. DEAK; State Univ. of New York, Binghamton, Binghamton, NY

Abstract: The rate of ethanol metabolism depends on the activity of several enzymes including alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Recent evidence suggests glial expression of these enzymes can also influence behavioral effects of ethanol due to local actions within the CNS, thereby contributing to the development of tolerance. Thus, the overarching goal of the present studies was to determine the role of glia in brain ethanol metabolism and the development of tolerance following Adolescent Intermittent Ethanol (AIE) exposure. First, we investigated the development of chronic tolerance to ethanol-induced motor impairment following AIE using the inclined plane. During adolescence, male and female Sprague Dawley rats were exposed to water or 4 g/kg ethanol i.g. for 3 days followed by two days off. This pattern was repeated 4 times for a total of 12 ethanol exposures. Rats were then tested on the inclined plane 24 hours (Experiment 1) or 30 days (Experiment 2) after AIE. Prior to ethanol administration, rats were tested for baseline latency to turn 180. Rats then received

either saline or 2 g/kg ethanol i.p. and were tested three times (15, 30, and 60 min post-injection). Tail blood samples were collected after the 60 min time point to measure blood ethanol concentrations (BECs). AIE-exposed males showed reduced latency to turn compared to water exposed males 24 hours after AIE. AIE-exposed females trended toward reduced latency to turn compared to water exposed females 30 days after AIE. There was no significant effect of AIE on BECs at 24 hours or 30 days following AIE. These findings suggest that males displayed chronic tolerance to ethanol-induced motor impairment 24 hours after AIE, but these effects recovered in adulthood, whereas females showed minimal chronic tolerance effects 30 days after AIE. Next, we investigated the cell-type expression of ethanol metabolizing enzymes in the cerebellum and hippocampus following AIE. Immediately following testing, rats were perfused and brains were collected to assess the expression of enzymes in microglia and astrocytes using immunofluorescence. There were no long-term effects of AIE on ALDH2 expression in microglia and astrocytes in males or females. However, males showed a significant reduction in % ALDH2/IBA1 colocalization area in the hippocampus and % ALDH2/GFAP colocalization area in the cerebellum following a 2 g/kg ethanol challenge. Ongoing work is investigating the expression of ethanol metabolizing enzymes within these cell-types to determine sex-specific effects of AIE on ethanol tolerance.

Disclosures: **S. Trapp:** None. **A.S. Vore:** None. **A. Lutzke:** None. **P. Marsland:** None. **A. Gano:** None. **T. Deak:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P50AA017823
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NIH Grant T32AA025606

Title: A comparison of Blood-Brain Barrier (BBB) permeability after acute and chronic ethanol challenges in both sexes

Authors: *A. LUTZKE, A. S. VORE, P. MARSLAND, T. DEAK;
SUNY - Binghamton Univ. Behavioral Neurosci., Binghamton, NY

Abstract: Adolescent binge drinking compromises many features of CNS function, with recent work showing that Adolescent Intermittent Ethanol (AIE) led to sex-specific changes in Blood-Brain Barrier Permeability (BBBP) within corticolimbic regions including the amygdala and prefrontal cortex (Vore, 2022). The goals of the current studies were to (a) develop a procedure that would enable assessment of BBBP at multiple molecule sizes in the same rat; and to (b) evaluate changes in BBBP after both acute and chronic ethanol exposure. In Experiment 1, adult

male Sprague Dawley rats (P136-138) were challenged with 4.0 g/kg ethanol (i.g.), 4.0 mg/kg i.p. Poly I:C, or vehicle and BBBP was assessed with 20 kDa and 70 kDa dextran 24 hours later. Prior ethanol exposure increased BBBP exclusively in the amygdala, at 20 kDa but not 70 kDa. No other ROIs showed altered BBBP to either ethanol hangover or Poly I:C challenge. These changes suggested that acute ethanol exposure was insufficient to produce the widespread change in BBBP previously reported. In Experiment 2, rats were exposed to AIE wherein rats received 3 days of ethanol intubation (4.0 g/kg) followed by 2 days undisturbed in homecage, which produces Blood Ethanol Concentrations (BECs) in the range of 175-225 mg/dl. This procedure was repeated for a total of 4 cycles. After a 21-day abstinence period (P70-72), rats were injected with 4.0 mg/kg Poly I:C (i.p.) challenge followed by test of BBBP (70 kDa) assessments 24 hours later. Male but not female rats with a history of AIE showed significantly increased dextran permeability in the amygdala after both saline and Poly I:C challenge. Similar changes occurred in the cingulate prefrontal cortex in male rats with a history of AIE that received saline injection. Finally, Experiment 3 used a chronic ethanol consumption procedure starting in adolescence (P28-32) in which rats were given a single bottle of ethanol (10% v/v) for 2 days, followed by a 2-day period of tap water access, a procedure that produces BECs in the range of 40-100 mg/dl. This 4-day procedure was repeated for a total of 12 cycles terminating at P76-P80 and BBBP was assessed with a 20 kDa dextran probe after a 21-day abstinence period (P97-101). No significant differences in BBBP were noted in any ROIs in either sex. Together these data suggest that changes in BBBP may depend upon the ethanol exposure model and relate to the magnitude of peak BECs produced. Given the propensity of adolescents to engage in high levels of binge drinking, these findings could have significant implications for future drug and alcohol exposures as well as general CNS health.

Disclosures: **A. Lutzke:** None. **A.S. Vore:** None. **P. Marsland:** None. **T. Deak:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

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Program #/Poster #: PSTR232.21/SS22

Topic: G.09. Drugs of Abuse and Addiction

Support: R25GM056929
DP2 NS132372-01

Title: Uncovering ethanol-mediated glutamate receptor regulation and memory deficits across aging

Authors: K. L. BRANDEL-ANKRAPP, R. N. AREY, *K. L. B. A. BRANDEL-ANKRAPP; Ctr. for Precision Envrn. Hlth., Baylor Col. of Med., Houston, TX

Abstract: UNCOVERING ETOH-MEDIATED GLUTAMATE RECEPTOR REGULATION AND MEMORY DEFICITS ACROSS AGING IN *C. ELEGANS*

Brandel-Ankrapp KL, Arey R
Chronic ethanol (EtOH) consumption is linked to memory deficits and worsened cognitive aging trajectories. Thus, it is critical to identify molecular pathways disrupted by chronic alcohol that contribute to persistent memory deficits. EtOH targets conserved memory regulators, including the ionotropic glutamate receptor a-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPAR). *In vivo* and in cell culture, chronic EtOH exposure and withdrawal (WD) enhances AMPAR-mediated signaling and expression. However, **it remains unknown which AMPAR regulatory pathway is impacted by chronic ethanol exposure that leads to memory deficits across aging.** Due to the complexity of the mammalian brain, it is difficult to unravel how EtOH regulates AMPARs in mammals to modify behavior, let alone across aging. In *C. elegans*, GLR-1, an AMPAR ortholog, has tightly defined expression in relatively few neurons and is linked to molecularly conserved memories including those disrupted by EtOH. Previous studies demonstrated exposing worms to 400mM EtOH is sufficient to induce 40-50mM internal concentrations that correspond to .2% BAC in humans and induce WD behaviors upon 1h removal from EtOH. Using this exposure protocol in *C. elegans*, we assessed how EtOH affects associative olfactory memory. Memory was measured using a positive olfactory associative memory assay, in which pairing a neutral odorant with a food source yields long-lasting and measurable attraction towards that odor. This assay can delineate distinct forms of memory (learning, short-term associative memory/STM, intermediate-term memory/ITM, forgetting), each of which requires conserved molecular processes. We find that chronic EtOH exposure and WD during early adulthood causes significant ITM defects. Furthermore, EtOH treatment accelerates age-related deficits in associative behavior. Chronic EtOH also increases surface area of GLR-1 expression at timepoints corresponding to behavioral defects in young adult animals. Overall, these results suggest that 1) Adult chronic EtOH exposure and WD disrupts ITM and 2) Adult chronic EtOH and WD alters AMPA-type receptor expression, which has been previously associated with associative memory deficits and 3) Chronic EtOH and WD worsens cognitive aging trajectories. Future experiments will examine the role of multiple pathways regulating GLR-1 in the observed behavioral defects following EtOH treatment, and will determine if there are windows of susceptibility to exacerbation of cognitive aging by ethanol exposure.

Disclosures: K.L. Brandel-Ankrapp: None. **R.N. Arey:** None. **K.L.B.A. Brandel-Ankrapp:** None.

Poster

PSTR232. Alcohol: Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR232.22/SS23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA AA026999
NIAAA AA028549
NIAAA 006420
NIAAA T32 AA007456

Title: Alternative use of suvorexant (Belsomra®) for the prevention of alcohol drinking and seeking in rats with a history of alcohol dependence

Authors: *F. J. FLORES RAMIREZ[1], J. M. ILLENBERGER[2], G. E. PASCASIO[2], A. MATZEU[3], B. J. MASON[2], R. MARTIN-FARDON[4];
[2]Dept. of Mol. Med., [1]The Scripps Res. Inst., La Jolla, CA; [3]The Scripps Res. Inst., La Jolla, CA, ; [4]TSRI, La Jolla, CA

Abstract: Alcohol use disorder (AUD) is one of the most treatment-resistant medical conditions globally. The orexin (Orx) system regulates diverse physiological processes, including stress, and is a system of interest for the development of pharmaceuticals to treat substance use disorders, particularly AUD. The present study tested the ability of the dual orexin receptor antagonist suvorexant (SUV), marketed by Merck as Belsomra®, for the treatment of insomnia, to decrease alcohol self-administration and the stress-induced reinstatement of alcohol-seeking behavior in male Wistar rats with a history of alcohol dependence. Rats were trained to orally self-administer 10% alcohol (30 min/day for 3 weeks) and were either made dependent via chronic intermittent alcohol vapor exposure (14 h ON, 10 h OFF) for 6 weeks or exposed to air (nondependent). Starting on week 7, the effect of SUV (0-20 mg/kg, p.o.) was tested on alcohol self-administration at acute abstinence (8 h after vapor was turned OFF) twice weekly. A separate cohort of rats that were prepared in parallel was removed from alcohol vapor exposure and then subjected to extinction training for 14 sessions. Once extinction was achieved, the rats received SUV (0 and 5 mg/kg, p.o.) and were tested for the footshock stress-induced reinstatement of alcohol-seeking behavior. Suvorexant at 5, 10, and 20 mg/kg selectively decreased alcohol intake in dependent rats. Furthermore, 5 mg/kg SUV prevented the stress-induced reinstatement of alcohol-seeking behavior in dependent rats only. These results underscore the significance of targeting the Orx system for the treatment of substance use disorders generally and suggest that repurposing SUV could be an alternative approach for the treatment of AUD.

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Poster

PSTR233. Orbitofrontal Cortex

Location: WCC Halls A-C

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Program #/Poster #: PSTR233.01/SS24

Topic: H.03. Decision Making

Support: NIH NIDA Grant RO1DA047870
NIH NIDA Grant RO1DA047870-S1
NIH NIMH Grant MH122800

Title: Reward expectancy violation signals in OFC following single-photon calcium imaging in freely-behaving rats

Authors: *J. L. ROMERO SOSA, H. BLAIR, A. IZQUIERDO;
Psychology, UCLA, Los Angeles, CA

Abstract: Learning is one of the essential building blocks of cognition. Individuals with mental health conditions, including substance use disorder and schizophrenia, often show symptoms related to flexible learning when adapting to changing reward environments. Substantial evidence indicates that the orbitofrontal cortex (OFC) is involved in flexible learning under uncertainty; yet, its putative role gathered from electrophysiological studies has mostly been encoding expected value (Schoenbaum et al., 2011) and learning a causal structure or 'cognitive map' of the environment or task (Costa et al., 2022). To better understand the role of OFC in dynamic learning under uncertainty, we designed a task requiring rats to adjust to both *unexpected uncertainty* or abrupt changes in reward expectation that generate a steep increase in the frequency of prediction errors and *expected uncertainty* or the changing probabilistic nature of the reward outcomes. Learning under expected and unexpected uncertainty engages systems in the prefrontal cortex (Soltani and Izquierdo, 2019), yet it is unclear how OFC may be involved in these different adjustments. A group of rats (n=3) expressed GCaMP6f in OFC and had GRIN lenses implanted directly above the ventrolateral area so that we could utilize miniscopes to record calcium traces while they learned the better-rewarded action within and across sessions. Rats learned reversals of action-reward contingencies within 40-70 trials, with expectedly more trials required to reach 75% accuracy under the most uncertain block (100/0: 42 trials; 90/10: 53 trials; 80/20: 48 trials; 70/30: 68 trials), and exhibited evidence of stable or improved initial learning of probabilities, even in the most uncertain block (trials to 75%: 100/0: 50 trials; 90/10: 56 trials; 80/20: 26 trials). Preliminary analyses of neural data from OFC around reward events show approximately 1/3 of the population encoding reward expectation- increasing activity prior to reward delivery, 1/3 of the population encoding reward response- with increased activity on receipt and 1/3 with non-selective activity. In the population of reward-predicting cells, during incorrect trials, we found a reliable increase in reward-predicting population activity when animals would have experienced reward receipt. This increase in activity was observed in fully predictive but also probabilistic reversals. Ongoing work involves direct comparisons of how the uncertainty representations in OFC differ from other frontocortical regions, such as the anterior cingulate cortex, which has also been linked to expected uncertainty and trial-by-trial strategy during learning.

Disclosures: J.L. Romero Sosa: None. **H. Blair:** None. **A. Izquierdo:** None.

Poster

PSTR233. Orbitofrontal Cortex

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR233.02/SS25

Topic: H.03. Decision Making

Support: National Institutes of Health Grant K01-DA-036659-01
Busch Biomedical Foundation
Whitehall Foundation

Title: Estimating subjective values and decision intent from primate orbitofrontal cortex: linear vs. non-linear methods

Authors: *T. ROUSE[1], S. LUPKIN[2], V. MCGINTY[3];
[1]Rutgers Univ. Behavioral and Systems Neurosci., Piscataway, NJ; [2]Rutgers, The State Univ. of New Jersey, Teaneck, NJ; [3]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ., Newark, NJ

Abstract: Neural activity in orbitofrontal cortex (OFC) has been shown to maintain representations related to the value of choice options during decision-making. Prior investigations have demonstrated that single cells in posterior OFC encode value through monotonic changes in firing rate, suggesting an approximately linear neural code for value . Assuming linear encoding, recent work has also identified value signals at the level of simultaneously recorded neural populations, and has shown that variability in these value signals can predicts choices with modest accuracy in trials where the options have equivalent values. However, despite these recent advances, deriving a clear estimation of single-trial value representations is still challenging. Deviating from the linear assumption made in previous studies of population level value encoding, we seek to investigate whether value estimation is improved by leveraging non-linear methods. Concurrently, we seek to investigate whether changes in the dimensionality of the value representation affect the ability to estimate value. We show that changes in dimensionality, characterized by varying the number of principal components , has no impact on the accuracy of value signal estimation. We also show that any improvements gained from leveraging nonlinear models for value estimation (e.g. Kernel methods or tree methods) do not improve our ability to predict the choice outcomes. These findings have implications for understanding the basic mechanisms of value representation in OFC, as well as for the development of cognitive neural prosthetic devices for inferring subjective value and choice intent in real time.

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Poster

PSTR233. Orbitofrontal Cortex

Location: WCC Halls A-C

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Program #/Poster #: PSTR233.03/TT1

Topic: H.03. Decision Making

Support: National Science Foundation Graduate Research Fellowship Grant No. DGE-2034835
R01 DA047870

UCLA Division of Life Sciences Recruitment and Retention Fund
Howard Hughes Medical Institute Gilliam Fellowship

Title: Stimulus discrimination, retention, and foraging behavior following rat medial orbitofrontal cortex inhibition using DREADD actuator DCZ in rats

Authors: *Z. M. G. RIVERA[1,2], P. GANUPURU[1], A. G. GOMAN[1], A. IZQUIERDO[1], A. M. WIKENHEISER[1];
[1]Psychology, [2]Neurosci. Interdepartmental Program, UCLA, Los Angeles, CA

Abstract: Orbitofrontal cortex (OFC) and perirhinal cortex (PRh) have been implicated in cue-based learning and decision-making. OFC is thought to aid in learning and adopting strategies to obtain reward, particularly in situations involving cues. Concurrently, PRh also processes information regarding object values and object reward context. To understand its connections to OFC, we first tagged tracers to/from PRh revealing that cell bodies in medial OFC (mOFC) selectively projected to PRh (N= 11; 6 females). We studied the role of mOFC for discrimination learning and long-term retention of reward-predicting stimuli. In a touchscreen equipped operant chamber, adult Long-Evans rats (N=12; 6 females) discriminated between two distinct equiluminant visual stimuli with the rewarded stimulus alternating sides. Once rats learned the discrimination, they were put on rest for 3 weeks. Rats were then re-tested on the same contingencies as before to assess retention. We transfected rats with hM4Di DREADDs in mOFC and administered deschloroclozapine (DCZ) or vehicle 20 min prior to each session during either the discrimination or retention phase. We found completely intact discrimination learning and retention following mOFC inactivation. mOFC has also been implicated in decisions where subjects must evaluate immediately available reward relative to not presently available reward that might be obtained elsewhere. We used a patch foraging task (N=6; 3 female) where rats earned food pellets in two arenas connected by a corridor. The arenas had distinct foraging patches, and doors at each end of the corridor allowed rats alternating access to one patch at a time. Rats could collect food in one patch indefinitely, but the rate of reward decreased as they stayed, incentivizing a switch to the opposite arena. The rats waited in a corridor (containing no food access) to simulate travel time, making patch-switching costly. The speed the reward rate decreased differed for each patch, forcing rats to flexibly adjust foraging strategy. Following mOFC inhibition, rats increased the time they spent foraging in nearly all patches, suggesting mOFC may play an important role in patch-leaving decisions. In ongoing studies, we are further validating the efficacy of DCZ *in vivo* through electrophysiological recordings in anesthetized rats using Neuropixel probes (N=2; 1 female). DCZ has not been extensively used in *in vivo* rat chemogenetic studies but is potentially advantageous given its purported reduced off-target effects. Using DCZ may provide refined targeted inhibition of cortical areas including PRh during stimulus-based learning and reward-guided decisions.

Disclosures: Z.M.G. Rivera: None. **P. Ganupuru:** None. **A.G. Goman:** None. **A. Izquierdo:** None. **A.M. Wikenheiser:** None.

Poster

PSTR233. Orbitofrontal Cortex

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Program #/Poster #: PSTR233.04/TT2

Topic: H.03. Decision Making

Support: STI2030-Major Projects: 2021ZD0203700/2021ZD0203703
the National Natural Science Foundation of China: 32171030
Shanghai Municipal Science and Technology Major Project: 2018SHZDZX05

Title: Role of mediodorsal thalamus to orbitofrontal cortex pathway in reversal learning

Authors: *Z. LE, C. HUANG, H. YAO;
Ctr. for Excellence in Brain Sci. and Intelligence Technology, CAS, Shanghai, China

Abstract: Abstract: *Rationale and objective*: The orbitofrontal cortex (OFC) plays a crucial role in flexible behaviors, such as reversal learning. However, the circuit mechanism by which the OFC facilitates adaptive cue-reward associations in reversal learning remains unclear. The mediodorsal thalamus (MD), a higher-order thalamic region that provides inputs to the OFC, also contributes significantly to adaptive behaviors. Here we investigated whether the MD projections to OFC support neural plasticity essential for behavioral flexibility. *Methods:* We trained head-fixed mice to performed an olfactory reversal learning task, in which the cue-reward association was switched after the discrimination performance reached 90%. We tested the effects of lesioning OFC neurons defined by MD inputs or inactivating MD axon terminals in the OFC. Electrophysiological recordings in the OFC were performed during the reversal learning task, with or without inactivating MD axons. *Results*: We bilaterally injected AAV2/1-hSyn-mCherry-Cre in the MD and AAV-CAG-Dio-taCaspase3 (or AAV-CAG-FLEX-EGFP as a control) in the OFC. We found that lesioning OFC neurons receiving inputs from MD did not impair the initial discrimination learning but significantly slowed the reversal learning process (control group: $n = 13$, Caspase group: $n = 13$, $p = 0.03$, two-way ANOVA). In a second set of experiment, we bilaterally injected AAV-CamKIIa-NpHR-mCherry (or AAV- CaMKIIa-EGFP as a control) in the MD and implanted optic fibers in the OFC. Optogenetic inactivation of MD axons in the OFC also significantly slowed the reversal learning (control group: $n = 16$, NpHR group: $n = 18$, $p = 0.04$, two-way ANOVA). The mice used in the electrophysiological recordings were divided into two groups, a fast group ($n = 7$) and a slow group ($n = 5$), which differed significantly in reversal learning. By comparing the stimulus selectivity before and after the reversal, we calculated the percentage of neurons that maintained, reversed, or lost selectivity. We found that the percentage of reversed neurons in the fast group (56%) was significantly higher than that in the slow group (33%) ($n = 59$ and 36 neurons in fast and slow groups, $p < 0.05$, Fisher's exact test). Inactivation of MD axons significantly reduced the fraction of reversed neurons in the OFC (with inactivation: $n = 29/119$ neurons; without inactivation: 45/95 neurons, $p < 0.001$, Fisher's exact test). *Discussion*: These results suggest that MD inputs to the OFC contribute to the flexibility in stimulus selectivity essential for reversal learning. Further experiments will be conducted to examine the activity of OFC-projecting MD neurons during reversal learning.

Disclosures: Z. Le: None. **C. Huang:** None. **H. Yao:** None.

Poster

PSTR233. Orbitofrontal Cortex

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Program #/Poster #: PSTR233.05/TT3

Topic: H.03. Decision Making

Support: Israel Science Foundation Grant #1269/20

Title: Orbitofrontal cortex contributes to flexible context adaptation in mice

Authors: *G. MARMUR, H. RAJABI, J. SCHWARCZ, R. REINER, E. LOTTEM;
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Abstract: In an ever-changing environment, we constantly need to adjust our behavior, yet the neural mechanisms underlying flexibility are poorly understood. Here, we developed a novel auditory change detection task to study flexible context adaptation. In this task, on each trial, head-fixed mice hear a sequence of intermittent beeps followed by a response window in which only consecutive beeps are played. The duration of the beep sequence (the stimulus) is exponentially distributed and the transition to the response window (the change point) is un-cued. Reward is only available during the response window, so the likelihood of a reward increases with the length of the consecutive beep that is played. The optimal response time (defined as the beep duration before lick) is modulated by the probability of misleading beeps during the stimulus (P_{beep}). Lower P_{beep} should correspond to lower response times, as shorter beeps are sufficient to indicate that a reward is available. Behavioral data collected from 17 mice, 10 sessions each, indicate that mice succeed in detecting the change points and adapt their response times to different P_{beep} contexts. In each session we introduced 2-3 different P_{beep} values (out of 10 possibilities) in blocks of 30-40 trials and found that mice flexibly adapt to the new context within approximately 2 trials. Importantly, in this task, the same sequence of beeps can occur in different P_{beep} contexts seeing as the stimulus is probabilistic. We find that mice respond differently to identical inputs in different P_{beep} blocks indicating the mice employ different policies in these different contexts. Next, we set out to study the neural mechanisms underlying this flexible behavior. To test the involvement of the prefrontal cortex in the task, we used muscimol (a GABA-A agonist) to reversibly silence the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) in behaving mice. In the first exploratory study, we administered bilateral injections of muscimol and saline on intermittent days to the OFC (n=4) and the ACC (n=4). We found that muscimol injections into the OFC reduced the mice's adaptation to the different P_{beep} contexts compared to the ACC and saline injections. This finding was replicated in a subsequent study (OFC: n=5, ACC: n=2) supporting the hypothesis that OFC contributes to the flexible adaptation in response times mice exhibit in this task. By uncovering the behavioral strategies and neural circuitry involved in this task, this research advances our understanding of the cognitive processes underlying adaptive behavior and decision-making.

Disclosures: G. Marmur: None. **H. Rajabi:** None. **J. Schwarcz:** None. **R. Reiner:** None. **E. Lottem:** None.

Poster

PSTR233. Orbitofrontal Cortex

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR233.06/TT4

Topic: H.03. Decision Making

Support: Royal Society Research Grant RGS\R2\202155
Wellcome Trust institutional strategic awards
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Title: Trial-resolution neural representations of behavioral strategies during tactile reversal learning

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Abstract: Animals continuously track past and current sensory and contextual information to make appropriate decisions during behavior. Understanding how animals employ specific behavioral strategies on a trial-by-trial basis during learning and how neural responses guide such strategy remains understudied. We trained mice on a tactile reversal learning task and implemented a Bayesian evidence accumulation model to measure the probability of strategies used during behavior. We analyzed multiple exploratory strategies across key task-learning phases and subsequent rule reversal. During initial task learning, mice shifted from choice-driven strategies (e.g., hit-stay-choice) to cue-driven strategies (e.g., hit-stay-cue). Following the rule switch, mice reused a cue-driven strategy for reward-guided exploration. Furthermore, animals seem to rely on specific relevant strategies dependent on task phases to adapt their behavior dynamically. Silencing of a key prefrontal brain area, the lateral orbitofrontal cortex (lOFC), resulted in delayed choice-to-cue transition and impairments in flexibility. To study neural representations of behavioral strategies, we measured functional responses from excitatory layer 2/3 neurons in the primary somatosensory cortex (S1) using two-photon Ca2+ imaging. We employed tensor component analysis (TCA) to reveal functionally distinct neuronal populations. Additionally, using associated trial factors, a novel method of temporal decoding revealed that neural fingerprints and history-dependent processes for key strategies (e.g., cue-driven) could be decoded for reward-guided learning across mice. Our study sheds light on how animals employ distinct exploratory strategies during flexible behavior and reveals cognate neural signatures of strategy implementation in S1. It further highlights the role of lOFC in leveraging prior knowledge supporting reward and error-guided learning.

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Poster

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Topic: H.03. Decision Making

Support: Whitehall Foundation Fellowship
Busch Biomedical Foundation

Title: Selective Disruption of Target Value and Target Location Signals in Primate Orbitofrontal Cortex: Effects on Binary Choice

Authors: *E. ALBAYRAK[1,2], S. M. LUPKIN[1,2], V. B. MCGINTY[2];
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Abstract: During economic decision-making, the primate orbitofrontal cortex (OFC) encodes multiple decision-related variables, including the value and location of decision targets. While the role of value signals in decision-making is well-characterized, the role of target location signals is still unclear. Here, we compared the role of these two signals by selectively disrupting them with the electrical micro-stimulation of the OFC. One monkey performed a two-alternative value-based decision-making task (Lupkin & McGinty, 2022, BioRxiv) while stimulation pulse trains were delivered unilaterally to the posterior OFC. The aim of the stimulation was to disrupt neural activity concurrent with the encoding of either the target values or target locations in separate sessions. To disrupt value signals, stimulation was delivered in a 200ms epoch following the moment that the monkey fixated upon either the first or second target in each trial. Likewise, to disrupt target location signals, we stimulated during an earlier 200ms epoch following the moment the first target was initially shown on display - an epoch when only the target location, but not the value, was encoded in OFC. Stimulation was delivered through linear recording/stimulation arrays (Plexon V-Probes), in the form of 20-25µA biphasic current pulses delivered to five channels at once (200µm channel separation) at a rate of 200 pulses per second. We found that stimulation at different times in the trial had distinct effects on the monkey's choices. As expected based on previous studies, stimulation following fixation onto the first target (when its value was encoded) decreased the monkey's probability of choosing the first target, consistent with a decrease in its subjective value (Ballesta, Conen, & Padoa-Schioppa, 2020). Stimulation after fixation onto the second target did not change the net fraction of choices in favor of the first offer but did make the monkey's choices more dependent on the first target value and less dependent on the second target value. Interestingly, stimulation of the epoch containing the first target location signal increased the probability of choosing the first target, consistent with an increase in its subjective value - showing the opposite effect as stimulation during the first target value-encoding epoch. This was an unexpected result, given that in this epoch value signals were not yet evident in OFC. Though preliminary, these results suggest the possibility of a causal role for non-value-related OFC activity in economic choice.

Disclosures: **E. Albayrak:** None. **S.M. Lupkin:** None. **V.B. McGinty:** None.

Poster

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Topic: H.03. Decision Making

Support: KAKENHI 20K16471
KAKENHI 23K06788

Title: Single unit activity in the monkey orbitofrontal cortex related to reward value history

Authors: ***M. HIGUCHI**[1], T. IZAWA[1], J. MATSUMOTO[1,2], H. NISHIMARU[1,2], N. MATSUMOTO[3], M. SHIDARA[4], T. SETOGAWA[1,2];
[1]Univ. of Toyama, Toyama, Japan; [2]Res. Ctr. for Idling Brain Sci. (RCIBS), Toyama, Japan; [3]AIST, Tsukuba, Japan; [4]Med., Univ. of Tsukuba, Tsukuba-Shi, Japan

Abstract: When making a choice from several alternatives, we normally choose more valuable options than less valuable ones. It is well known that neuronal activities in orbitofrontal cortex (OFC) encode the subjective values of offered options. Previous studies have also reported that neuronal activities in OFC representing the values of currently presented choice options are affected by the value of the past options. This activity is interpreted as carrying the reward history information, and this reward history is thought to be important for facilitating optimal and adaptive choice. In this study, we examined whether 1) the neuronal activities in the OFC representing reward history can be observed when multiple factors related to reward value calculation (reward amount and workload) are introduced, and 2) these activities are also observed even when a certain time has passed after the choice was made. We reanalyzed the activity of 256 OFC neurons that we recorded previously from two monkeys while they performed a decision-making task (Setogawa et al., 2019). In this task, two choice targets were presented one on each side of the fixation point at the monitor (choice phase). Each target represented a schedule of 1, 2 or 4 trials of a simple visual color discrimination (workload) to be rewarded with 1, 2 or 4 drops of liquid reward after schedule completion. The monkey indicated its choice by touching the corresponding, left or right, bar in the monkey chair. Following a choice of one target, the chosen reward schedule task was started. After the reward acquisition, 2 seconds of inter-trial-interval (ITI) was inserted before the next trial initiation. For 38/256 (14.8 %) of the neurons, we found a significant correlation between the neuronal firing in ITI and the chosen target value calculated from reward amount and workload. Furthermore, over 20 % of neurons (41/256) showed a significant correlation between the neuronal firing in the choice phase in the current trial and the reward value of the chosen target in the previous trial. The relationship between the neuronal activity during the choice phase and target values was evaluated using a model selection procedure. We assessed 14 models: 14 new models with term of the reward value of the chosen target in the previous trial. The activity of more than 50% of

the neurons was well explained by the new models in which previous reward value term is included. These results suggest that OFC neurons maintain the reward value information even after animals received the reward, and these reward value history neurons might affect the animals' future choices.

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Poster

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Program #/Poster #: PSTR233.09/TT7

Topic: H.03. Decision Making

Support: Projet-ANR-19-CE37-0004

Title: Contribution of insular cortex to value- versus cue-guided choice

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Abstract: Every day individuals are faced with choices between different actions. The factors influencing these choices are multifaceted. While our choice is often driven by the current value or desirability of the consequences associated with each action, there is also a strong influence of environmental cues on action selection. For instance, cues associated with food can direct us toward different sources of nourishment and can also trigger cravings, even in the absence of hunger. Cues can also contribute to the development of maladaptive behaviors, such as drug-related cues triggering relapse in individuals battling addiction. Here, we studied the role of the insular cortex (IC) in both value-guided and cue-guided choice. We used two behavioral paradigms to dissect choice situations that are driven by the value of outcomes (outcome devaluation) versus situations where choice is instead guided by predictive cues (Pavlovian-to-Instrumental Transfer or PIT). Using chemogenetics and Deschloroclozapine, a new high-affinity and selective Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) agonist, we first demonstrated that inhibition of the IC impairs both value- and cue-guided choice. Importantly, the IC sends direct projections to mediodorsal thalamus (MD) and the core region of the nucleus accumbens (NAcC), both of which have been implicated in value- and cue-guided behaviors. We therefore hypothesized that IC input to these regions may be required for choice behaviour. Specifically, we predicted that value-guided choice may be highly reliant on a cortico-striatal pathway (IC to NAcC) whereas cue-guided choice may depend on a cortico-thalamic pathway (IC to MD). Using an innovative trans-synaptic anterograde viral approach, we expressed an inhibitory DREADDs in NAcC or MD cells receiving IC inputs. Chemogenetic

inhibition of NAcC cells receiving IC input impaired value- (outcome devaluation) but not cue-guided choice (PIT) and the reverse was true when we inhibited MD cells receiving IC input. These results show that IC is required for both value- and cue-guided choice however, these behaviors are mediated by distinct and dissociable IC pathways. Specifically, a cortico-striatal (IC to NAcC) pathway is required for value-guided choice, whereas a cortico-thalamic (IC to MD) pathway is necessary for cue-guided choice.

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Poster

PSTR233. Orbitofrontal Cortex

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Topic: H.03. Decision Making

Support: ANR Grant CE37-0019 NORAD
FRM Grant ECO20160736024

Title: Unraveling flexible behavior through orbitofrontal noradrenaline

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Abstract: Just like humans, animals use their knowledge of an environment to engage in behaviors that meet their basic needs and desires. In order to survive and thrive in a dynamic environment, an animal must also be able to update its understanding of the setting, particularly when the outcomes of its actions change. Numerous studies indicate that goal-directed behaviors are supported by the prefrontal cortex (PFC), with a high parcellation of functions existing within different prefrontal regions in rodents. Specifically, the prelimbic region or Area 32 (A32) of the medial PFC is needed to learn the relationship between distinct actions (A) and their outcomes (O), whereas the ventral and lateral parts of the orbitofrontal cortex (vlOFC) are required to learn that a specific O associated with a given A has changed, and to recall this information to guide action selection. Behavioral flexibility also requires activity in noradrenergic (NA) neurons of the locus coeruleus (LC), which are thought to track uncertainty in the current situation. Most notably, compelling theoretical models hypothesize that the LC interacts with PFC subregions, including the vlOFC, to support behavioral flexibility. In our most recent work, using both lesions and chemogenetics, we demonstrated that NA inputs from the LC to the vlOFC are indeed necessary for instrumental reversal learning. Specifically, rats were trained to perform different actions for distinct rewards, then these rewards were reversed. Rats with NA inhibition were unable to act according to these new, reversed associations. Notably, we showed that NA inputs to other prefrontal regions known to be involved in goal-

directed behavior, like the abovementioned A32, or dopaminergic inputs to the vlOFC, are not involved in such updating process, thereby suggesting a very specific *regional* and *neurochemical* effect. Interestingly, recent fiber photometry recordings we carried out with the NA-specific sensor $GRAB_{NE}$ revealed a huge increase in vlOFC-NA activity exclusively at the very first reward delivery following reversal, suggesting the existence of a highly specific *temporal* definition in the encoding and updating of reversed A-O associations. Again, this increase in NA activity was not present in other prefrontal regions (i.e. A32). Notably, the magnitude of the vlOFC-NA burst positively correlated with the rats' ability to efficiently encode and update the new associations. Altogether, our results not only add to the overarching theory that NA is involved early on in network reset and adaptive behavior, but also support the growing idea of a modular locus coeruleus architecture.

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Poster

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Topic: H.03. Decision Making

Support: R01-DA032758
R01-MH104494

Title: Neuronal activity in the orbitofrontal cortex of monkeys choosing between three options varying on three dimensions

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Abstract: An established literature has shown that neurons in the orbitofrontal cortex (OFC) encode the subjective values of offered and chosen goods during economic choice. However, the vast majority of studies to date have focused on binary choices. Thus the neuronal mechanisms underlying multinary choices remain unclear. Furthermore, in most previous studies, offers varied on two dimensions (e.g., juice type and quantity, or quantity and probability) and/or all dimensions were quantized. It thus remains unclear whether previous findings generalize to more complex choice tasks. To examine these important questions, we recorded neuronal activity in the OFC of monkeys performing a trinary choice task. In each trial, the animal chose between up to three juices (labeled A, B, and C) offered in variable quantities and variable probabilities. Offers were represented by "pie" symbols displayed on a computer monitor; in each symbol, the color represented the juice type, the radius represented the quantity, and the filled angle represented the probability. Monkeys indicated their choice with a saccade; at the end of the trial, they obtained (or did not obtain) the chosen juice according to its probability. Across trials, both

the quantity and probability of each juice varied continuously and uniformly within a fixed range. We used logistic regressions to analyze choices made by two animals in >250 sessions. In particular, we examined whether choices between two given juices depended on the presence or the expected value of the third juice. Remarkably, we did not find any evidence for such dependence. Confirming previous reports, monkeys were generally risk seeking. Interestingly, risk attitude and choice variability were significantly correlated across sessions in both animals. Using standard techniques, we recorded the spiking activity of >800 cells. Preliminary analyses indicated that different neurons encoded different decision variables, including the subjective values of individual offers, the choice outcome, and the chosen value. In particular, neurons encoding individual offer values integrated quantity and probability, and reflected the risk attitude. Neurons encoding the chosen value also reflected the relative values of the three juices (derived from logistic analyses). Neurons encoding the choice outcome responded in a binary way depending on whether a particular juice type (A, B or C) was chosen or not chosen. All decision variables were represented in a non-spatial way (good-based representation). These results shed light on the neuronal mechanisms underlying choices between multiple options.

Disclosures: **M. Barretto Garcia:** None. **J. Tu:** None. **C. Padoa-Schioppa:** None.

Poster

PSTR233. Orbitofrontal Cortex

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Program #/Poster #: PSTR233.12/TT10

Topic: H.03. Decision Making

Title: The neural mechanism of value-based decision making between multiple-item options

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Abstract: In real life, value-based decisions are often made between two options comprising multiple items. One good example is to choose between two combo meals on the menu. The value of each combo meal may be determined from the total value of its items, and the evaluation process of the individual items may be accompanied by eye movements. However, the dynamic process of value computation, eye movements, and the underlying neural circuitry has not been well explored. In this study, we trained two macaque monkeys to perform a similar value-based decision-making task. The monkeys viewed two combo options, each consisting of three shapes. Each shape was associated with a certain amount of liquid reward, and the monkeys had to choose the option with a larger total value to receive the corresponding reward. During the shape viewing period, the monkeys were allowed to freely move their eyes to examine the shapes. We show that the monkeys were able to make appropriate choices based on the total value of each option. We further recorded single-unit activities from the orbitofrontal cortex (OFC) and the ventrolateral prefrontal cortex (VLPFC) when the monkeys were

performing the task. When the monkeys were examining the shapes, both neurons in the OFC and the VLPFC encoded the value of the shape that the monkeys were fixating on as well as the total value of the corresponding option. The dynamics of the value encoding in the OFC and the VLPFC reflected eye fixation shifts and a transition from a salience-based to a choice-based value encoding, Moreover, the chosen value information arose in the VLPFC earlier than in the OFC, suggesting that the OFC may not be where the choice was first computed. Together, these results provided a new perspective on how value-based decisions are computed in the brain.

Disclosures: R. Su: None. **W. Zhang:** None. **T. Yang:** None.

Poster

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Topic: H.03. Decision Making

Support: Wellcome Trust grant 206207/Z/17/Z
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Title: Primate amygdala neurons signal oral-sensory and nutrient reward components during value-based decisions

Authors: *F.-Y. HUANG, F. GRABENHORST;
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Abstract: Nutrients and sensory food qualities influence the subjective valuation of foods. We recently showed that monkeys prefer sugar- and fat-rich foods, consistent with subjective integration of sensory and nutrient components into economic values that guide choices (Huang et al., 2021, *PNAS*) and reinforcement learning (Huang & Grabenhorst, 2023, *JNeurosci*). Previous studies showed that neurons in primate amygdala encode subjective values during economic choice (Grabenhorst et al., 2012, *PNAS*, Jezzini & Padoa-Schioppa, 2020, *JNeurosci*). However, it is unknown how amygdala neurons process information about specific nutrients and sensory food components for decision-making. Here, we recorded the activity of 289 neurons from the amygdala of two adult male rhesus macaques (*Macaca mulatta*) when they repeatedly chose from eight dairy-based liquid rewards varying in nutrients (fat, sugar) and oral-sensory properties (viscosity, sliding friction). In each trial, the monkeys were sequentially presented with two visual conditioned stimuli (CS) that cued the available rewards and made a touch choice to receive their chosen reward. Each reward was associated with two distinct CS to rule out visual responses. Mixed-effects logistic regressions (n = 18,704 and 22,170 choices from monkey Ya and Ym, respectively) confirmed that both monkeys preferred liquids high in fat and sugar content irrespective of CS sets, similar to our previous findings (Huang et al., 2021, *PNAS*). Oral texture variables (viscosity, sliding friction) mediated the effect of fat content on the monkeys' choices. We found that significant proportions of amygdala neurons encoded the

subjective value of offered options (offer value), the subjective value of chosen options (chosen value), and the monkeys' trial-by-trial choices. Importantly, these variables reflected the animal-specific, subjective integration of specific nutrients and sensory food properties. Separately from these integrated values, some amygdala neurons encoded particular nutrient and oral-sensory value components associated with the cued food rewards. The performance of a classifier that decoded subjective value levels of choice options from amygdala population activity generalized when training and testing on different CS sets. However, classification of specific nutrients and sensory components did not generalize well across CS sets. Our findings suggest that amygdala neurons encode both the nutrient and oral-sensory components of food rewards and their integration into subjective values underlying economic decision-making.

Disclosures: F. Huang: None. **F. Grabenhorst:** None.

Poster

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Support: Wellcome Trust grant 206207/Z/17/Z
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Wellcome Trust grant 208363/Z/17/Z

Title: A neural mechanism in the orbitofrontal cortex for preferring dietary fat based on food texture

Authors: P. KHORISANTONO[1], F.-Y. HUANG[2], M. SUTCLIFFE[1], P. FLETCHER[1], S. FAROOQI[4], ***F. GRABENHORST**[3];
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Abstract: Overconsumption of high-fat food is a major driver of weight gain. Oral food processing elicits sensory signals that inform about a food's fat content and reward value. Here we combined food-engineering and functional-MRI approaches to show that the human orbitofrontal cortex (OFC) translates oral sensations produced by high-fat foods into subjective, economic valuations that guide eating behavior. Healthy volunteers (N = 22) sampled liquid foods varying in nutrients (sugar, fat) and oral-texture qualities. Activity patterns in mid-to-lateral OFC encoded a key oral-texture parameter that mediated the influence of fat content on subjective value: the coefficient of sliding friction (CSF). We measured CSF using our recently developed tribometer (Huang, Sutcliffe & Grabenhorst, 2021, PNAS) with parallel-sliding pig tongues that approximate softness and surface of the human tongue. OFC responses to foods in the mouth reflected low sliding friction produced by fatty liquids. Neural encoding of CSF in

OFC was partly independent from its encoding of subjective value, suggesting separate but overlapping representation. These effects were not explained by viscosity, a distinct texture parameter that separately influenced behavior and neural activity. Neural integration of food-texture parameters with subjective value was not found in oral somatosensory cortex or pregenual anterior cingulate cortex, which separately encoded food texture and value, respectively. Importantly, oral-texture sensitivity of OFC predicted subjects' fat preferences in a naturalistic eating test performed on a separate testing day. These findings suggest a key role for the human OFC in evaluating food textures to mediate preference for dietary fat.
Reference: Huang, F.-Y., Sutcliffe, M.P.F. & Grabenhorst, F. Preferences for nutrients and sensory food qualities identify biological sources of economic values in monkeys. Proc Natl Acad Sci U S A 118, e2101954118 (2021).

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Poster

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Topic: H.03. Decision Making

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Economic and Social Research Council [grant number ES/P000649/1]

Title: Neural encoding of oral-sensory food properties and economic values during food choice

Authors: *T. I. TRAUT, F. GRABENHORST;
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Abstract: Food oral processing involves a chain of sensory and neural events in which a food's physical structure elicits oral sensations and subjective valuations that guide eating behavior. For example, foods high in sugar and fat typically produce a characteristic 'mouthfeel' in the form of a sweet taste and thick, smooth texture. These sensory signals are thought to contribute to the reward value of food, to the near-universal preferences for foods high in sugar and fat, and to the development of obesity. Previous human neuroimaging studies identified neural value signals underlying food choices in response to visual cues. However, the neural mechanisms linking food oral processing to subsequent choices remain unclear. Healthy human participants (N = 30) orally sampled and evaluated (using willingness-to-pay bids) eight different liquid foods that varied in nutrient (sugar, fat) content and sensory properties (coefficient of sliding friction, viscosity; Huang et al., 2021). Participants later made consumption choices between these foods, cued by abstract, conditioned, visual stimuli, while undergoing functional magnetic resonance imaging (fMRI) scanning. Willingness-to-pay bids and food choices were positively correlated

and well explained by participant-specific integrations of particular nutrient and sensory components of offered foods. Subjective evaluations of oral-texture variables (coefficient of sliding friction, viscosity) partially mediated the effect of fat content on food choices. The orbitofrontal cortex, ventromedial prefrontal cortex, and amygdala signalled the subjective economic value of offered food rewards in response to conditioned visual stimuli. At the time of choice, ventromedial prefrontal cortex activity signalled the value of the chosen option. These integrated value signals could be decomposed into components relating to specific nutrient and sensory food properties. Our findings show that nutrients and oral-sensory food qualities structure food preferences measured from choices and willingness-to-pay bids, and that neural reward and decision systems integrate orally sensed nutrients and food qualities into economic valuation and decision signals.
References: Huang, F.-Y., Sutcliffe, M.P.F. & Grabenhorst, F. Preferences for nutrients and sensory food qualities identify biological sources of economic values in monkeys. Proc Natl Acad Sci U S A 118, e2101954118 (2021)

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Poster

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Pew Biomedical Scholars Program
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Title: Decision making in the context of multi-attribute options

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Abstract: Often decisions are made between options that have multiple features, or attributes, that are relevant to one's choice. For example, when deciding between snacks to purchase, one might factor cost and taste into a selection. The orbitofrontal cortex (OFC) has an important role in decision-making and OFC neurons represent associations between stimuli and their overall values. However, it is still unknown whether OFC only evaluates options on the basis of their integrated value, as suboptimal decision-making effects such as the attraction effect indicate that within-attribute comparison may also contribute to decision-making. To investigate how multi-attribute options are represented in neural activity, we trained two rhesus macaques on a multi-

attribute decision making task, in which two simultaneously-presented options were represented by stimuli reflecting the sweetness of that option's sucrose reward, and the probability of receiving that reward. These composite stimuli represented information about the attributes of the options with separate bars that either increased *or* decreased with increasing attribute value, allowing us to investigate both free-viewing gaze behavior and changes in choice behavior due to perturbations in attribute presentation. We recorded neurons in OFC and frontal eye fields (FEF) using acute electrodes and multi-contact linear probes. We found that when comparable attributes did not share a presentation mode (e.g., reward bar A increased in size with increasing sweetness, while reward bar B decreased), choice behavior became suboptimal, implying a role for within-attribute comparison. Likewise, analysis of gaze transitions reveals a preference for within-attribute over within-option comparisons, even as similar proportions of either transition are made across trial types. Neuronal analysis indicates a greater presence of independent information relating to attribute than integrated value of the chosen option in OFC and FEF firing rates. Our interim results support the notion that value-based decisions take place, at least partially, in the space of individual attributes, and may depend on attribute value representations in OFC.

Disclosures: A.Q. Perkins: None. **E.L. Rich:** None.

Poster

PSTR233. Orbitofrontal Cortex

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR233.17/TT16

Topic: H.03. Decision Making

Support: NIH Grant R21 MH131900
NIH Grant T32 AG049688

Title: Neural mechanisms of the influence of reward expectations on perception and behavior

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Abstract: Expectations of reward play a critical role in cognition and decision-making, allowing us to predict the outcomes of certain actions. These expectations can influence which actions we take and also inform how we interpret sensory input. However, when our expectations are wrong, the ability to flexibly shift our behavior in response to unanticipated circumstances is critical for our well-being. For example, recognizing that the milk in the fridge that you thought was still good has actually spoiled protects you from an unfortunate episode of food poisoning. In order to better understand the neural dynamics involved in these processes, we recorded neurons from the orbitofrontal cortex and the gustatory cortex while monkeys performed a task in which different image cues predicted the taste of a fluid bolus (from sweet to bitter). Each trial

could be either forced-choice (where only one image was presented for selection) or free-choice (where a choice between two images was presented). In order to select an image, the monkey was required to fixate the image and release a touch-sensitive bar, which was followed by an initial bolus of fluid. Following image selection, there was a four-second period during which the monkey could tap the touch-sensitive bar to receive additional small deliveries of the same fluid. Monkeys consistently chose sweeter options, and tapped more for them. Once associations were well established, a small percentage of trials became "mismatch" trials, in which the fluid delivered did not match the cue image. On these trials, tapping behavior initially reflected the expected outcome, but shifted over the four-second window in accordance with the actual fluid received. To understand the neural dynamics of expectation in taste perception and motivated behavior, we are simultaneously recording from the orbitofrontal cortex and the gustatory cortex while monkeys perform the task. Preliminary data have suggested that the activity of the orbitofrontal cortex is flexibly modulated by expectations of taste.

Disclosures: S. McConnell: None. **E.L. Rich:** None.

Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR234.01/TT17

Topic: H.03. Decision Making

Support: NIH Grant R01 MH115035
COBRE 5-27132

Title: Neural Mechanisms of Credit Assignment in the Prefrontal Cortex (PFC): Unraveling the Roles of ventrolateral PFC and orbitofrontal cortex

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Abstract: The Prefrontal Cortex (PFC) plays a key role in complex cognitive tasks requiring temporal integration of information, attentional control, and reinforcement learning. These processes become particularly vital when addressing credit assignment problems, which causally link the outcomes of choices to antecedent stimuli across multiple features and time lags. However, our understanding of how different regions within the PFC cooperate while maintaining specific roles in resolving these problems remains elusive. To investigate this issue, we conducted a study in which two monkeys performed a credit assignment task. They were chronically implanted with large arrays of independently movable electrodes (96 and 128 electrodes respectively) in the PFC, including the ventrolateral PFC (vlPFC) and the orbitofrontal cortex (OFC). Our primary objective was to disentangle the unique roles of these different PFC regions in this process. In the task, animals were required to learn, by trial and

error, which of four simultaneously-presented cues was relevant to obtaining reward. To indicate their choice, after a blank delay of one second, animals performed a saccadic movement to the former location of the selected cue. Critically, they received only general feedback indicating whether the choice was correct or incorrect, necessitating the inference of a causal link between the outcome and the identity of the earlier cue. We implemented a hybrid reinforcement learning model paired with Poisson generalized linear models to unpack the neural representations pertaining to cue identities, locations, feature values, and feature-specific reward prediction errors (fRPEs) in each area. We then employed "partial mutual information" to quantify the nonlinear direct associations between diverse variables, such as the spike activity and the fRPEs conditioned by subsequent outcomes. Our results revealed that both the vlPFC and OFC were involved in representing cue information (identity, location, and cue value) during the initial cue presentation. However, the vlPFC showed an early representation of cue identity and fRPEs during the feedback phase, highlighting a potentially critical role in resolving credit assignment problems. These findings suggest distinctive roles of the vlPFC and OFC in resolving credit assignment problems during reinforcement learning.

Disclosures: E. Lee: None. **W.F. Asaad:** None.

Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

Location: WCC Halls A-C

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Program #/Poster #: PSTR234.02/TT18

Topic: H.03. Decision Making

Support: IDEX - IRS

Title: Cortical networks encoding pleasantness and goal during value-based tasks

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Abstract: From the moment we wake up, we are presented with a series of decisions, whether it be on what to wear, what to eat, or which task to start working on. We are therefore confronted on a daily basis with a variety of choices, some more pleasant than others, which may further have differences in their underlying goals (e.g., “what would I prefer to eat” vs “what would be the least worst task to check off first”). So far, most research has asked participants to choose the

preferred option (“choose best”) from sets of neutral (or pleasant) items, leading to the identification of a core network of brain regions involved in the valuation and choice stages of decision-making. Here, we aim to build on this existing literature by exploring whether the subjective values of pleasant and unpleasant stimuli are encoded differently, and whether this encoding is modulated by the task goal. We collected stereo-electroencephalography recordings from 29 patients with refractory epilepsy while they performed a decision-making task on hypothetical life situations, organized in a 2 (pleasant, unpleasant) x 2 (“choose best”, “choose worst”) factorial design. Neural results demonstrated an anatomically-dissociable encoding of pleasant and unpleasant subjective values in key decision-making regions. Furthermore, the encoding of chosen and unchosen values during the choice stage was modulated by the task goal.

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Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR234.03/TT19

Topic: H.03. Decision Making

Support: IBS-R002-A1

Title: The Contribution of Distinct Types of Motor Cortical Projection Neurons to Value-Based Action Selection

Authors: *E. J. SHIN[1], J. JU[1], J. GHIM[2], M. W. JUNG[1];
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Abstract: Previous studies in rodents have underscored the significance of the secondary motor cortex (MOs) in value-dependent choice behavior. However, the specific neural circuit processes within MOs that underpin value-based action selection remain unclear. In this study, we investigated the roles played by three different types of MOs projection neurons: superficial-layer intratelencephalic (IT) neurons, deep-layer IT neurons, and pyramidal tract (PT) neurons. We conducted experiments using head-fixed mice performing a dynamic foraging task, in which the animal’s choice behavior was well accounted by model-free reinforcement learning. Before the onset of the animal’s choice behavior, deep-layer IT and PT neurons carried advanced choice signals earlier than superficial-layer IT neurons. Moreover, during the outcome period, deep-layer IT and PT neurons conveyed stronger chosen value and outcome signals than superficial-layer neurons. These results indicate a more important role of deep-layer MOs neurons than superficial-layer neurons in value-dependent action selection in a simple dynamic foraging situation. Within the deep layers, IT neurons primarily represented the value associated with the contralateral target, while PT neurons exhibited activity that favored contralateral choices. Also,

unilateral optogenetic stimulation of deep-layer IT neurons disrupted value-dependent choice behavior only when the value of the contralateral target was greater than the ipsilateral target, while optogenetic stimulation of PT neurons enhanced contralateral choice irrespective of value. These results suggest that deep-layer IT neurons preferentially encode the value of the contralateral target, which may in turn influence PT neurons to facilitate contralateral target selection. Collectively, our findings shed light on distinct contributions of different types of projection neurons within MOs to value-based action selection.

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Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

Location: WCC Halls A-C

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Program #/Poster #: PSTR234.04/TT20

Topic: H.03. Decision Making

Title: Social construction of value explains partisan alignment in behavior and the brain

Authors: *B. B. LU, B. SHEN, J. J. VAN BAVEL, P. W. GLIMCHER;
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Abstract: Although it is widely accepted that partisan affiliations have a significant impact on the political preferences of individuals, the social-psychological and neurobiological mechanisms for this phenomenon are less understood. Many US voters, for example, show strong political preferences that appear to be more tied to their partisan affiliations than to their direct self-interest. We developed a behavioral task designed to measure the strength and structure of how partisan affiliations influence preferences. Our behavioral data showed a strong partisan cuing effect such that learning whether their political party supported a given law led partisans from the left and right rapidly and significantly shift their preferences for that law to align with party leaders. Similarly, knowing the degree of the opposing political party's support for that law also strongly impacted preferences. In addition to partisanship, the degree and direction of these shifts were notably influenced by a subject's psychological trait measures related to social and interpersonal relationships, such as desire for social esteem and approval of social hierarchies. In the present study, we examined the neural activations associated with this rapid partisan-evoked shift in political preferences. Subjects viewed short synopses of real proposed laws that have appeared before US Congress. They were additionally asked to report their preference for the law based on the synopsis. In later blocks, they viewed and evaluated the laws again after learning the true percent of members of Congress in each political party who voted in favor of the law. This allowed us to search the neural data for the loci at which subjects' political preferences were represented, as well as neural activations correlated with the degree of partisanship expressed by individuals. We found areas that track political preferences in known economic value-related areas including ventromedial prefrontal cortex. These preference ratings were associated with the social cognition network of the brain involving temporoparietal junction

and cingulate cortex. Finally, preliminary data suggest the dorsolateral prefrontal cortex is engaged when partisans encountered unexpectedly high out-partisan support, which may point to the role of cognitive control in political judgments. These findings indicate that political preferences use the common currency network for economic value and establish the central role of social cognition in evaluating these preferences. This value-based framework may explain idiosyncratic political behavior in the context of decision-making biases such as the decoy effect and others.

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Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

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Topic: H.03. Decision Making

Support: NIH Grant R01HD097619

Title: Neural mechanisms underlying the effects of monetary wage on effort provision

Authors: *A. KIM[1], V. S. CHIB[2];
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Abstract: The expectation of increased wages is that these raises will illicit an increase in a worker's motivation, thereby increasing their work output. In this study, we examined how changes in monetary wages serve as a motivating influence for effortful exertion at the level of brain and behavior. Participants completed a reward-based effort task while we scanned their brain with functional magnetic resonance imaging (fMRI). During each trial, we presented participants with a risky option that could yield either fixed monetary payment, independent of their effort exertion, or payment based on the amount of effort exerted, called piece-rate payment. Each of these options had an equal probability of occurring. The piece-rate payment was determined by one of two conditions: Low (maximum exertion resulted in $2) and High (maximum exertion resulted in $6), where the maximum payment was lower or higher, respectively, than the maximum fixed payment ($4). We varied the fixed payment and piece-rate condition so that participants had different reward expectations. The payment was received after individuals had a chance to exert effort, enabling us to infer how the brain encoded expectation as a reference point in relation to payment outcome. We found that participants exerted more effort for the High than Low piece-rate condition, but overall effort exertion did not vary based on the fixed payment, and there was no interaction between the piece-rate condition and fixed payment. These results suggest that individuals' expectations and effortful output are primarily driven by the offered piece rate and not the experimentally defined fixed payment. Our subsequent neuroimaging analyses will examine how the brain processes expectations from

related piece-rate and fixed payments offered, and how these sources of information serve to motivate effort exertion.

Disclosures: A. Kim: None. **V.S. Chib:** None.

Poster

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Topic: H.03. Decision Making

Support: JSPS KAKENHI Grant Number JP22J20134
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Title: Amygdala rather than dopamine neurons processes unconscious value information in macaque monkey

Authors: *O. TOYOSHIMA[1], J. KUNIMATSU[2], M. MATSUMOTO[2];
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Abstract: An intriguing idea in psychology is that unconscious information, such as sensory information that are not perceived, can influence our behavior. In the present study, we aimed to understand how the brain processes unconscious information at the single-unit level. Especially, we focused on reward value information that influence many kinds of behavior such as motivation, learning and decision-making. We developed a visual discrimination task in which a macaque monkey was required to discriminate a visual information that was less perceptible due to visual masking. In each trial, a circle-shaped visual stimulus or a visual stimulus in which a part of the circle-shaped stimulus was missing (i.e., C-shaped stimulus) was presented. The position of the missing part of the C-shaped stimulus varied (top or bottom of the circle) in each trial. The monkey was required to discriminate whether the presented stimulus was circle-shaped or C-shaped. After the brief presentation of the stimulus, a mask stimulus was presented with a delay called “stimulus onset asynchrony” (SOA: 50, 100, or 200 ms). In general, shorter the SOA, less visible a presented stimulus. Actually, when the SOA was shorter, the monkey was more likely to report that the presented stimulus was circle-shaped even if the actual stimulus was C-shaped, indicating that the monkey did not perceive the missing part. More importantly, the position of the missing part (top or bottom) predicted the amount of a liquid reward. The C-shaped stimulus missing the top part was associated with a small reward, whereas that missing the bottom part was associated with a large reward. The circle-shaped stimulus was followed by the small or large reward with 50% probability. Using this task, we investigated neuronal activity representing the reward value predicted by the missing part of the C-shaped stimulus especially when the monkey did not perceive the missing part. We recorded single-unit activity from two different components of the reward circuitry, midbrain dopamine neurons and the amygdala. We

found that both dopamine and amygdala neurons represented the value even when the monkey did not perceive the missing part, suggesting that these neurons represented the “unconscious” value information. However, whereas the amygdala neuronal modulation by the unconscious value information was almost the same as the modulation by the conscious value information, the dopamine neuronal modulation by the unconscious value information was significantly smaller than the modulation by the conscious value information. These findings suggest the gradient of capacity to represent the unconscious value information in the reward circuitry.

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Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

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Creative-Pioneering Researchers Program from SNU

Title: Cognitive flexibility representationin the primate putamen

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Abstract: Cognitive flexibility is a fundamental aspect of cognition that enables individuals to adapt behaviors to changing environments. Current models suggest that cognitive flexibility is processed in the caudate, while the neighboring putamen (PUT) mainly engages in habits. However, we hypothesized that PUT might be involved in cognitive flexibility as it is a primary target of dopamine neurons that flexibly update the reward change.To test this, we recorded neurons in PUT of two monkeys while they performed an object value reversal task. In this task, monkeys learned through trial and error which of the two fractal objects was associated with a reward. The object-reward contingency remained constant over a block of 24 trials, but then, the values of objects reversed. While performing the task, monkeys flexibly changed their behaviors based on the updated contingency. Saccades were significantly faster for a high-valued object than a low-valued object. 376 neurons in PUT responded to objects. 247 of 376 object-responsive neurons (65%) adapted firing rates to changing object values (flexible value-coding neurons). Among the 247 value-coding neurons, 138 neurons (56%) responded more strongly to high-valued objects(Positive value-coding), while 109 neurons (44%) exhibited higher firing rates to low-valued objects (Negative value-coding).To examine the role of PUT in habit, monkeys learned values of fractal objects stably associated with reward outcomes, and their long-term memory was tested with a passive-viewing task. To generate habitual saccade based on the long-term value memory, the learning repeated for more than 4 days. Their habitual

behavior and neural response were tested several days after the last learning. Monkeys showed gaze bias towards previously learned rewarded objects in the free-looking task, but a few neurons encoded the long-term value memory in the passive-viewing task. 19 PUT neurons (19/376 neurons, 0.05%) showed a value discrimination response. 15 of 19 stable value-coding neurons encoded both stable and flexible values, and the other 4 neurons exclusively encoded stable values. Our findings demonstrate that neurons in PUT, excluding its small tail part, updated object-outcome associations, indicating its role in cognitive flexibility.

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Poster

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Topic: H.03. Decision Making

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Title: Age-related differences in neural responses to trust

Authors: *A. DACHS[1], C. J. SHARP[1], E. YANILMAZ[1], J. B. WYNGAARDEN[1], O. ZAFF[1], T. TROPEA[1], D. SAZHIN[1], R. LUDWIG[1], T. GIOVANNETTI[1], D. S. FARERI[3], D. V. SMITH[2];
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Abstract: Social relationships influence our behaviors and may modulate our neural responses to reward. However, the effect of relationships on behavior and reward may change as we age. For example, reciprocated trust from a friend compared to a stranger is associated with enhanced ventral striatal (VS) activation (Fareri et al., 2015), though our recent work has suggested that this striatal response is blunted in older adults (Fareri et al., 2022). It remains unclear whether age-related differences in responses to trust are associated with maladaptive outcomes such as financial exploitation. To address this gap, we recruited participants (N = 22; ages 21-73 years; mean = 48 years) to play an economic trust game while undergoing fMRI. We also collected self-report questionnaires on risk for financial exploitation. In each round of the trust task, participants were allotted a sum of money ($8) and were presented with choices to invest a predetermined amount in one of three possible partners (friend, stranger, and computer). The money that participants decided to invest into their partner was then tripled, and the participant was shown whether their partner had split the money evenly with them (i.e., reciprocate) or kept the money for themselves (i.e., defect). Consistent with prior work from our group (Fareri et al., 2015), we found that participants invested more with friends relative to strangers and computers ($z>3.1$, FWE<0.05). Further, our imaging results replicated past work (Schultz et al., 2003 PTRSL), finding increased fusiform face area (FFA) activation when participants were making

decisions involving human relative to computer partners (z>3.1, FWE<0.05). Preliminary fMRI analyses also showed increased activation in the VS during reciprocate relative to defect outcomes (p<.05, uncorrected). We also found enhanced responses within components of the default mode network (i.e., posterior cingulate cortex, temporoparietal junction, and medial prefrontal cortex) to reciprocated trust from friends relative to from computers. Taken together, these preliminary findings replicate prior work and suggest that social relationships modulate neural responses to trust. Future analysis will explore how these effects differ across the lifespan and may be linked to self-reported risk for financial exploitation.

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Poster

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Topic: H.03. Decision Making

Support: NIH R01MH097061

Title: Value-guided Time Investments: Bridging reinforcement learning, behavioral economics and mesolimbic dopamine

Authors: T. OTT[1], ***A. ARORA**[3], S. REN[4], V. VEGA[2], T. S. GOUVEA[5], A. KEPECS[6]; [2]Bernstein Ctr. for Computat. Neurosci. Berlin, [1]Humboldt Univ. of Berlin, Berlin, Germany; [3]Neurosci., Washington Univ. In St. Louis, Saint Louis, MO; [4]Washington Univ. Sch. of Med., Saint Louis, MO; [5]German Res. Ctr. for Artificial Intelligence: Kaiserslautern, Rheinland-Pfalz, Germany; [6]Neurosci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Whether deliberating how long to wait in line for tickets to a Beyonce concert or how many years to invest in a medical degree, the time we are willing to commit reveals the subjective value we place on these choices. The more we value an option, the more time we are willing to invest in it. Time investment serves as a 'revealed preference' that directly reflects subjective value, as studied in behavioral economics. This contrasts with the approach often used in neuroscience and learning theory, which algorithmically infers value from reinforcement history. Both approaches aim to quantify subjective value, yet the relationship between ‘model-inferred value’ and ‘revealed value’ remains elusive.
To integrate the perspectives of behavioral economics and learning theory, we devised a novel, probabilistic reward learning task for rats. Rats made choices between two options, each unpredictably ‘baited’ with rewards that varied over time. The task also required rats to invest time to obtain uncertain, delayed rewards, mimicking real-world decision-making. The model-inferred value of each choice, derived from the rats' reinforcement and choice history, not only

predicted which option they would pick but also how long they would invest in it. The time rats invested predicted their selection of higher-valued options, providing a trial-by-trial, graded measure of revealed value.
We next probed the relationship between these subjective value measures and mesolimbic dopamine by using fiber photometry to track fluorescence from a genetically-encoded dopamine sensor in the ventral striatum. We observed phasic dopamine release around the moment of choice. Dopamine release was not consistently correlated on a trial-to-trial basis with the model-inferred value derived from reward history. However, dopamine release robustly predicted how long rats would invest in an option seconds later. Thus, mesolimbic dopamine appears to encode the rats' revealed value for subjective valuation of each option, which can be measured behaviorally in their single-trial time investment decisions. Our study introduces a novel behavioral task that unites reinforcement learning theory with behavioral economics, and identifies a role for mesolimbic dopamine in guiding time investment decisions.

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Poster

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McKnight Memory and Cognitive Disorders Award

Title: Dorsal raphe neurons signal integrated value during multi-attribute decision-making

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Abstract: The dorsal raphe nucleus (DRN) is implicated in psychiatric disorders that feature impaired sensitivity to reward amount, impulsivity when facing reward delays, and risk-seeking when grappling with reward uncertainty. However, whether and how DRN neurons signal reward amount, delay, and uncertainty during decision-making is unclear. We recorded discharge from single DRN neurons as monkeys chose between offers whose attributes, namely reward expected amount, delay, and uncertainty, varied independently. We identified many DRN neurons that signaled offer attributes. Remarkably, many neurons integrated offer attributes in a

manner that reflected monkeys' overall preferences - the subjective value of amount, delay, and uncertainty. Moreover, after decision-making, these same neurons signaled quantitative reward prediction errors, suggesting a broader role in tracking value across task epochs and behavioral contexts. Our data illustrate how the DRN can be involved in integrated value computations, guiding theories of DRN in decision-making and psychiatric disease.

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Poster

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Program #/Poster #: PSTR234.11/TT27

Topic: H.03. Decision Making

Support: R00 DA047419

Title: Elevating endocannabinoid tone influences the dopaminergic substrates of cognitive flexibility

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Abstract: Dopamine (DA) in the mesolimbic system is crucial for reward-based learning. A reward prediction error is adapted and encoded via mesolimbic DA neurons to associate a cue with the value of a reward. During early reversal learning when stimulus-reward associations are altered, accumbal phasic DA release is essential for flexibly updating these associations and improving choice performance. The endocannabinoid system has emerged as a critical modulator of the mesolimbic DA system. Endogenous cannabinoids, such as 2-AG, can influence cue-motivated behavior via midbrain dopaminergic projections to the nucleus accumbens (NAc). Further, extracellular DA levels are shown to be reduced in the NAc following CB1 antagonism and increased following direct and indirect CB1 agonism. Therefore, endocannabinoid signaling may play a role in reward processing and reversal learning performance on a reward-motivated task via regulation of mesolimbic DA function. The current experiment used fiber photometry to measure transient DA signals in the NAc via the genetically-encoded dopamine sensor, GrabDA, during an operant probabilistic reversal learning (PRL) task. Male and female mice (N = 43) were first trained to discriminate between two levers of differing reinforcement probabilities (80% vs. 20%); this was followed by a reversal phase in which the reinforcement probabilities were inversed across levers. During the reversal session, mice were pre-treated with a monoacylglycerol lipase (MAGL) inhibitor, JZL-184 to increase synaptic levels of 2-AG. DA was recorded during early and late acquisition, and early, mid, and late reversal sessions. Performance on the PRL task was analyzed to assess mean differences various performance

metrics on the first day of the reversal phase. Analysis of error probabilities (win-stay and lose-shift) were also conducted. Finally, DA release was compared between and within subjects across sessions to determine differences in DA transients following different trial outcomes. Results demonstrate that systemic manipulations of endocannabinoid signaling impair reversal learning performance and dysregulate associated NAc DA release. These findings give critical insight into the role of the endocannabinoid system in flexible reward-based learning and may have significant implications for the use of cannabinoids for recreational or therapeutic purposes.

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Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR234.12/TT28

Topic: H.03. Decision Making

Support: NIDA Grant R01DA054201
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Title: Neural mechanisms of scaled value under craving: relationship to drug and food-related addictive symptomatology

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Abstract: Craving is commonly experienced by both healthy individuals and those with addictive disorders. Previous work has identified a putative craving circuit comprised of canonical value and emotion/interoceptive processing regions of the brain. However, this work is limited in that it has focused on cue-reactivity paradigms that do not examine subjective value computations understood to dictate - and potentially bias - decision making under craving (i.e. that capture the behavioral intent component of craving). We previously showed that craving scales subjective value in a multiplicative-gain manner along a similarity dimension: people value disproportionately more craved items and similar ones, but craving is not reflected in changes in the value of dissimilar options. To understand how these scaling and similarity effects are generated, here we investigated BOLD activity within the putative craving circuit in an incentivized decision task. Additionally, we examined if addictive-like symptomatology (captured by screening measures for problematic drug and food-related behaviors) potentiated these effects. Participants (N=32) repeatedly reported on their willingness-to-pay (WTP, indexing current subjective value) and desire for various palatable snack foods both before and

after a multisensory craving induction designed to elicit craving for one of the snacks. Behaviorally, all participants demonstrated a significant increase in desire and WTP for the 'craved snack', especially when offered in higher quantities. Neurally, we found canonical value regions (ventromedial prefrontal cortex and ventral striatum) tracked these changes in value and psychological state; however, there were no differences by addiction symptomatology. What did differ by symptomatology was the similarity effect: low symptomatology individuals only increased WTP for the target 'craved snack', whereas individuals with more problematic and addictive-like eating displayed greater WTP across all snacks offered post-induction, suggesting overgeneralization of craving. Moreover, the amygdala tracked similarity contingent on symptomatology, with higher symptomatology individuals displaying increased activation post-induction. These preliminary results tie in the putative craving circuit to value-based decision making and implicate the amygdala in differentially tracking similarity effect, which is integrated into computed subjective value. Such neuroeconomic procedures enable probing of choice mechanisms that may underlie clinically relevant individual differences unavailable in simpler cue-reactivity paradigms.

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Poster

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Program #/Poster #: PSTR234.13/UU1

Topic: H.03. Decision Making

Support: JST CREST JPMJCR1853

Title: Dopamine signals transmitted to different subregions of the primate striatum during economic decision-making

Authors: *Z. DUO[1], Y. WANG[2], M. NEJIME[2], J. KUNIMATSU[2], H. AMITA[3], K. INOUE[3], M. TAKADA[3], H. YAMADA[2], M. MATSUMOTO[2];
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Abstract: Economic decision-making consists of several subprocesses, such as option recognition, option evaluation, option selection, choice execution, outcome evaluation and so on. Midbrain dopamine (DA) neurons are thought to play a key role in the economic decision-making by signaling reward prediction error (RPE) that reinforces choices leading to better-than-expected rewards for future decision-making. On the other hand, impairments in the DA system disrupt various behaviors that are not directly related to rewards, suggesting that DA neurons are divided into functionally distinct subgroups. However, how these diverse roles represented by DA neurons shape the economic decision-making is still unknown. To tackle this issue, we

focused on DA neuron projections to the striatum that is also divided into functionally distinct subregions (i.e., the caudate, putamen, and accumbens), and compared DA signals transmitted to these subregions in a rhesus monkey performing an economic decision-making task. We utilized a cutting-edge DA biosensor, dLight1.1, to measure DA release in the striatal subregions of the monkey. In the task, one of the six possible options that were associated with different amounts of a liquid reward was offered at a time, and the monkey had to decide whether to accept this option during its presentation. If the monkey accepted, it obtained the reward associated with that option but only with 50% probability (i.e., the reward was sometimes not delivered). We so far found that DA signals transmitted to the putamen and caudate were different. In the putamen, the DA release increased in response to the reward and decreased in response to the omission of the reward, but it did not exhibit clear response to the option. These responses were enhanced when the option was associated with a larger reward, suggesting that the DA signal in the putamen represents the RPE that is important for future decision-making. On the other hand, although the data are still preliminary, the DA release in the caudate increased in response to the option. The magnitude of this response was correlated with the expected value of the option, which was an important signal to decide whether to accept the currently presented option. Our data suggest that two modes of DA release in the putamen vs. caudate signal distinct information and regulate different subprocesses of decision-making.

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Poster

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Topic: H.03. Decision Making

Support: NIH Grant RF1-AG067011 (to DVS)

Title: Associations Between Age and Neural Response to Reward in Social Contexts

Authors: *O. ZAFF[1], D. V. SMITH[2], J. Y. YANG[1], J. WYNGAARDEN[3], A. DACHS[3], C. SHARP[1], M. COLLINS[1], D. SAZHIN[3], R. LUDWIG[1], T. GIOVANETTI[1], D. FARERI[4]; [1]Psychology & Neurosci., [2]Dept. of Psychology & Neurosci., [3]Temple Univ., Philadelphia, PA; [4]Adelphi Univ., Garden City, NY

Abstract: Previous studies examining neural activation and functional connectivity have found links between social context and corticostriatal responses to reward. However, relatively less is known about how these effects may differ across the lifespan, from young adulthood to older adulthood. In the current study, participants (N = 23, Male = 9, White = 15) from age 21 to 73 (mean = 47.7) underwent fMRI while playing a card guessing game for monetary outcomes that could be shared with three partners - a close friend of the participant, a stranger, or the computer

(adapted from Fareri et al., 2012). After completing fMRI, a subset of participants (n = 19) were asked to subjectively rate the experience of winning or losing with each partner. Participants' ratings were significantly different across outcome and partner ($F(2,36)=20.392$, $p<0.001$), with ratings for wins shared with friends being significantly more positive than those shared with strangers ($z(18)=3.325$, $p<0.05$). Our preliminary neuroimaging analyses indicate increased activation in the fusiform face area (rFFA; $z>3.1$, FWE<0.05) when making decisions while partnered with human (friends + strangers) relative to non-human agents (computer). Additionally, we found activation in the precuneus in response to outcomes known to be shared socially (friend vs computer; $z>3.1$, FWE<0.05). We also observe increased striatal activation when participants experienced shared rewards relative to shared losses, across partners ($p<0.05$, uncorrected). These preliminary results replicate previous research on FFA activation in response to faces as well as precuneus in response to social context (Schultz et al., 2003; Eckstrand et al., 2017). Future analyses will explore interactions between striatal activation, whole-brain connectivity, and age differences. Overall, this study will help elucidate age-related differences in how social context modulates responses to reward and decision making. Understanding how these processes differ across the lifespan may provide insight into maladaptive social decision making in older adults, such as risk for financial exploitation.

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Poster

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Topic: H.03. Decision Making

Title: Dopamine in the nucleus accumbens core signals confidence in decision-making as well as reward in a visual signal detection task

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**Abstract: Dopamine in the nucleus accumbens core signals confidence in decision-making as well as reward in a visual signal detection task Authors:L.J.F. Wilod Versprille[1]*, C. McKenzie[1], B.J. Alsiö[1,2], K. Yano[3], J.W. Dalley[1,4], T.W. Robbins[1]
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Author Disclosure Information: L. Wilod Versprille: None. **C. McKenzie:** None. **B.J. Alsio:** None. **K. Yano:** None. **J.W. Dalley:** Research grants from Boehringer Ingelheim. None. **T.W. Robbins:** Research Grant from Shionogi & Co., Ltd.
Abstract:A general role of dopamine (DA) in executive functions such as attention and working memory is well established. However, time-dependent fluctuations of DA during attentional and working memory performance have been difficult to determine due to the low resolution of conventional neurochemical sampling methods. The present study investigated time-resolved dopaminergic signalling in the nucleus accumbens core during the performance of a visual sustained attention task using the fluorescent dopamine receptor, DLight1.3b.
Rats were trained to criterion on a Signal Detection Task (SDT) to detect and respond to the absence or presence of visual light to obtain food reward. Trial initiation in response to a visual discriminative cue immediately triggered signal presentation/omission. Rats responded 1s later to indicate either presence or omission of the visual target, immediately followed by reward or punishment (5s time-out) feedback. Following task acquisition, rats were infused with a viral vector encoding DLight1.3b and implanted with an optic fibre to allow for *in vivo* DA recordings with high temporal resolution. Three weeks post-surgery DLight1.3b expression and functionality was confirmed using a sucrose reward probe, and recordings during SDT commenced (n = 12).
DA peaked upon the correct detection of either signal presentation or omission, with a rewarding food pellet. DA levels increased prior to correct responses but remained low prior to incorrect responses, possibly reflecting high versus low confidence. The introduction of a visual distractor impaired performance on the SDT and produced greater variability in DA signals.
These findings indicate that the DA levels in the nucleus accumbens core as well as signalling rewarding feedback may also accurately reflect expectancy of reward and hence the confidence in rewarded outcomes.

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Poster

PSTR234. Neural Mechanisms of Decision-Making: Value

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR234.16/UU4

Topic: H.03. Decision Making

Support: NIH Grant R01 DA047870

Title: Contribution of amygdala to dynamic model adoption under uncertainty

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Abstract: Intrinsic uncertainty in the reward environment requires the brain to run multiple models simultaneously to predict reward outcomes based on cues, stimuli, or actions that precede them, and moreover, determine the most reliable model that should control behavior at a time. To investigate the rather perplexing contributions of amygdala to reward learning, we reanalyzed choice behavior of amygdala-lesioned and control monkeys during two probabilistic learning tasks that involved different levels of uncertainty about the correct model of the environment. In the first task, macaque monkeys were trained to perform a stimulus-based reversal learning (*What-only*). The second task (*What/Where*) had additional uncertainty about the correct model (stimulus- vs. action-based) of the environment. Using information theoretic metrics to measure concurrent learning, we first found evidence for dynamic competitive interactions between stimulus- and action-based learning. To reveal neural mechanisms underlying this interaction, we constructed a reinforcement learning (RL) model with two parallel learning systems for stimulus and action and an arbitration mechanism. On every trial, a parameter capturing the relative weight of the two systems was dynamically adjusted by comparing the reward prediction errors as measures of reliability for the two systems. Across both control and amygdala-lesioned groups, this model explained the choice behavior better than an RL with fixed static weighting of the two systems. Further, we found that for control monkeys, the learning rate for arbitration in favor of the correct system (for a given environment) was significantly higher than that of the incorrect system. To capture the mechanism underlying this asymmetric update rates, we next equipped the arbitration component of the model with metaplastic synapses (Farashahi et al., 2017). Metaplasticity further improved the fit of choice behavior in both groups and accounted for the observed asymmetry. In contrast, amygdala-lesioned monkeys showed similar arbitration learning rates, suggesting a deficit in reliability-based weighting. Thus, amygdala lesion seems to diminish the benefits of metaplasticity in reducing noise in the reliability signals. Together, our results illustrate that primates utilize multiple learning systems to predict outcomes in uncertain environments and suggest a novel role of amygdala in arbitration between these systems based on metaplasticity.

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Poster

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Title: Two types of locus coeruleus norepinephrine neurons drive reinforcement learning

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Abstract: Two types of locus coeruleus norepinephrine neurons drive reinforcement learning
Zhixiao Su, Jeremiah Y. Cohen
To make decisions in a dynamic world, animals adjust their behavior in response to environmental feedback. To understand how this flexibility is achieved, we seek to understand how the nervous system learns from choice outcomes. Theories proposed that norepinephrine (NE) neurons in the locus coeruleus (LC) modulate learning from reinforcement. We tested this hypothesis by measuring activity from identified mouse LC-NE neurons during a behavioral task requiring ongoing learning from reward prediction errors (RPE). Mice were trained to make choices to lick leftward or rightward for reward with changing probabilities. Because reward probabilities were unknown to the animals, they used reward history to make future choices. Reward history was also reflected in their tongue kinematics. We made electrophysiological recordings from identified LC-NE neurons by "tagging" channelrhodopsin-2-expressing LC-NE neurons in *Dbh*-Cre mice. We found two types of biophysically distinct LC-NE neurons with different action potential shapes, dorsal-ventral distribution, excitability and correlation with pupil diameter. These two types of neurons also responded differently to decision outcomes. Type I neurons were excited by RPE while type II neurons were excited by lack of reward. To test the hypothesis that LC-NE neurons modulate learning from decision outcomes, we silenced their activity using the inhibitory opsin GtACR2. The inactivation caused increased probability of switching choices on the trials following no reward and introduced a negative shift to policy update, which can be captured by modeling inhibition as a negative shift of RPE. Our data indicate biophysically and anatomically distinct modules in LC and reveal a function for LC-NE neurons in modulating learning from experience.

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Poster

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Topic: H.03. Decision Making

Support: IBS-R002-A1

Title: Value-dependent neural activity in the medial entorhinal cortex

Authors: *M. SEO[1,2], J. KIM[1,2], M. JUNG[1,2];
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Abstract: Even though the hippocampus has long been postulated to process primarily cognitive variables related to spatial navigation and episodic memory, value signals have been found in the hippocampus of mice, rats, monkeys, and humans. A recent study in mice, in particular, found that population activity of dorsal CA1 neurons increases monotonically as a function of value. Little is known, however, about the source of hippocampal value signals. One candidate is the entorhinal cortex, which provides strong afferent inputs to the hippocampus. To investigate whether and how the entorhinal cortex processes value signals, we examined value-related activity of pyramidal neurons in layer 2 of the medial entorhinal cortex (MEC) which innervate inhibitory interneurons in the CA1 region. We trained Wfs1-Cre mice in a probabilistic classical conditioning task in which three different odor cues were paired with three different reward probabilities (0%, 25%, 75%), and monitored MEC layer-2 pyramidal neuronal activity using *in vivo* Ca^{2+} imaging. We found that the population activity of MEC layer-2 pyramidal neurons decreases monotonically with value. Thus, an inverse relationship was found between value-dependent activity of CA1 and MEC pyramidal neurons. Our results raise the possibility that the MEC contributes to shaping value-dependent activity of CA1 neurons via feedforward inhibition.

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Poster

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Topic: H.03. Decision Making

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Title: Investigating the effect of value bias on sensory evidence encoding in perceptual decision making

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Abstract: When we make perceptual decisions under uncertainty we must prioritize outcomes that are more rewarding. This has been shown to be mainly effected by shifting the starting point in optimal accumulation-to-bound models. Recent work also found biases in drift rate (mean accumulation rate). However, it is still unclear whether the latter bias involves altering the encoding of sensory evidence itself or its readout further downstream.To address this question, we devised a value-biased decision task in which we could separately measure sensory evidence encoding for the two alternatives, the accumulation rate, and starting point bias using EEG. Subjects viewed two orthogonally-oriented gratings and reported immediately which had higher

contrast. A preceding cue indicated the higher-value alternative. The gratings flickered on and off in alternation, allowing us to trace the encoding of sensory evidence by measuring Steady State Visual Evoked Potentials (SSVEP). Rate of accumulation was measured via the Centro-parietal Positivity (CPP), previously established to reflect evidence accumulation, and starting point bias was measured via motor preparation signals. We now report on data of 11 healthy humans (3080 trials per condition) collected so far.The value cues had a significant effect on the speed and accuracy of responses. As expected, motor preparation signals indicated a starting point bias. The CPP exhibited an enhanced build-up rate not only with increasing contrast but also to higher-value sensory alternatives, indicating a drift rate bias. However, the SSVEP showed only very small signs of modulation. To benchmark this modulation size against what is possible for top-down control, we designed a task that explicitly incentivized top-down attention modulations using the same stimuli. In this case, the same preceding cue instructed the subjects to which orientation they attend, to detect a randomly-timed spatial frequency transient (SFT) and report after stimulus offset (8 participants so far, 3584 trials per condition). SFT detection rate was higher in the relevant grating but far from zero in the irrelevant grating, confirming a distraction effect overcome by attention. A much larger (x 19.32 in the current dataset) cue-dependent modulation was observed in the SSVEP in this attention task compared to the value-biased decision task.Results from the two experiments suggest that drift rate biases may be enacted by enhancing the readout of valuable sensory representations rather than direct modulation in SSVEP. The robustness of these findings will be verified with further data and computational modeling will be conducted to affirm the conclusion.

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Poster

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Program #/Poster #: PSTR234.20/UU8

Topic: H.03. Decision Making

Title: Neural Representations of Decision Values for Task Difficulty and Effort

Authors: *Y. WANG[1,3], A. CASAMENTO-MORAN[1,3], J. LEE[1,3], V. S. CHIB[1,3,2];
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Abstract: The perceived effort level and difficulty of an action shape our everyday decisions. If a task is very effortful and requires a great deal of exertion, or is very difficult and requires precise performance, we may be less likely to engage. Despite the ubiquity of effort and difficulty as factors in our decisions, there is a limited understanding of how they are represented in the brain. In this study, we investigated how individuals evaluate effort level and difficulty when making decisions, at the levels of behavior and brain. Participants first performed a motor task in which effort level and difficulty were independently manipulated. The task required

participants to squeeze a hand-grip dynamometer to move a cursor into a target on the screen. The position of the target was associated with the level of effortful grip exertion, while the size of the target was associated with the difficulty of the task. After the performance of the task, participants rated their perceived effort and difficulty levels. We found that participants' success rates increased with tasks requiring less effort (less exertion) and less difficulty (larger target size). To investigate how effort and difficulty were encoded in the brain, and influence choices, participants performed a decision-making task in which they weighed choice between performing tasks of varying effort and difficulty for monetary reward, while their brain was scanned with functional magnetic resonance imaging (fMRI). Participants preferred lower effort and difficulty levels and exhibited a trade-off between the two components. We also observed an interaction effect with reward, as participants were motivated to choose more effortful and difficult options when offered larger rewards. We found that activity in ventromedial prefrontal cortex (vmPFC) was greater for lower effort levels, while the ventral striatum (VS) activity was greater for lower difficulty and higher reward levels of the chosen target. These results suggest that effort and difficulty are encoded as costs within different regions of the value network.

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Poster

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Topic: H.03. Decision Making

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Title: Age-related differences in social reward processing

Authors: *J. WYNGAARDEN, III, O. ZAFF, D. SAZHIN, A. DACHS, M. J. COLLINS, C. R. JOHNSTON, T. GIOVANNETTI, J. M. JARCHO, D. V. SMITH;
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Abstract: Decision making often occurs in a social context. Processing of social rewards (e.g., positive peer feedback) can elicit responses within traditional reward processing brain regions, including the striatum and ventromedial prefrontal cortex (vmPFC), as well as connectivity between these and other regions. These findings have been well documented in young adults and adolescents, but it remains unclear whether these relations vary as a function of age into older adulthood. To examine these relationships, the current study is leveraging matched monetary and social reward tasks in which participants (N = 23; mean age = 47.03, SD = 18.57) choose between two stimuli in search of a reward (Quarmley et al., 2019, Frontiers in Neuroscience). In the monetary task, participants choose between two doors to find a monetary prize and avoid a monetary cost (win = $1.00 gain; loss = $0.50 loss). In the social task, participants choose between the faces of two peers who have purportedly indicated whether they like or dislike the

participant (win = peer 'like;' loss = peer 'dislike'). Except where noted, all results are reported with a cluster-forming threshold of $z > 3.1$ and $p < 0.05$. Preliminary analyses showed significant vmPFC activation for wins relative to losses (i.e., win > loss) across domains and activation in the anterior cingulate cortex for social rewards relative to monetary rewards [i.e., social (win > loss) > monetary (win > loss)] ($p < .05$, uncorrected). Future analyses will examine variation in these relationships related to aging and cognitive impairment. These results will help characterize differences in neural mechanisms of social reward processing between different stages of the lifespan. Taken together, these findings may shed light on maladaptive decision making in social contexts, which may increase the risk of being financially exploited by another person in older adulthood.

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Poster

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FCT

Title: Dopamine neurons reveal an efficient code for a multidimensional, distributional map of the future

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Abstract: Causal inference based on temporal relationships is fundamental for adaptive, intelligent behavior. However, standard value-based reinforcement learning (RL) models learn estimates of temporally discounted average future reward, leading to ambiguity about future reward timing and magnitude. Extending these algorithms to learn a set of values that differ in their sensitivity to reward prediction errors (RPEs) and temporal discounting can support inference. Midbrain dopamine (DA) neurons, known to encode RPEs, display signatures of learning about distributions of reward magnitude. However, it is unclear whether they also display signatures of learning distributions of reward timing, how such encoding might be multiplexed with reward magnitude information, and whether the joint encoding of these two dimensions allows for distributional readout of reward over time at the start of an episode. Here, we generalize distributional RL learning rules to the time domain by proposing a population coding model that optimally represents such information under resource constraints, adapting to

the temporal statistics of experienced rewards. If the brain implements such a code, neural RPEs should 1) express variable sensitivity to future reward timing, 2) carry future reward distribution information and, 3) adapt to changes in the temporal statistics of reward. We tested these predictions by recording responses of optogenetically identified midbrain DA neurons to stimuli that predict rewards at variable delays and amounts. Temporal discounts varied among DA neurons, providing future reward timing information. Additionally, we observed that in trials wherein animals commenced licking earlier or later, the decoded distribution over future rewards exhibited a qualitatively similar shift in time, which suggests that estimates of the timing of future reward decoded from the DA neuron population reflected temporal expectations that animals used to guide behavior. When removing the longest delay, DA RPE responses adapted, increasing encoding accuracy on shorter reward time scales. Finally, we show that a 2D map of future reward amount over time can be decoded from the DA neuron population. Our work suggests that midbrain DA neurons reflect a simple model-free distributional time, in addition to distributional value RL, that paradoxically allows for complex computations such as context dependent risk attitudes and impatiency, planning and flexible adaptation to reward revaluation, as we demonstrate in simulated environments.

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Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

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Program #/Poster #: PSTR235.01/Web Only

Topic: H.03. Decision Making

Title: The richness of the current and past environment influences human risk preference in sequential economic risk choices

Authors: *Y. MOCHIZUKI[1], M. AGGARWAL[2], N. HARASAWA[2], C. CHEN[3], H. FUKUDA[4];
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Abstract: Decision-making involving probabilistic outcomes (i.e., under risk) is an integral part of our daily lives. Empirical investigations into human decisions regarding these outcomes have revealed that human behavior often deviates from standard economic theory, exemplified by framing and decoy effects. While behavioral economics has developed empirical mathematical models for each of these effects, there is no unifying principle that can explain why these effects occur in human economic decision-making. In this study, we demonstrate that one such context effect can be well explained by the ethological principle of fitness maximization, providing insight into the deviations of human behavior from standard economic theories. Our findings

suggest that these deviations are adaptive and that a better understanding of human economic decisions can be achieved by considering the single ethological principle of fitness maximization.

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Poster

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Topic: H.03. Decision Making

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Title: Preserved neural encoding of subjective valuation under uncertainty in human opioid addiction

Authors: *F. M. LOFARO[1,2], M. C. M. GUEGUEN[2], A. KAPOOR[2], E. E. ALVAREZ[2], D. BONAGURA[2], A. B. KONOVA[2];
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Abstract: Opioid use disorder (OUD) is associated with increased risk-taking and an increased tolerance for uncertainty. The computation of a reward's subjective value (SV) under uncertainty is modulated by the individual idiosyncratic tolerances all people maintain. Researchers have posited that the decision to take opioids could be the result of faulty subjective valuation such that their internal value function mapping objective value to SV is 'broken'. Using functional brain imaging, we examined whether subjective valuation in people with OUD reflects normative differences due to idiosyncratic tolerances *or* a breakdown of SV computations in the brain's valuation system.Treatment-engaged OUD patients (n=32; 6 females; mean [SE] age=44.7 [2.19] years) and matched controls (n=27; 11 females; age=45.0 [2.87] years) completed a decision-making fMRI task targeting two types of uncertainty, known-risk and ambiguity. A modified utility model parameterizing uncertainty was used to compute trial-by-trial SV of the chosen option. Model-based fMRI analyses were used to identify regions encoding SV and assess for a brain-behavior match. Multivariate analyses were also used to ascertain that specific patterns of activation were decodable based on individual uncertainty tolerances. Behaviorally, most subjects were averse to uncertainty (76%; consistent with previous research) with no significant group differences; this ensures that any observed neural differences cannot be explained by differences in idiosyncratic uncertainty tolerance. Neurally, we found that canonical value areas encode SV similarly across the OUD and control groups ($p<0.001$; ventromedial prefrontal cortex, $t=4.42$; ventral striatum, $t=3.95$; posterior cingulate cortex, $t=4.50$). In addition, there were no differences in these correlates or between groups when

uncertainty was split by type, either known-risk or ambiguity. Multivariate analyses indicated that SV could be significantly decoded above chance levels (permutation test) in all three regions, and in both groups separately, further demonstrating shared patterns across uncertainty types consistent with a domain-general SV signal (also in both groups). Contrary to prevailing assumptions, these results imply that the encoding and computation of SV is preserved in people with OUD. Instead, risk-taking observed in OUD may arise from changes in the subjective integration of variables used to compute value. That is, people with OUD do *not* have 'faulty' valuation or use different decision strategies, but rather differences emerge from subjective (biased) encoding of decision variables consistent with idiosyncratic preferences.

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Poster

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Topic: H.03. Decision Making

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Title: Effects of systemic administration of oxytocin and oxytocin antagonist on risky decision making in female and male rats

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Abstract: Oxytocin (OT) is a neuropeptide primarily known for its role in parturition, lactation, and pair-bonding, and can act via both peripheral and central nervous system mechanisms. Recent studies indicate that exogeneous OT can attenuate addiction-related behaviors such as drug self-administration in preclinical models. OT signaling is, however, understudied in the context of cost-benefit decision making, which is frequently altered in the context of drugs of abuse. Here we investigated the role of this hormone in modulating decision making under risk of punishment. Female (n=16) and male (n=8) Long-Evans rats were trained on a risky decision-making task (RDT), in which they made discrete choices between a large "risky" food reward accompanied by a probabilistic footshock (0%, 25%, 50%, 75%, 100% chance of punishment), and a small "safe" food reward without punishment. Drug administration began when stable performance in the RDT emerged. Rats were initially tested on the RDT following receipt of acute intraperitoneal injections (1.0 ml/kg, 15 min before testing) of OT (0, 0.3, 1.0, 3.0 mg/kg) following a randomized, within-subject design, with at least a 48h washout period between successive injections. Upon completion of OT administration, rats continued testing on the RDT until stable performance reemerged. Next, rats received intraperitoneal injections (1.0 ml/kg, 40 min before testing) of the OT receptor antagonist L-368,899 hydrochloride (OT-A, 0, 1.0, 3.0

mg/kg) under a similar randomized, within-subject design.In males, neither OT nor OT-A administration had statistically significant effects on choice of the large, risky reward. In females, however, both OT and OT-A administration led to a significant reduction in preference for the large, risky reward. In addition, evaluation of shock reactivity thresholds in females showed no difference in reactivity between vehicle and OT or OT-A conditions. Together, these results are indicative of sex differences in OT signaling mediating risky decision making in the RDT. Ongoing experiments are investigating the mechanisms that regulate these effects.

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Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

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Program #/Poster #: PSTR235.04/UU13

Topic: H.03. Decision Making

Support: DSTLX 1000128890

Title: Eeg decoding of confidence in multistep decision making

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Abstract: Many decisions are complex multistep processes involving updating internal representations from a stream of changing information. While confidence in decision making has been shown to reflect a marker of this internal updating process, the P300, this study explores how this marker of context updating responds to changes in evidence in multistep decision making. Using a probabilistic reasoning task in which participants can sample information to improve their decision-making, EEG data was collected and analysed for changes in context updating relating to the changing available evidence. 22 participants (13 female, 9 male, age: mean = 27.5, standard deviation = 6.97) took part in an EEG study. Data was collected with a 64-channel BioSemi ActiveView Two set. Behavioural results showed that confidence tended to closely track evidence, and that the confidence calibration received a boost after a decision was made. Using linear spatial integration, it was possible to decode the neural response to fluctuating evidence strength and extract a P300-like component. We found that the P300-like component response to evidence was larger at the point a decision was made. It was also larger in response to larger changes in evidence. The context in which the change in evidence is observed also influences the P300-like component. In more ambiguous contexts without sufficient information to choose between two options, larger changes in evidence correspond to larger neural activity. However, in contexts with clear evidence for one choice over the other, larger changes in evidence elicit less activity than smaller changes in evidence, possibly due to

the reduced information content in the evidence. This work extends the work in the decoding of confidence and shows that it is possible to use decoding to extract a neural response to available evidence that traces fluctuating confidence during multistep decision-making.

Disclosures: J. Tan: None. **N. Yeung:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

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Title: Prefrontal-limbic circuits during approach-avoidance conflict in a Pacman game

Authors: *B. STAVELAND[1], O. KIM-MCMANUS[4], J. T. WILLIE[5], P. BRUNNER[6], M. DASTJERDI[7], J. J. LIN[8], M. HSU[2], R. T. KNIGHT[3];
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Abstract: Choosing to approach or avoid actions or stimuli that represent both rewarding and aversive outcomes is characteristic of everyday decisions and frequently induces anxiety. In humans, excessive avoidance is a feature of generalized anxiety disorder, PTSD, and agoraphobia. Research in rodents and primates has implicated the hippocampus (HC) in approach-avoidance conflict (AAC), while human fMRI studies have characterized frontal cortex activity elicited by AAC, including PFC structures such as the orbitofrontal cortex (OFC), lateral prefrontal cortex (lPFC), and anterior cingulate (AC). Despite this, BOLD activity in the PFC and HC in humans is challenging to relate to circuit dynamic accounts, given the spatiotemporal resolution. Here we tested 7 adult presurgical epilepsy patients on a novel, continuous-time approach-avoidance conflict decision-making game inspired by the arcade game Pacman (240 trials). The decision to move towards the center of the corridor was associated with potential gains (eating “dots”, resulting in points) and potential losses (ghost attack, resulting in loss of the Pacman life). We focused on correlations in theta band power (3-8Hz) between the hippocampus

and prefrontal regions, specifically the AC and lPFC, and hypothesized we would see strong connectivity between the hippocampus and AC, but not between the hippocampus and lPFC. Within regions, we first found that lPFC theta power transiently increases at the beginning and end of each trial but quickly drops back to baseline (150-950 milliseconds post-onset; 950 to 300 milliseconds pre-onset, $p < .05$, corrected), while theta power in the hippocampus (300-2500 milliseconds post-onset, $p < .05$, corrected) and anterior cingulate (350-1450 milliseconds post-onset, $p < .05$, corrected) rises within the first second of the task and maintains its elevation for approximately two seconds. Next, power envelopes between pairs of electrodes were correlated trial-by-trial during approach/avoidance behavior and compared to a trial-shifted null distribution and corrected for the number of pairs. We found the majority of pairs of electrodes in the hippocampus and AC to be correlated during approach/avoidance behavior (7/7 patients; 173/206 electrodes; $p < 0.05$, corrected). However, there were also many correlated electrodes within the hippocampus and lPFC (5/7 patients; 215/308 electrodes) though the average strength of the correlation with the hippocampus was stronger with the AC (Wilcoxon nonparametric test, $p = 0.011$). The results provide evidence for distributed cortico-limbic system interactions underlying approach-avoidance conflict.

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Poster

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Topic: H.03. Decision Making

Support: NIMH IRP

Title: Rats risk more when dealing with decisions framed as losses: The reflection effect

Authors: *M. H. LOWRIE[1], V. VISOCKIS[1], S. P. BRADLEY[2], Y. CHUDASAMA[1,2]; [1]Section on Behavioral Neurosci., [2]Rodent Behavioral Core, NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD

Abstract: It is well known that humans view choice options not in absolute terms but rather in relation to salient reference points. For example, when considering options for combating a deadly disease, people are more risk-accepting when the options are presented in terms of losses (i.e., lives lost) but more risk-averse when the outcome is considered a gain (i.e., lives saved; Tversky and Kahneman 1981). This pattern of risk-taking behavior, known as the “reflection effect,” is defined by a context-dependent reversal of risk preference. The reflection effect is also present in nonhuman primates suggesting that the bias toward framing may be conserved across species. Here, we explore whether rats exhibit a similar reflection effect. We used an operant touchscreen platform to implement the rat Reflection Effect Task. In this task, a pair of computer

graphic stimuli were presented as options to the left and right side of the touchscreen. The rat made a nose-poke touch response to indicate its choice. In each trial, rats were presented with a choice between a safe and risky option for which the payoff could be framed as a gain (reward bonus) or a loss (reward reduction). Two blocks of trials were implemented. In the first block (Gain), the rat was presented with 60 choices between a certain 50µl gain of liquid sucrose, and a risky gain of 25% chance of a large (170µl) reward or a 75% chance of a small (10µl) reward. In the second block (Loss), there was a certain loss option of 3.5s wait-time to receive 50ul of liquid sucrose, or a risky loss option of 0s to wait (25%) or a 5s wait (75%) to receive 50µl of liquid sucrose. In both blocks, the certain and risky options were of equivalent expected value. Rats that display a reflection effect will prefer the safe option in the gain block and risky option in the loss block. Our preliminary data showed that rats (n=8) did indeed choose the risky option more often during the Loss Block (46% ± 2%) relative to the Gain Block (37% ± 3%), consistent with the reflection effect. In the Loss Block, rats omitted more trials and exhibited longer trial initiation latencies, suggesting their motivation may have been affected by the choice context. Ongoing studies using fiber photometry to record from cells within the hippocampus and prefrontal cortex may reveal potentially distinct neural dynamics in those structures that might orchestrate risky decision-making behavior observed in psychiatric and psychopathological disorders in which patients engage in behaviors that are disruptive to their normal lifestyle.

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Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

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Topic: H.03. Decision Making

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Title: Optogenetic inactivation reveals temporally-distinct contributions of ventral hippocampus to risky decision making in rats

Authors: *M. FARAJI[1], O. A. VIERA[2], L. CAO[2], B. SETLOW[1,3], J. L. BIZON[2,3];
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Abstract: The hippocampus has been extremely well studied in contexts related to memory and learning. In contrast, the contributions of this structure to cost-benefit decision making are less well understood. While the dorsal hippocampus is known to be more involved in cognitive processes, the ventral hippocampus (vHPC) is more closely tied to affect and motivated behavior. In this work, we employed an optogenetic approach to dissect the contributions of vHPC to temporally distinct phases of a task that assesses decision making under risk of punishment, and that engages multiple elements of emotional and motivated behavior. Young

adult (6 mo.) male and female Fischer 344 x Brown Norway F1 hybrid rats were surgically implanted with guide cannulae targeting the vHPC (AP: -5, ML: ±5, DV: -6), through which pAAV-CaMKIIa-eNpHR3.0-mCherry (halorhodopsin) was injected and optic fibers were implanted. After recovery, rats were trained in standard operant chambers on a risky decision-making task, in which they made discrete choices between a small, "safe" food reward and a large "risky" food reward that was accompanied by varying probabilities of footshock punishment. Upon reaching stable task performance, a within-subjects design was used to evaluate the effects of optogenetic vHPC inactivation during discrete phases of the task. The results show that vHPC inactivation during receipt of the large reward on trials when it was punished caused rats to reduce their preference for the large, risky reward, whereas vHPC inactivation during receipt of the large reward on trials when it was unpunished did not affect choice behavior. These findings suggest that vHPC is critical for using information about the punishment to evaluate the subjective value of the large reward, such that deficits in integrating that information into the decision structure leads to overestimation of the risk associated with the large reward, and ultimately attenuated choice of this option (i.e., risk aversion). In addition, inactivation during the intertrial interval caused a similar decrease in choice of the large, risky reward, suggesting a role for vHPC in maintaining/consolidating memory for previous choices and outcomes that is necessary for assessment of the subjective value of the risky choice in future trials. Future work is needed to elucidate the circuitry through which vHPC is involved in mediating risky decision-making.

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Poster

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Title: Effects of locus coeruleus norepinephrine signaling on cortical neurons, neuronal populations, and astrocytes during reinforcement learning

Authors: *G. DRUMMOND[1], J. SHIH[1], M. CELOTTO[3], Y. OSAKO[1], Y.-N. LEOW[1], J. PARK[2], V. BRETON-PROVENCHER[4], S. PANZERI[5], M. SUR[6];
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Abstract: The locus coeruleus (LC) is a small brainstem nucleus and the primary source of the neuromodulator, norepinephrine (NE), in the brain. Through a widely divergent set of projections, LC neurons release NE throughout the brain to regulate arousal and attention. However, our recent work has also indicated two distinct functions for LC-NE in reinforcement learning. In an instrumentally conditioned go/no-go task with graded auditory stimulus detection, phasic LC-NE activity is critical for task execution under high uncertainty conditions, and for optimizing task performance after surprising outcomes. It is unknown how LC-NE activity alters neuronal responses and population dynamics in target regions to facilitate task execution and to promote reinforcement encoding. Here, we explored the effects of LC-NE on cortical neurons and astrocytes in prefrontal cortex (PFC) and motor cortex (MC) during our reinforcement learning task. We used neuropixels probes to generate high density recordings of single units in PFC and MC in mice performing the task while silencing LC-NE neurons on a subset of trials. Using mutual information analyses, we find that LC-NE silencing reduces the amount of information about the stimulus and the choice in individual cortical neurons. Using population analyses, we find that LC-NE release following a false alarm changes population dynamics to improve discrimination between go and no-go tones on the next trial. Phasic LC-NE activity lasts only milliseconds, while the effect of a trial outcome on the next trial requires that the signal persists for several seconds. LC-NE has been shown to act on astrocytes, the major glial cell of the brain, which can integrate and alter neuronal signals over diverse timescales, presenting a means by which the information from phasic LC-NE signals can be sustained through the next trial. Thus, we used 2-photon calcium imaging to record astrocyte and neuron calcium dynamics in the cortex during the task. We find that astrocytes show reliable and long-lasting increases in calcium following a false alarm. When we manipulate astrocyte calcium, we no longer see an improvement in performance following a false alarm, indicating that astrocyte dynamics are important for this behavioral outcome. Finally, by silencing LC-NE neuronal activity while imaging astrocyte calcium, we are exploring the role of NE action on astrocytes in mediating this sustained increase in astrocyte calcium and its impact on behavior. Together, our results indicate that phasic LC-NE during pre-execution and post-reinforcement epochs alter neuronal population dynamics and astrocyte calcium to facilitate task execution and optimization.

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Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

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Program #/Poster #: PSTR235.09/UU18

Topic: H.03. Decision Making

Title: Improving the correlation between survey and task-based measures of preferences.

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Abstract: Preferences have been studied by both psychologists and economists, although using disparate methods. While both surveys and incentive-compatible tasks aim to capture the same constructs, recent studies have shown only a weak correlation between these methods. Two explanations for the low correlation have been proposed - that the two methods measure different mental processes or the low test-retest reliability of behavioral tasks obscures an underlying correlation. To determine which is correct for risk, ambiguity, and delay discounting preferences, we developed a new experimental protocol to enhance test-retest reliability. Using this approach, we reexamined the correlation between survey and task measures of these important preferences. Our major improvements were the use of multiple incentive-compatible measurements in a longitudinal experiment with a well-stratified sample. We used a standard lottery task to study risk and ambiguity preferences and a standard delay discounting task. We compared these to the standard Domain-Specific Risk-Taking Scale (DOSPERT), the standard Intolerance of Uncertainty Scale (IUS), and the standard Urgency-Premeditation-Perseverance-Sensation Seeking (UPPS) impulsivity scale. We conclude that the DOSPERT does measure the same underlying mechanism as behavioral risk and ambiguity tasks, at least in young adults. However, we find no evidence that the mechanisms accessed by the standard Intolerance of Uncertainty (IUS) are connected to those accessed by incentive-compatible lottery choice tasks. Examining time-preferences, we found that the standard UPPS scale for measuring impulsivity was completely uncorrelated with intertemporal impulsivity as measured with a standard discounting task. While surprising, this suggests that financial impulsivity, measured with a discounting task, and behavioral impulsivity, measured with the UPPS, reflect fundamentally different constructs.

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Poster

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Title: Multidimensional risk profiles of impulsive decision-making for substance use disorders: similarities and differences between stimulants and opiates

Authors: *H. KIM[1], E. PSEDERSKA[3], J. VASSILEVA[4], W.-Y. AHN[1,2];
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Abstract: Impulsive decision-making is a multidimensional risk factor associated with substance use disorders (SUD). However, there is still limited understanding of similarities and differences in decision-making across various substances, such as stimulants and opiates, and whether it can serve as potential endophenotype for SUD. To address these questions, we constructed a multidimensional risk profile of impulsive decision-making by employing several decision-making tasks. We compared these profiles between different groups using a sibling comparison design. The decision-making tasks included the Iowa Gambling Task (IGT), Cambridge Gambling Task (CGT), Delayed Discounting Task (DDT), and Balloon Analog Risk Task (BART). Through hierarchical Bayesian analysis, we computationally modeled the tasks and identified latent decision-making parameters that constituted the multidimensional profiles. Participants included individuals with SUD (heroin: N=157, amphetamine: N=140), healthy controls (N=179), unaffected siblings of heroin (N=67), and amphetamine (N=52). Individuals with SUD were dependent on a single substance and had maintained protracted abstinence. After determining the multidimensional risk profiles, we calculated Euclidean distances between the groups to assess similarities and differences. Our findings revealed shared risk factors across the heroin and amphetamine groups, characterized by deterministic betting choices on the CGT and quick forgetting on the IGT. These factors were also observed in the unaffected siblings. Additionally, unique risk factors were identified in the heroin group, including higher delay aversion on the CGT, lower sensitivity and learning rates for losses on both the CGT and the IGT, and a higher preference for recently chosen options on the IGT. Among these factors, lower sensitivity and learning rates for losses were also observed in the unaffected siblings. Meanwhile, the amphetamine group exhibited a unique risk factor of selecting options more randomly on the DDT. Based on the Euclidean distances, the heroin and amphetamine groups displayed the closest proximity, followed by the siblings of heroin users and siblings of amphetamine users. The healthy control group exhibited the greatest distance from the other groups, with the order being siblings of heroin users, siblings of amphetamine users, heroin users, and amphetamine users. In conclusion, our results suggest that multidimensional impulsive decision-making risk profiles could be potential endophenotypes for stimulant and opioid use disorders. These findings also highlight shared and unique characteristics among these substances.

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Location: WCC Halls A-C

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Program #/Poster #: PSTR235.11/UU20

Topic: H.03. Decision Making

Title: Identifying the influence of risk preferences on incentivized reports of perceptual confidence.

Authors: *C. N. RAYMOND[1], R. PIZARRO[1], A. RENFRO[1], C. PLATE[1], Z. BOUNDY-SINGER[2], C. M. ZIEMBA[2], S. LOPEZ-GUZMAN[1];
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Abstract: Objective: Perceptual decisions are accompanied by a sense of confidence. In the laboratory, perceptual confidence is often studied by simply asking human subjects to report their decision. In natural contexts, decisions generally have meaningful consequences and confidence can provide an important guide for our choices and actions. Studies of confidence in animal subjects require incentivization to elicit confidence —for example, through post-decision wagers or time costs— but incentivized confidence reports have been less prevalent in human research. Although incentivization could provide a more objective assessment of confidence, some studies indicate that incentives can bias confidence reports. Because choosing to report higher confidence to win a higher reward could be equated to playing a lottery, for which the probability of winning is the probability of a correct decision, risk preference may influence this choice. Economic risk preferences, or the willingness to incur risks, are known to vary widely across people and may have a differential effect on confidence across individuals. Here, we quantified both perceptual confidence reports and risk preferences, to investigate whether individual risk preferences can account for incentive-induced confidence bias. Methods: Online participants (N = 149), enrolled through CloudResearch, completed two computerized decision-making tasks: an orientation discrimination and confidence reporting task and a risk preference task. The orientation discrimination task was a 4-alternative forced choice task with clockwise/counterclockwise and high/low confidence options, with high- and low-incentive blocks in which high confidence/correct trials were differentially rewarded. The risk preference task was a 2-alternative forced choice task with lotteries versus certain gain options. A random trial from either task was paid out to achieve incentive compatibility. Behavior from both tasks was evaluated non-parametrically and by applying computational models of confidence and economic utility. Results and Conclusion: Preliminary results show a significant increase in confidence reports in the high-incentive blocks compared to the low-incentive blocks, consistent with prior research. Additionally, risk tolerance is associated with high-incentivized confidence. Our study has potential implications for the interpretation of incentivized confidence and metacognitive measures at the individual level. Dissociating risk preferences from confidence may improve our ability to characterize behavior in clinical populations that may vary differentially in these two dimensions.

Disclosures: C.N. Raymond: None. **R. Pizarro:** None. **A. Renfro:** None. **C. Plate:** None. **Z. Boundy-Singer:** None. **C.M. Ziemba:** None. **S. Lopez-Guzman:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.12/UU21

Topic: H.03. Decision Making

Title: Uncovering cognitive processes underlying risky decision-making in a rat gambling task

Authors: *M. KWAK[1], W. KIM[2], J. KANG[3], J.-H. KIM[2];
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Abstract: Excessive risk-seeking behavior is a major characteristic of gambling disorder. The Iowa gambling task (IGT) has been widely used to study risky decision-making in humans by simulating complex real-world decision-making. Similarly, a rat version of the gambling task (rGT) allowed researchers to identify 'risky decision-makers' in rats, thereby expanding the scope of neurobiological study on decision-making. While computational modeling based on reinforcement learning has been widely applied to gain a deeper understanding of how individuals process multiple rewards and punishments in the context of the IGT, its application to the rGT remains relatively scarce and limited. To address this gap, we applied three different reinforcement models that used in IGT studies to analyze 20 days of trial-by-trial rGT sessions. We found that value-plus-perseverance (VPP) model best fits rGT data. By examining the posterior estimates of the model parameters, we observed notably distinct patterns in the risk-seeking rats compared to the risk-averse group. The risk-seeking rats exhibited parameter values indicative of a greater reliance on expected value and increased exploration during the decision-making process. These findings can provide insight into the precise cognitive mechanisms underlying risky decision-making and contribute to our understanding of the applicability of computational models in the rGT paradigm.

Disclosures: **M. Kwak:** None. **W. Kim:** None. **J. Kang:** None. **J. Kim:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.13/UU22

Topic: H.03. Decision Making

Support: NIH R01 EB026945
NSF GRFP DGE1845298

Title: Suboptimal human decision-making can reflect an efficient information bottleneck

Authors: *J. PARKER[1], A. FILIPOWICZ[2], V. BALASUBRAMANIAN[3], J. KABLE[4], J. I. GOLD[5];
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CA; [3]Dept. of Physics, [4]Dept. of Psychology, [5]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Decision-making behavior often varies widely across both tasks and individuals, in many cases differing substantially from the behavior expected from optimal (i.e., reward-maximizing) strategies. One possible reason for this variability is that people try to use optimal strategies but are subject to limitations in resources such as time, effort, and computational costs. This idea, known formally as bounded rationality, has been the subject of study for several decades. However, the specific resources that are limited, the effect of such limitations, and how these resources are flexibly allocated under different conditions remain unclear. We propose that one important form of resource limitation is the amount of information the brain is able or willing to use to make decisions. In principle, the effect of limiting, or compressing, the available information can be quantified via a model-agnostic framework known as the information bottleneck (IB), which defines an upper bound on choice accuracy that increases asymptotically as compression decreases. Here we used the IB to assess the theoretical and empirical relationships between accuracy and compression for simple forms of decision-making.
We considered simple inference tasks that require people to make inferences about future events based on sequences of past observations. We first used both analytic approaches and simulations to show that for these tasks, maximizing "information efficiency" - that is, maximizing accuracy with respect to any degree of compression of the information contained in the past observations about future events - is equivalent to corrupting the optimal inference process with logistic noise. Notably, this equivalence implies that optimal compression necessarily leads to noisy, but otherwise unbiased, decision-making behavior. In contrast, non-optimal compression induces biases that correspond to strategies that fall below the IB bound. We then tested 67 human observers (25 female; Age mean 28.21, SD 8.04) on these tasks. We found that most people failed to maximize accuracy but used strategies that fell near the IB bound. We used model fitting and comparisons to confirm that these information-efficient strategies tended to correspond to use of an optimal strategy corrupted by logistic noise. These results: 1) highlight the possibility that information compression leads to a fundamental type of choice variability often attributed to noise; and 2) provide a new, principled way to assess the bounded rationality of decision-making behavior.

Disclosures: J. Parker: None. **A. Filipowicz:** None. **V. Balasubramanian:** None. **J. Kable:** None. **J.I. Gold:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.14/UU23

Topic: H.03. Decision Making

Support: Max Planck Florida Institute for Neuroscience
Max Planck Free Floater Program

NIH New Innovator Award (NINDS and OD; 1DP2NS132108)
Searle Scholars Program
Klingenstein-Simons Fellowship
McKnight Scholar Award

Title: Neural dynamics in mouse frontal cortex underlying temporal discounting task

Authors: *Y. JUNG, H. J. INAGAKI;
MPFI, Jupiter, FL

Abstract: Value-based decision-making is essential for animal survival. Previous studies identified neural correlates of value across brain areas, primarily in the prefrontal cortex, yet how these value representations bias action selection remains elusive. To bridge this knowledge gap, we have established a temporal discounting task in head-restrained mice, in which water-restricted mice are presented with two lick ports. Licking one lick port leads to an immediate but small water reward, whereas licking the other port leads to delayed but large reward. Contingency switches between two ports in a block of trials. When there is no delay, mice lick the port associated with a larger reward, and the preference changes as a function of the ratio of delivered water amount (1:10, 1:4, 1:2, and 1:1). In contrast, when there is a delay before the large reward (0.75 - 4 seconds), preference for the large reward decreases. Thus, mice evaluate reward size and cost of delay to decide lick direction, consistent with the temporal discount observed in primates. Anterior lateral motor cortex (ALM) plays a key role in motor planning and execution of lick. We bilaterally silenced ALM activity during the task (Vgat-ChR2 mice, n =3), which reduced the percentage of choice to the large reward from ~100% to the chance level, indicating that ALM is necessary for the action selection during the task. To study how this action selection is made, next we recorded activity of ~5,500 units simultaneously from ALM and medial prefrontal cortex (mPFC; a region implicated for value coding) in 6 male and female behaving mice (C57BL/6J). Both brain areas contained units selective for lick directions (~31% of total recorded units), and/or different reward delays (~52%; delay selectivity). While both ALM and mPFC showed strong delay selectivity (~39% and ~26% cells, respectively), ALM showed significantly more cells with direction selectivity (~34% vs. ~14% cells). We questioned whether delay selectivity in mPFC influences selectivity in ALM to determine action. To this end, we injected Cre-dependent ChrimsonR virus in mPFC of Vgat-Cre mice (4 animals). Red-light illumination to the surface of the brain in non-behaving mice activated local inhibitory neurons and inactivated pyramidal neurons in mPFC (67% cells inactivated with 15% activated). Simultaneous recording showed that some ALM neurons were indirectly modulated by this manipulation (28% activated and 22% inhibited). We will leverage this method to manipulate mPFC activity during the temporal-discount task to test its effect on the behavior and ALM activity, which may identify key multiregional interactions underlying value-based decision.

Disclosures: Y. Jung: None. **H.J. Inagaki:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.15/Web Only

Topic: H.03. Decision Making

Title: Effect of acute restraint stress on behaviour in a rat gambling task

Authors: *L. CALDERHEAD[1], C. A. WINSTANLEY[2];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Univ. British Columbia, Univ. British Columbia, Vancouver, BC, Canada

Abstract: Stressful experiences are known to effect cognitive processes. Previous research has shown that administration of stress related hormones can alter risk-based decision making in rats. However, previous studies have relied on injections of stress hormones rather than evoking a full stress response. A rat gambling task (rGT) has previously been developed, that allows animals to choose one of four options that differ in the magnitude and frequency of sugar pellet rewards and the length of time-out punishments periods. Risks and rewards are structured so that risky high-reward/high-punishment options are suboptimal compared to safer low-reward/low-punishment options over multiple trials. Here, we utilize a single exposure to one hour restraint stress to induce stress in female Long-Evans rats that are well-trained in the rGT. We found that exposing animals to restraint stress before testing on the rGT altered risk preference and reduced overall performance in the task, but animals returned to baseline performance the day after stress exposure. These findings indicate that acute stressful experiences can alter risk-based decision making temporarily.

Disclosures: L. Calderhead: None. **C.A. Winstanley:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.16/UU24

Topic: H.03. Decision Making

Title: Timing of sensory reward-paired cues modulates their risk-promoting effects

Authors: *M. C. POTTS[1], H. BRODIE[2], E. FLYNN[3], B. RUSSELL[4], P. KROM[5], J. J. BARTON[2], C. A. WINSTANLEY[6], M. V. CHERKASOVA[5];
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Abstract: Reward cues can powerfully influence behavior. We have previously demonstrated that audiovisual reward-paired cues promote riskier decision-making in gambling tasks both in rodents and in humans (Barrus et al, 2016; Cherkasova et al, 2018). However, the mechanisms

by which cues encourage riskier choice remain unclear. We investigated whether the timing of the cues in the task impacts their effects on decision making. Human participants (n=80) were randomly assigned to perform 2 versions of the binary choice Vancouver Gambling Task (VGT), in which the riskier option features a lower probability of winning a larger reward relative to the alternative safer option, and gambles differ in their risk-worthiness based on the relative expected values of the two options. The 2 versions of the VGT differed in cue-timing: 1) audiovisual reward cues accompanied the winning feedback (feedback-concurrent cues), or 2) visual cues (gold coins) were used to represent prospective rewards at the time of choice (choice-concurrent cues). Both versions were compared to a version that included no reward-predictive cues at either time point in a within-subjects design. Both feedback-concurrent cues and choice-concurrent cues encouraged risker choice. However, feedback-concurrent cues promoted irrationally risky choices, i.e. choices that resulted in selecting the option with both a lower expected value and a lower chance of obtaining it. Choice-concurrent cues, on the other hand, encouraged riskier choices across all types of gambles, regardless of the expected values of the two options. These findings suggest that cues accompanying reward delivery preferentially encourage risk taking that is unwarranted and maladaptive. This has implications for understanding how addiction-related reward cues help maintain addictive behavior and encourage relapse. In particular, in the context of electronic gambling games, the prominence of salient audiovisual cues at the time of winnings outcomes may facilitate maladaptive and irrational risk taking.

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Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.17/UU25

Topic: H.03. Decision Making

Support: CIHR PJT-162312 - C. A. Winstanley
NSERC CGS M - C. S. Chernoff

Title: The role of the noradrenergic locus coeruleus in gambling-like behaviour: Interactions with biological sex and baseline impulsivity.

Authors: *C. S. CHERNOFF[1,3], D. K. AVRAMIDIS[2,4], S. RAMAIAH[2,5], T. J. HYNES[3], A. KHOSHNEVIS[2], K. M. HRELJA[1], C. A. WINSTANLEY[6];
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Abstract: The stimulating sounds and lights of modern casinos might be more insidious than once thought. When audiovisual cues are paired with wins on rodent and human gambling tasks, a greater proportion of subjects develop a preference for the unfavorable high-risk high-reward options. Noradrenaline (NA) neurons in the locus coeruleus (LC) fire phasically to salient cues, and the balance between phasic and tonic LC-NA release can promote shifts between exploitative and exploratory task strategies. Our previous pharmacological experiments show that noradrenaline significantly influences decision making and impulsivity on the cued rat gambling task (crGT), a paradigm during which rats sample between probabilistic outcomes to maximize sugar pellet wins and minimize time-out punishments. However, it is uncertain how LC firing itself may facilitate the development of maladaptive risk preference on the crGT. I therefore sought to chemogenetically alter the activity of NAergic LC cells while rats learn the crGT using cell-type specific manipulations. I infused a Cre-dependent viral vector to express a Gi-coupled designer receptor (DREADD) in the TH+ LC neurons of male and female TH:Cre rats. I then administered the DREADD ligand, CNO (1 mg/kg ip), prior to each crGT acquisition session to selectively dampen NAergic LC cell activity as the rats learn the task. Rats were categorized as high or low impulsivity (HI/LI) based on premature responses made during CNO-free sessions, as past work suggests unique effects of NAergic manipulations depending on levels of baseline impulsivity. LC inhibition improved decision making selectively in LI rats. Inhibiting NAergic LC neurons also transiently increased motor impulsivity of LI rats in a sex-dependent manner. Our results reveal that the LC is a critical mediator of gambling-like behaviour in rats. Further, these data suggest that LC noradrenaline differentially regulates decision making and impulse control depending on biological sex and baseline impulsivity, and that the LC may modulate behaviour differently in individuals that are more vs less impulsive at baseline.

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Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

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Program #/Poster #: PSTR235.18/UU26

Topic: H.03. Decision Making

Support: CIHR Grant F17-03934

Title: Risk-promoting effects of reward-paired cues in human sign- and goal-trackers with problem gambling behaviour

Authors: *B. RUSSELL[1], L. CLARK[2], J. STOESSL[1], J. J. BARTON[1], C. WINSTANLEY[3], M. V. CHERKASOVA[4];
[2]UBC, [1]Univ. of British Columbia, Vancouver, BC, Canada; [3]Univ. British Columbia, Vancouver, BC, Canada; [4]Psychology, West Virginia Univ., Morgantown, WV

Abstract: The incentive sensitization theory of addiction proposes that reward-related stimuli acquire a powerful motivational influence on behaviour through repeated associations with rewards. Animal research suggests that there is trait-like individual variation in the degree of incentive salience attribution to reward-predictive cues, defined phenotypically as sign-tracking (high incentive salience attribution) and goal-tracking (low incentive salience attribution). While these phenotypes have been linked to addiction-related behaviour in rodents, there are few studies investigating their translational validity. Here, we examined whether sign- and goal-tracking modulates the effects of reward-paired cues on cost-benefit decision-making in human volunteers from the community with problem gambling behaviour (n=60) and healthy age-matched controls (n=60). Individuals in the problem gambling group had a score within the moderate range on the Problem Gambling Severity Index (PGSI), whereas those within the control group had scores within the low to non-risk range. All participants also completed the Structured Clinical Interview for DSM-5 (SCID-5), as well as a demographic questionnaire. Sign-tracking was measured in a Pavlovian conditioning paradigm as the amount of eye gaze fixation on the reward-predictive cue versus the location of impending reward delivery. Participants ages 19 to 65 performed two versions of the two-choice lottery Vancouver Gambling Task in which rewards were either accompanied (cued VGT) or unaccompanied (uncued VGT) by money images and casino jingles. Results from the SCID-5 indicated that 85% of participants within the problem gambling group met DSM-5 criteria for one or more psychological disorder over their lifetime; gambling disorder (57%) and substance use disorders (55%) were the most prevalent among this group. In comparison, 17% of participants in the control group met criteria for a psychological disorder across their lifetime, with past substance use disorders being the most common among this group (13%). Based on previous studies, we hypothesize that cues will promote riskier choice in both groups, and that goal-tracking in the control group will be associated with greater risk-promoting effects of cues; however, sign-tracking in the problem gambling group will be associated with greater risk-promoting effects of cues. These findings would support the notion of sign-trackers being preferentially susceptible to the influence of reward cues on behavior and suggest cues exert their effect on decision making through incentive salience.

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Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.19/UU27

Topic: H.03. Decision Making

Support: CIHR PJT 162312

Title: Dissociable effects of low versus high doses of psilocybin in gambling-like behaviour in male and female rats

Authors: *D. K. AVRAMIDIS[1], C. S. CHERNOFF[2], S. RAMAIAH[1], C. A. WINSTANLEY[1];
[1]Psychology, [2]Neurosci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The use of sub-hallucinogenic doses of psilocybin, colloquially known as microdosing, has gained popularity for its potential mood-enhancing and cognitive benefits. However, the bulk of psilocybin research focuses on hallucinogenic doses ("macrodoses") whereby the lasting benefits of the compound on affective and depressive-like behaviours are thought to be partially underpinned by increased cognitive flexibility. Previous research from our lab found that antagonism at the serotonin 2C receptor decreased risk taking in the cued rat gambling task (crGT), an assay of cost-benefit decision making in rats. In this task, rats choose between small, more probable rewards and larger, less likely payouts. As psilocybin is a potent 5-HT2A agonist, and that 5-HT2A and 5-HT2C receptors have functionally opposing effects, we predicted psilocybin should also decrease risky decision making on the crGT. We tested this by administering various microdoses of psilocybin (0, 0.003, 0.01, and 0.03 mg/kg), in a Latin square design, to male and female Long-Evans rats before the crGT. In a subsequent phase, the same rats were given one macrodose of psilocybin (0.3 mg/kg i.p.) prior to crGT performance. Post-macrodose drug-free baseline crGT behaviour was assessed in a subset of rats. Microdoses of psilocybin acutely improved decision making score, selectively in female rats, along a biphasic dose-response curve. An acute macrodose of psilocybin enhanced decision making score in risk-preferring rats of both sexes. These effects were driven by increased choice of advantageous options and decreased preference for disadvantageous risky options. The macrodose also decreased motor impulsive premature responding, with this effect persisting throughout the five post-macrodose baseline sessions. Collectively, our data reveal the potential benefits of micro- and macro-doses of the serotonergic psychedelic psilocybin on risk taking and impulse control, and suggest this effect may be more potent in females.

Disclosures: D.K. Avramidis: None. **C.S. Chernoff:** None. **S. Ramaiah:** None. **C.A. Winstanley:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.20/UU28

Topic: H.03. Decision Making

Title: Linking Neural Activity to Biased Action Selection: Insights from Mouse Study

Authors: *Y. PAN, X. XIAO, Y. DENG;
Chinese Acad. of Sci., Shanghai, China

Abstract: In the pursuit of human-level performance in AI research, identifying relevant inductive biases is crucial. Many theories suggest that animals prefer default options during decision-making under uncertainty, such as "loss aversion." The ventral striatum (VS) has been

implicated in selecting default choices, but a direct causal link between VS neural activity and default bias is missing. Here, we trained mice to perform a two-alternative forced choice task with varying tone frequencies, where they exhibited a preference for one side. Mice showed higher selection probability and accuracy for the preferred side, with their psychometric subjective error biased towards the non-preferred side. Higher neural activity of indirect striatal projection neurons (iSPNs) in the VS during action selection correlated with mice's choice for the biased side. Optogenetic activation and inhibition of iSPNs in the VS on randomly chosen trials resulted in an increase or decrease of the biased side, respectively, without inducing any motor programs. Our findings provide a direct link between neural activity of VS iSPNs and biased action selection.

Disclosures: **Y. Pan:** None. **X. Xiao:** None. **Y. Deng:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.21/VV1

Topic: H.03. Decision Making

Support: CRCNS RO1DA053014

Title: Modulation of Decision Policy by Environmental Uncertainty & Striatal Stimulation

Authors: *J. K. BADYNA, E. A. YTTRI;
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Abstract: How does the brain control decision policy to maximize rewards across dynamic environments? The world is always changing, and individuals must take environmental constraints into account when selecting decision strategies. One way in which the environment can change is through *conflict* - how reliably a reward signal indicates an optimal decision. Flexible decision-making and adaptation to environmental changes are crucial for survival. Optimal decisions are not always explicitly indicated, and internal variables, such as decision bias, may influence decision-making. To study how the striatum contributes to flexible decision-making, we ran mice on a two-armed bandit y-maze task, in which they show an innate bias to turn left or right, across varying conflict levels. Using regression analysis and fitting a q-learning model, we analyzed the decision behavior to study how environmental constraints and striatal activity alter decision policy. In high conflict (low certainty) environments, the mice tended to resort to their bias to guide decision-making, whereas in no conflict (high certainty) environments, the mice tended to utilize outcome information to guide decision-making. Each decision strategy optimized rewards for that specific environment without affecting cognitive energy cost. We manipulated activity of either direct (dSPN) or indirect (iSPN) dorsomedial striatal neurons via optogenetic stimulation to determine how increased activity drives changes in decision strategy (using cohorts of both male and female d1-cre or a2a-cre mice, respectively).

dSPN stimulation promoted a responsive (driven by reward signal), optimistic (decreased weights to unrewarded actions), unbiased decision policy that increased cognitive energy cost without affecting task performance. dSPN stimulation also decreased variability in decision time (DT) and slightly increased deliberation prior to turn execution. iSPN stimulation induced a random (near-zero weight to both outcome and choice regressors), pessimistic (decreased weights to rewarded actions), unbiased exploration policy that slowed down decision-making. iSPN stimulation also increased DT variability while slowing down both DT and kinematics throughout the turn execution. This work reveals that, just as changes in the environment drive changes in decision policy, increases in dSPN/iSPN activity cause decision policy shifts as well. Modulating the balance between dSPN and iSPN activity likely drives changes in decision strategy between responsive/optimistic and explorative/pessimistic, respectively, when confronted with environments where each policy would prove optimal.

Disclosures: **J.K. Badyna:** None. **E.A. Yttri:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.22/VV2

Topic: H.03. Decision Making

Support: NIH R01 EY032999
NSF Career #2146369
NIH K99EY032102

Title: Recipes for improving perceptual confidence with practice

Authors: *Z. BOUNDY-SINGER, C. ZIEMBA, R. GORIS;
Univ. of Texas at Austin, Austin, TX

Abstract: Observers are aware of the fallibility of perception. When we experience a high degree of confidence in a perceptual interpretation, that interpretation is more likely to be correct. This meta-cognitive ability to judge the reliability of perceptual events is imperfect. It is well established that practicing difficult perceptual tasks can improve perceptual capabilities (i.e., perceptual learning). This raises the question of whether meta-cognitive ability can similarly improve. Previous investigations yielded inconclusive results, potentially because the confidence judgments were not difficult enough (i.e., the baseline level of meta-cognitive ability typically was high, leaving little room for improvement). To test this idea, we leveraged insights offered by CASANDRE, a process model of perceptual confidence. In this model, confidence reflects a subject's noisy estimate of the reliability of their perceptual decisions. The quality of this estimate is limited by the subject's uncertainty about the variable that informs their decision ('meta-uncertainty'). Thus, the difficulty of a confidence judgment is determined by the ease with which a subject can estimate their perceptual uncertainty. This can be manipulated

experimentally by increasing the number of levels of stimulus reliability within a single experiment or by making the stimuli stochastic. We conducted three psychophysical experiments in which 26 subjects judged the orientation of ambiguous stimuli and additionally reported their confidence in these decisions. Subjects completed 3,000 trials over two sessions. The first experiment involved two levels of stimulus reliability and no stimulus stochasticity. We fit CASANDRE to each subject's data and studied the temporal evolution of meta-uncertainty. Some subjects exhibited meta-cognitive learning, but these effects were modest and inconsistent. The second experiment involved six levels of stimulus reliability. As expected, this resulted in a higher baseline level of meta-uncertainty and in more prominent meta-cognitive learning. The third experiment involved stochastic stimuli (two levels of stimulus reliability). This similarly yielded higher baseline levels of meta-uncertainty and prominent meta-cognitive learning. To test the generalizability of these findings, we conducted a fourth experiment in which 8 subjects judged the texture of stochastic visual stimuli and we obtained similar results. Together, these results demonstrate that meta-cognitive ability can improve with practice, provided that the confidence judgments are difficult enough.

Disclosures: **Z. Boundy-Singer:** None. **C. Ziemba:** None. **R. Goris:** None.

Poster

PSTR235. Neural Mechanisms of Decision-Making: Risk and Ambiguity

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR235.23/VV3

Topic: H.03. Decision Making

Title: Computational model-based analysis of spatial navigation strategies under stress and uncertainty using place, distance and border cells

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Abstract: Decision-making occurs during navigation and learning. It is widely studied in choice behaviors, but less well understood in natural and more continuous settings, especially under stress and uncertainty. This process could be investigated in rodent spatial navigation, which has been modeled with place-cell-based models. However, traditional models usually ignored detailed trajectories or kinematics. Here we extended a place cell-based reinforcement learning model to include detailed kinematics and used it to investigate the role of motivational stress in Morris Water Maze. We performed experiments with two strains of mice learning two versions of the task under different water temperatures: the task with a fixed platform location and the task where platform location varied randomly between two positions. Using computational modeling and parameter estimation, we were able to not only reproduce detailed mouse

behaviors but also reveal computational correlates of temperature-based behavioral differences. We then extended the model to include a wall-distance-based component, where spatial learning would be guided not just by place information but also by a cue-like signal, distance to the wall, which reproduced mouse behavior in tasks with uncertain platform positions better than place-cell-based strategies alone. Based on that, we further implemented a more biologically plausible model that uses a combination of border (boundary) cells and place cells. We finally compared performance of models with only place cells, place and distance cells, and place and border cells in different experimental conditions and genetic strains of mice and performed parameter estimation to find the best fitting model settings and parameters for each animal. Our findings provide insights into computational mechanisms underlying spatial navigation in mice and how various modulators influence it.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.01/VV4

Topic: H.05. Working Memory

Title: Working memory-related DLPFC and hippocampal recruitment studied longitudinally across puberty in typically developing children

Authors: *D. WRIGHT[1,2], S.-M. WEI[3,5], J. KIPPENHAN[3], K. M. COLE[5,3], M. D. GREGORY[3], C. A. RECTO[3], I. M. WILDER[3], M. N. GOLDBERG[3], L. K. NIEMAN[6], J. A. YANOVSKI[7], P. J. SCHMIDT[5], K. F. BERMAN[4];
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Abstract: Background: Throughout adolescence, there is a notable improvement in working memory (WM), which is the ability to retain and manipulate information. To gain insight into the underlying neural mechanisms, we used a longitudinal approach to test for concurrent changes in recruitment of key neural substrates associated with WM, specifically focusing on the dorsolateral prefrontal cortex (DLPFC) and the hippocampus. Our aim was to document typical neurodevelopmental trajectories of WM in healthy children aged 8-18 years within these brain regions.
Methods: fMRI scans were obtained approximately every 9 months from 121 healthy children for 473 total timepoints (53 girls, mean age=12.2±2.7 years; 68 boys, age=12.1±2.7 years) as

they performed a spatial 2-back WM task and a corresponding 0-back sensorimotor control task in a 3T GE scanner. Using a binary mask of the main effect of task across all participants and all visits (2-back versus 0-back, $p<0.05$), average beta-values for each participant were extracted from DLPFC and hippocampal regions of interests. For both DLPFC and hippocampal regions, mixed-effects penalized-spline modeling of age, sex, and age-by-sex interactions was performed. **Results:** Significant sex differences were identified in the developmental trajectories of both the bilateral DLPFC (left, $p=0.007$; right, $p=0.012$) and the hippocampus (left, $p=0.015$; right, $p=0.019$). Specifically, boys exhibited overall greater activation in DLPFC and less deactivation in hippocampus compared to girls. We also found an age-by-sex interaction in the left DLPFC ($p=0.014$), where the rate at which activation increased with age was higher in boys than in girls. **Conclusions:** Our findings revealed sex differences in developmental trajectories of DLPFC and hippocampal recruitment during spatial working memory across the pubertal transition. Future longitudinal investigations will address the effects of sex hormones as well as the roles of pubertal stage and tempo on WM-related brain development throughout this dynamic developmental period

Disclosures: D. Wright: None. **S. Wei:** None. **J. Kippenhan:** None. **K.M. Cole:** None. **M.D. Gregory:** None. **C.A. Recto:** None. **I.M. Wilder:** None. **M.N. Goldberg:** None. **L.K. Nieman:** None. **J.A. Yanovski:** None. **P.J. Schmidt:** None. **K.F. Berman:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.02/VV5

Topic: H.05. Working Memory

Support: 31827803

Title: Anterior cingulate-medial prefrontal circuit gates dual-task interference in working memory

Authors: *X. ZHANG[1,2], D. LI[1], Y. CHEN[1], R. HOU[1], T. CHEN[1], H. FAN[1], Z. CHEN[1], C. LI[1,2];
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Abstract: Humans and animals are significantly impaired in performing two tasks simultaneously than performing a single task, no matter how focused and attentive they are. This dual-task interference fundamentally reflects the bottleneck of the brain in processing and maintaining information online in working memory (WM). Although interference-related neuronal activities were observed in the prefrontal cortex (PFC) while performing dual tasks, circuitry mechanism that allows dual-task performance in the presence of interference remains unknown. Here we show that projections from anterior cingulate cortex (ACC) to medial prefrontal cortex (mPFC) are critical for gating distractor information in dual-task performance.

Mice were trained to perform an olfactory delayed paired-association (ODPA) and an olfactory delayed-response (ODR) task. In some trials the ODR task was inserted into the delay period of ODPA task, resulting in clear dual-task interference. The projections from ACC to mPFC are critical for dual-task performance, because the optogenetically silencing the projections during the delay period impaired behavior. Two-photon calcium imaging revealed that the activity of mPFC neurons exhibited higher coding ability for WM information but lower stability in coding the outer ODPA-task information than the ACC neurons. We then used the anterograde and retrograde tracer to identify the mPFC neurons receiving ACC inputs and the ACC neurons projecting to mPFC, respectively. Both types of neurons showed lower coding ability for distractors and higher cross-sample stability for the outer-task information comparing to the neurons not-labeled by the tracers. Only the mPFC neurons receiving ACC inputs showed significant correlation with behavioral performance. Optogenetic suppression of ACC inputs drastically reduced the coding ability for the outer-task information of mPFC neurons. Thus, ACC projections gate distraction information in mPFC and allow the brain to perform dual task simultaneously.

Disclosures: **X. Zhang:** None. **D. Li:** None. **Y. Chen:** None. **R. Hou:** None. **T. Chen:** None. **H. Fan:** None. **Z. Chen:** None. **C. Li:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

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Program #/Poster #: PSTR236.03/VV6

Topic: H.05. Working Memory

Support: NIH EY026924
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Research to Prevent Blindness

Title: Prefrontal-visual oscillatory interactions predict target selection during working memory

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Abstract: Dopaminergic activity within the Frontal Eye Field (FEF) has been shown to modulate the processing of sensory information in visual areas. Considering that FEF projections to visual areas carry the content of working memory (WM), we hypothesized that manipulating this activity can bias selection of WM targets. We specifically investigated the capacity of FEF dopaminergic manipulations to gate visual signals and guide target selection during WM.
Two monkeys performed a memory guided saccade task with distractors. In this task, the monkeys fixate on a central spot and a peripheral target appears to cue the memory location.

During the delay period, a distracting stimulus is presented at one of several locations. The monkeys must maintain the target information and ignore the distractor, to execute a saccade towards the target location at the end of the delay period. Targets and distractors were placed around the overlapping response field of simultaneously recorded FEF and V4 sites. Spiking activity and local field potentials in V4 and FEF were simultaneously recorded, before and after manipulation of dopamine D1 receptors (D1Rs) within the FEF using a local injection of 0.5-1 μL of D1R antagonist (SCH23390). The oscillatory coupling between the two areas was measured using a variety of methods, including phase-amplitude coupling, phase-phase locking, and spike-phase locking.
Before manipulating the FEF, the monkeys successfully ignored the distractors and selected the target location accurately. However, after D1R manipulation, the monkeys showed a significant bias towards selecting distractors appearing in the affected part of the visual field. We found the locking of FEF and V4 phases to be a key predictor of whether a visual stimulus is registered as a target of WM. These experiments will reveal the specific aspects of neural activity within these areas that govern the ability of visual stimuli to guide WM-dependent behavior.

Disclosures: I. Vanegas: None. **K. Clark:** None. **B. Noudoost:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

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Program #/Poster #: PSTR236.04/VV7

Topic: H.05. Working Memory

Support: ERC StG MEMCIRCUIT 758032

Title: Behavioral and neuronal signatures in delayed response and working memory tasks in freely moving mice

Authors: *V. HOHENDORF[1,2], R. C. BRINKMANN[2], S. HAJDUK[2], R. C. K. DEMANN[1], S. N. JACOB[1];
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Abstract: In order to deal with a large variety of diverse tasks, the brain's cognitive control centers such as the prefrontal cortex (PFC) facilitate representation, memorization and interpretation of sensory stimuli and orchestrate appropriate (re)actions. Many studies investigating cognitive behaviors use task designs that are heavily reduced in an attempt to understand specific components of complex behaviors and to increase reproducibility. However, even in highly controlled experiments, variability between individuals is frequently found. Further, simplified task protocols might not be representative of the complex problems we encounter in the real world. Here, we leveraged behavioral variability to distinguish between individual strategies and explore neuronal signatures that underlie different short-term memory

functions. Mice were trained to memorize spatial information in a touchscreen chamber, enabling the animals to move freely and develop distinct behavioral strategies to solve the task. Importantly, training proceeded in two steps. First, the animals were trained on a delayed response task in which they could use the location of a sample stimulus to fully predict the correct location of the subsequently presented test stimulus. Second, we introduced a working memory condition, where the animals had to memorize the sample location without being able to predict the test location and prepare an action. Mouse behavior was analyzed using DeepLabCut and Keypoint-Moseq. Individual animals could be characterized based on idiosyncratic behavioral signatures including distinctive running and turning behaviors. Faster mice with a more direct path performed better in delayed response trials, but their mean performance dropped strongly when starting on the working memory trials. In contrast, slower mice performed better in working memory trials, indicating that the two tasks have different behavioral demands and are met with distinct strategies by the animals. We hypothesized that these differences are reflected in the prefrontal neuronal representation of the memorized information. We therefore imaged large-scale mPFC activity at single-neuron resolution with GCaMP6f and Miniscopes during key training stages. Ongoing data analysis will enable us to decompose neuronal activity and possibly separate correlates of inter-individually varying behavioral strategies from conserved short-term memory coding principles, specifically, how the representation of the sample stimulus changes when behavioral demands dictate a shift from delayed responding to memorizing without the possibility of action preparation.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.05/VV8

Topic: H.05. Working Memory

Support: IBS-R002-A1

Title: Roles of prefrontal VIP neurons and dopamine in working memory

Authors: *J. BAE[1,2,3], J. YI[2,3], S. CHOE[2], M. JUNG[1];
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Abstract: VIP neurons, as the only cortical inhibitory neurons expressing dopamine D1 receptors (D1Rs), are thought to exert powerful influences on cortical circuit operations by modulating other inhibitory neuronal activity. We investigated roles of VIP neurons and their D1Rs in working memory in the medial prefrontal cortex (mPFC) in mice performing a delayed match-to-sample task. VIP neurons conveyed significant working-memory signals and their

inactivation led to a profound impairment in behavioral performance. Also, selective D1R knockdown in VIP neurons but not in pyramidal neurons impaired task learning only when working memory demand was imposed on the task. These findings highlight the critical role of VIP neurons and their D1Rs in working memory. Delay-period dopamine release was higher in contralateral than ipsilateral target trials, and optogenetic dopaminergic terminal stimulation disproportionately increased contralateral target-related delay-period activity and the animal's contralateral target choices. These results indicate dopamine's role in promoting contralateral target-related delay-period activity. Given the importance of working memory in various PFC cognitive functions, these findings shed light on neural circuit mechanisms underlying prefrontal cortical functions and dysfunctions.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

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Program #/Poster #: PSTR236.06/VV9

Topic: H.05. Working Memory

Support: Natural Science Foundation of China 2021ZD0204100

Title: Mental Sorting of Spatial Sequences in Working Memory in Macaque Prefrontal Cortex

Authors: J. CHEN[1], ***Z. TIAN**[1], C. ZHANG[1], B. MIN[2], L. WANG[1];
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Abstract: A fundamental problem in neuroscience is the elucidation of brain events underlying cognitive operations. Mental sorting refers to rearranging a series of items or events in a specific order in working memory (WM), yet its neural mechanisms remain largely unknown. Solution to the sorting problem was to involve the creation and mental reordering of population vectors representing items at each rank. Here, we investigated the neural dynamics of the sorting process using high-throughput electrophysiological recordings to record thousands of neurons (N=4193) in the prefrontal cortex of macaque monkeys memorizing and reordering a sequence of locations in the delay period. We found that items at each rank were gated and maintained in separate rank WM subspaces and then, depending on the sorting algorithm, swapped gradually between the rank WM subspaces. Crucially, the mental sorting was achieved by employing extra temporary subspaces ('temp'), where neural activities of each rank were first held in 'temp' and then transferred to the exchanged original WM subspaces. Furthermore, neural activities in these subspaces can faithfully predict monkeys' behavior in single trials. Thus, the well-coordinated neural dynamics in the prefrontal cortex underlie the cognitive operation of mental sorting in sequence WM.

Disclosures: **J. Chen:** None. **Z. Tian:** None. **C. Zhang:** None. **B. Min:** None. **L. Wang:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

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Program #/Poster #: PSTR236.07/VV10

Topic: H.05. Working Memory

Support: R01 MH121480
FBI Seed Funds

Title: Rostro-caudal gradient of structured representations in primate lateral prefrontal cortex

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Abstract: Self-organized sequential behaviors rely on the fundamentals of working memory to maintain and manipulate information in mind step-by-step. Although the cumulative information held in working memory is limited by capacity constraints, cognitive strategies such as chunking can structure the information and mitigate this constraint. Here, we investigated how neural ensembles in primate lateral prefrontal cortex (LPFC) structure information in sequential behaviors guided by different strategies. To do this, we trained two monkeys to perform a self-ordered target selection task. Monkeys were trained to saccade to eight identical targets on a screen, one at a time in any order, to collect a one-time reward from each target. This required them to use working memory to update reward-target contingency and prepare for the next target selection. From target selection patterns, we calculated the modularity index (MI) to quantify whether monkeys tended to separate the eight targets into subgroups, or chunks. Using this approach, we found that reaction times were significantly longer when transitioning between subgroups than within the subgroups. The MI was also negatively correlated with error rate, suggesting that monkeys used this chunking strategy to improve task performance. Strategy use in sequential behaviors has been linked to the cognitive functions of LPFC, so we recorded single-unit activities from four 64-channels Utah arrays implanted along with the dorsal LPFC, including dorsal principal sulcus and prearcuate gyrus, while monkeys performed the task. We assessed the hypothesis that LPFC is functionally heterogeneous, with a prominent rostro-caudal gradient corresponding to abstract-to-concrete information processing by investigating how ensemble codes and dimensionality change according to this proposed gradient. We found that ensemble activity represented target locations with more distinct structure in posterior compared to anterior LPFC, and this corresponded to the spatial locations of the targets. This indicates that concrete location information is more clearly represented by posterior LPFC neurons, which may be important for guiding saccadic eye movements. On the other hand, representations in anterior LPFC tended to merge with nearby targets, which might be evidence of abstract structure in the self-ordered task. Further work will assess how this structure relates to behaviorally-defined target chunks. Overall, our results are consistent with a rostro-caudal abstract-to-concrete

gradient of ensemble representations in LPFC that may be critical for flexible, intelligent behaviors.

Disclosures: F. Chiang: None. **E. Rich:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

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Topic: H.05. Working Memory

Support: Vassar College Internal Support to LAN
Beckman Foundation to DS
Sigma Xi to JB

Title: Chemogenetic activation of astrocytes using a GFAP promoter in prelimbic cortex and hippocampus can impair spatial working memory: A focus on activation of GFAP and sex differences

Authors: D. SERRANO[1], Z. DING[1], J. BONANNO[3], J. LIN[4], J. D'ORAZIO[2], K. U. TANG[2], E. LI[1], J. VITALE[1], F. RYAN[1], T. TSUKUDA[1], S. KUKRETI[5], ***L. A. NEWMAN**[1]; [2]Neurosci. and Behavior, [1]Vassar Col., Poughkeepsie, NY; [3]Yale Univ., New Haven, CT; [4]NIH, Bethesda, MD; [5]SUNY Binghamton, Binghamton, NY

Abstract: Astrocytes are glial cells that modify blood flow, energy production, neurotransmitter levels, ionic balance, and are integral in immune responses. All of these functions influence synaptic plasticity, a critical component of memory. However, the specific role of astrocytes in memory is still being characterized. Our study focuses on the relationship between astrocyte activity and spatial working memory. We employed a chemogenetic technique (Designer Receptors Exclusively Activated by Designer Drugs, or DREADDs) to activate astrocytes in the prelimbic cortex or dorsal hippocampus of male and female Long Evans rats during a delayed spontaneous alternation task. Bilateral injections of either pAAV-GFAP-hM3D(Gq)-mCherry or pAAV.GFAP.eGFP.WPRE.hGH control virus (AAV, serotype 5) were given. After 2 weeks to allow for viral expression, rats underwent delayed spontaneous alternation testing in which they received intraperitoneal injections of either the corresponding hM3D(Gq) receptor agonist, compound 21 (C21), or the vehicle 30 minutes before delayed spontaneous alternation testing in a counterbalanced order. Extramaze cues were changed to provide novel stimuli for each testing session. With injections of 1 mg/kg of C21 there is an interaction of sex and DREADDs activation on spatial working memory such that females receiving C21 showed impairments when astrocytes are activated. Morphological characterization of the astrocytes and GFAP staining suggest astrocytic activation by DREADDs is leading to increased GFAP. Our research indicates astrocytes play an integral role in memory processes in the prefrontal cortex and hippocampus and are a novel target for treatment in neurodegenerative diseases.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

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Topic: H.05. Working Memory

Support: EMBO ALTF 819-2020
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Title: Neural correlates of spatial working memory under different environments

Authors: *L. PENTOUSI[1], E. CHONG[2], V. PLATTNER[3], A. AKRAMI[1];
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Abstract: Spatial working memory (SWM) is the short-term maintenance and update of spatial information. SWM is important for a broad range of cognitive tasks that involve reasoning about physical objects and spatially-organized information, even in abstract non-spatial contexts. What are the neural computations underlying SWM? We trained rats to perform a novel, parametric SWM task across two separate environments: one where visual stimuli are projected onto the floor of a large behavioral arena, and another where stimuli appear on an upright touchscreen. Across both environments, animals learn to maintain and update stimulus locations in working memory over a delay period, and report recalled memory in continuous manner, by touching the remembered location. Rats display behavioral responses with dynamics that may reflect uncertainty or memory fidelity. We used silicon probes to record activity in posterior parietal cortex and hippocampal CA1 regions, revealing the neural representations that correspond to spatial working memory in continuous coordinates under different environments.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

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Topic: H.05. Working Memory

Support: CIHR grant FRN148365
BrainsCAN
NeuroNex
NSERC

Title: Spatial working memory in the presence of distractors in marmoset prefrontal cortex

Authors: *R. K. WONG, J. SELVANAYAGAM, K. D. JOHNSTON, S. EVERLING;
Univ. of Western Ontario, London, ON, Canada

Abstract: Persistent delay-period activity in prefrontal cortex (PFC) has been regarded as a neural signature of working memory (WM). Electrophysiological investigations in macaque PFC have provided much insight into WM mechanisms, however, a barrier to an understanding of circuits underlying persistent firing is the fact that a portion of PFC lies buried within the principal sulcus in this species rendering it accessible for laminar electrophysiology or optical imaging. The relatively lissencephalic cortex of the New World common marmoset *(Callithrix jacchus)* circumvents such limitations. Recently, we demonstrated that marmoset PFC neurons exhibit spatially tuned sample-, delay- and response-related activity that was linked to correct task performance in a touchscreen-based delayed-match-to-location task. To cope effectively with complex environments, organisms must be able to select relevant information and protect it from interference from irrelevant distractions. The role of PFC in processing and filtering salient but task irrelevant distractors has been investigated in detail in the macaque monkey. However, it remains unknown whether marmoset WM performance is robust to distractors presented during delay periods of WM tasks, and how such distractor filtering may be implemented in PFC circuits. Here, we addressed this gap by implanting a marmoset with a 96-channel Utah array (4 x 4 mm, 1 mm electrode length, 400μm pitch) in the left PFC and conducting wireless electrophysiological recordings while the animal performed a touchscreen-based delayed-match-to-location task in which a salient distractor was flashed at variable latencies and locations during the delay-period on a subset of trials. We observed no significant difference in task accuracy between distractor and no-distractor conditions (75.1% and 73.1%, respectively). We recorded the activity of 523 well-isolated single units. Of these, 231 units (44.2%) exhibited activity during the delay period. 7 of 523 units (1.3%) exhibited a delay activity, a distractor effect and a difference in activity between correct and error trials. In addition, we found 192 units (37.4%) that displayed sample-related activity. Of these units, we observed a larger magnitude of response for the sample compared to the distractor. These results indicate that marmosets can perform a spatial WM task with distractors and that similar effects on the no-distractor trials are consistent with previous work. Only a subpopulation of neurons had activity that was linked to task performance, suggesting that PFC may be implicated in filtering of task-irrelevant distractors.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

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Program #/Poster #: PSTR236.11/VV14

Topic: H.05. Working Memory

Support: NSF 2015276

Title: Glun2b antagonism in layer III dlpfc suggests a disinhibition mechanism on spatial working memory delay-related firing

Authors: *M. P. JOYCE[1], S. YANG[1], D. DATTA[2], J. I. ARELLANO[1], A. DUQUE[1], M. WANG[1], A. F. ARNSTEN[1];
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Abstract: The dorsolateral prefrontal cortex (dlPFC) is necessary for working memory and contains Delay cells that exhibit spatially tuned persistent firing across the delay epoch in a working memory task. This ability to represent specific visuospatial information in working memory is thought to arise from microcircuits in deep layer III, where pyramidal cells with extensive recurrent excitation generate persistent firing across the delay, and spatial tuning is refined by lateral inhibition from GABAergic interneurons. Delay cell firing depends on NMDA receptors with GluN2B subunits, as iontophoresis of moderate doses of GluN2B selective antagonists markedly reduced Delay cell firing, consistent with the prominent GluN2B receptor expression in the post-synaptic density of layer III spines (Wang et al, Neuron 77:736-49, 2013). The current study extended this previous research to examine the effects of very low doses of the selective GluN2B antagonist, TCN237, on Delay cell firing. Iontophoresis of a low dose of TCN237 onto Delay cells had no effect on the neurons' firing for their preferred direction, but increased firing for the nonpreferred directions, eroding spatial tuning. These effects may arise from GluN2B blockade on local interneurons. In contrast, a higher dose of TCN237 reduced firing for both the preferred and nonpreferred directions, similar to what has been reported before, and likely relating to the extensive GluN2B expression already reported on spines. However, their localization on interneurons in macaque dlPFC is not known. We used multiple label immunohistochemistry combined with confocal microscopy, and single label immunoelectron microscopy to investigate the distribution of GluN2B in layer III dlPFC inhibitory neurons in 2 female macaques (aged 8-10y). The calcium-binding proteins parvalbumin (PV), calbindin (CB), and calretinin (CR) are largely non-overlapping and label almost all cortical inhibitory neurons in dlPFC. We found that that GluN2B are robustly expressed in PV, CB, and CR somata in layer III, and confirmed expression of GluN2B in inhibitory-like aspiny dendritic shafts using immunoelectron microscopy. Thus, NMDA receptors with GluN2B subunits may to play a role in inhibitory circuitry as well as on pyramidal cells in the primate dlPFC.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

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Topic: H.05. Working Memory

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NIH Grant 1R01MH128293

Title: Dynamic and distributed spatial working memory representations in an eight-arm radial maze

Authors: *J. TALIAFERRO[1], J. GREENWALD[2], L. POSANI[1], S. FUSI[1], C. LACEFIELD[1,2], C. KELLENDONK[1,2];
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Abstract: Working memory (WM) is the cognitive capacity for temporarily holding information in mind for processing or use, and undergirds most of our actions and activities. Curiously, though rodent prefrontal cortex (PFC) activity is necessary for successful rodent spatial WM utilization[1,2], rodent spatial WM tasks do not always evoke robust PFC encoding of retrospective actions or stimuli[3,4]—theoretically a WM hallmark.
We hypothesized that increasing task spatial complexity might enable better elucidation of the role of the PFC in spatial WM. Inspired by a similar optionality increase in nonhuman primate work[5], we designed a novel spatial WM task in a custom-built, automated radial arm maze. Mice (n=9, female) were able to learn the task, performing well beyond chance. And indeed, cellular-resolution calcium imaging of the PFC during behavior revealed dynamic and distributed delay representations of retrospective spatial information, clarifying the nature of the PFC's role in spatial WM. Easily modifiable, this novel behavioral paradigm could fill a gap in the current neurobehavioral interrogation landscape.[6,7]

[1] Kellendonk et al., *Neuron*, 2006
[2] Vogel et al., *Cell Reports*, 2022
[3] Spellman et al., *Nature*, 2015
[4] Bolkan et al., *Nature Neuroscience*, 2017
[5] Funahashi et al. *Journal of Neurophysiology*, 1989
[6] Juavinett et al., *Current Opinion in Neurobiology*, 2018
[7] Krakauer et al., *Neuron*, 2017

Disclosures: J. Taliaferro: None. **J. Greenwald:** None. **L. Posani:** None. **S. Fusi:** None. **C. Lacefield:** None. **C. Kellendonk:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.13/VV16

Topic:

Support: CIHR
Neuronex

Title: Activity in the prefrontal cortex underlying working memory capacity in freely moving marmosets

Authors: *T. LO[1], S. VIJAYRAGHAVAN[1], L. E. MULLER[2], J. C. MARTINEZ-TRUJILLO[3]; [1]Dept. of Physiol. and Pharmacol., [2]Dept. of Mathematics, Western Univ., London, ON, Canada; [3]Dept. of Physiol. and Pharmacol. and Psychiatry, Schulich Sch. of Med. and Dentistry, Robarts Institute, Western Univ., London, ON, Canada

Abstract: Working memory (WM) enables the maintenance and manipulation of information necessary for achieving specific goals. WM is capacity limited: a limited number of items that can be maintained at a time in WM buffers. These limitations extend to various sensory modalities and vary across species. Studies in macaque monkeys performing visuospatial WM tasks show that prefrontal cortical neuronal activity is modulated with increasing WM load, in a manner consistent with divisive normalization. There has been a growing interest in using marmosets as a novel non-human primate model to study WM and other cognitive functions. Marmosets' lissencephalic cortex enables researchers to efficiently study the layer-specific cortical processing during cognitive tasks. In addition, studies have demonstrated that marmosets can perform WM tasks such as delay match to position/sample tasks. Yet, marmosets' WM capacity has not been extensively studied. Here, we undertook a behavioural and physiological investigation of WM capacity in marmosets. We trained seven marmosets on a delay non-match to position touchscreen task using a modified on-cage system that allows a marmoset to enter and exit the setup freely. In the task, marmosets are presented with an array of stimuli where the number of stimuli progressively increases. To receive reward, they had to choose the novel stimulus that was not present in the previous iteration of the stimulus array presentation. The number of items maintained in WM increases across trial iterations (span).
We implanted two marmosets with multi-shank "volume" probes (N-form array, Plexon Inc.). We implemented a wireless on-cage recording system that allows us to examine PFC neuronal activity while marmosets performed the WM task. We found that numerous PFC neurons differentially encoded the location of the target that was selected for the current touch. Some of these neurons showed persistent responses encoding the response location in the post-touch epoch, while other neurons showed location-tuned persistent activity during and after reward delivery that remained until the next trial iteration. Our results demonstrate the feasibility of studying the physiological underpinnings of WM capacity using a WM paradigm in freely moving marmosets.

Disclosures: **T. Lo:** None. **S. Vijayraghavan:** None. **L.E. Muller:** None. **J.C. Martinez-Trujillo:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.14/VV17

Topic: H.05. Working Memory

Support: Canada research chair
VISTA, York university

Title: Prefrontal ensemble dynamics in spatial working memory in marmosets

Authors: *N. KATYARE[1], R. WONG[2], S. EVERLING[2], L. MA[1];
[1]York Univ., Toronto, ON, Canada; [2]Western Univ., London, ON, Canada

Abstract: Neurons in the lateral prefrontal cortex (lPFC) are known to play a role in spatial working memory. Single unit recordings from the lPFC of marmosets during a delayed-match-to-location task confirmed that like Macaques, marmoset lPFC neurons exhibit directional tuning, which correlated with task performance (Wong et al., 2022) . Not much, however, is known about population coding in the lPFC of marmosets. Here, we re-analyzed data from Wong et al. (2022), focusing on the coding of spatial information in neuronal ensembles using support vector machines, using 2 different approaches: Best unit and Optimized (Leavitt et al., 2017). In the Best-unit approach, we first sorted neurons based on their decoding accuracy. We then calculated decoding accuracy of top-N neuron ensembles at each size. In the Optimized approach, we identified the N-1 neurons that together with the top neuron, achieved the best performance. As previously reported in macaques, the Optimized approach significantly out-performed the Best-unit approach; and ensembles of size 5-15 performed superior to full ensembles. We next analyzed the ensemble performance separately for broad-spiking and narrow-spiking neurons (BSNs and NSNs). Although BSNs dominated the ensembles, the NSNs contribute substantially to the decoding, especially in the Optimized ensembles, indicating a significant role of interneurons in spatial coding. Analysis of signal and noise correlations revealed a population of non-tuned neurons that were present in Optimized but not in the Best-unit ensembles, and 80-100 % of these exhibited significant noise correlations with tuned neurons. This observation supports a role of noise correlations in enhancing spatial coding. Finally, we observed that the average inter-unit distance for a 10-neuron ensemble was significantly shorter than the average inter-unit distance among the population. This indicates colocalization of neurons that play key roles in spatial coding in lPFC. Our results provide the first evidence that population-level coding properties in the lPFC can be generalized across primate species. References Leavitt ML, Pieper F, Sachs AJ, Martinez-Trujillo JC (2017) PNAS 114:E2494-E2503.Wong RK, Selvanayagam J, Johnston KD, Everling S (2022) Cerebral Cortex: bhac289.

Disclosures: **N. Katyare:** A. Employment/Salary (full or part-time):; York university. **R. Wong:** A. Employment/Salary (full or part-time):; Western University, London, ON, Canada. **S. Everling:** A. Employment/Salary (full or part-time):; Western University, London, ON, Canada. **L. Ma:** A. Employment/Salary (full or part-time):; York university.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.15/VV18

Topic: H.05. Working Memory

Support: MRC Grant MR/T033967/1

Title: Validation of a touchscreen-based task to assess spatial working memory in a marmoset model of schizophrenia

Authors: *A. R. HODGSON, H. F. CLARKE;
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Abstract: Impairment in spatial working memory (SWM) is a key cognitive symptom of schizophrenia, for which there is no effective treatment. To develop treatments for these deficits, we must understand how structural and functional alterations in the neural circuits underlying working memory produce SWM impairment. Research in rhesus monkeys has established that the dorsolateral prefrontal cortex (dlPFC) is a critical area for SWM. However, due to their lissencephalic cortex, the use of a marmoset model would permit more accessible pharmacological manipulations of dlPFC function. Specifically, development of a SWM task suitable for marmosets will allow us to establish which dlPFC manipulations result in SWM deficits, and whether these deficits can be ameliorated pharmacologically.
Common marmosets (*Callithrix Jacchus*; n=3) were trained on a delayed match-to-position (DMP) task to measure SWM. In each trial, the animal must touch a sample stimulus appearing in one of four locations on the touchscreen. They must then select a stimulus at the centre of the screen, which is rewarded with 2s of milkshake reward delivered through a central spout, bringing the animal's head and body to the centre and preventing the use of body position as a mediating strategy. The marmoset is then presented with stimuli in 3-4 locations, and must select the location presented in the sample stage to receive 5s reward. This task was modified for one animal such that the stimuli were on the right of the screen and the 'centre' stimulus on the left to preclude use of a hand-position strategy.
When performing significantly above chance, marmosets were surgically implanted with indwelling cannulae targeting the dlPFC (Brodmann areas 9 and 46). To establish whether the DMP task was dlPFC dependent, we investigated the effect of infusing GABA agonists muscimol/baclofen and NMDA antagonist MK-801 on DMP performance. Preliminary data suggests that MK-801 but not muscimol/baclofen impairs performance on the DMP task. This is consistent with electrophysiological data from rhesus monkeys suggesting a critical role for

NMDARs in the persistent firing of dlPFC ‘delay’ cells thought to underlie SWM.
In conclusion, the DMP is a suitable task for measuring SWM in marmosets, and marmosets can learn this task even when the use of body position strategies are prevented. Future experiments will examine dlPFC manipulations of relevance to schizophrenia and their pharmacological amelioration.

Disclosures: **A.R. Hodgson:** None. **H.F. Clarke:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.16/VV19

Topic: H.05. Working Memory

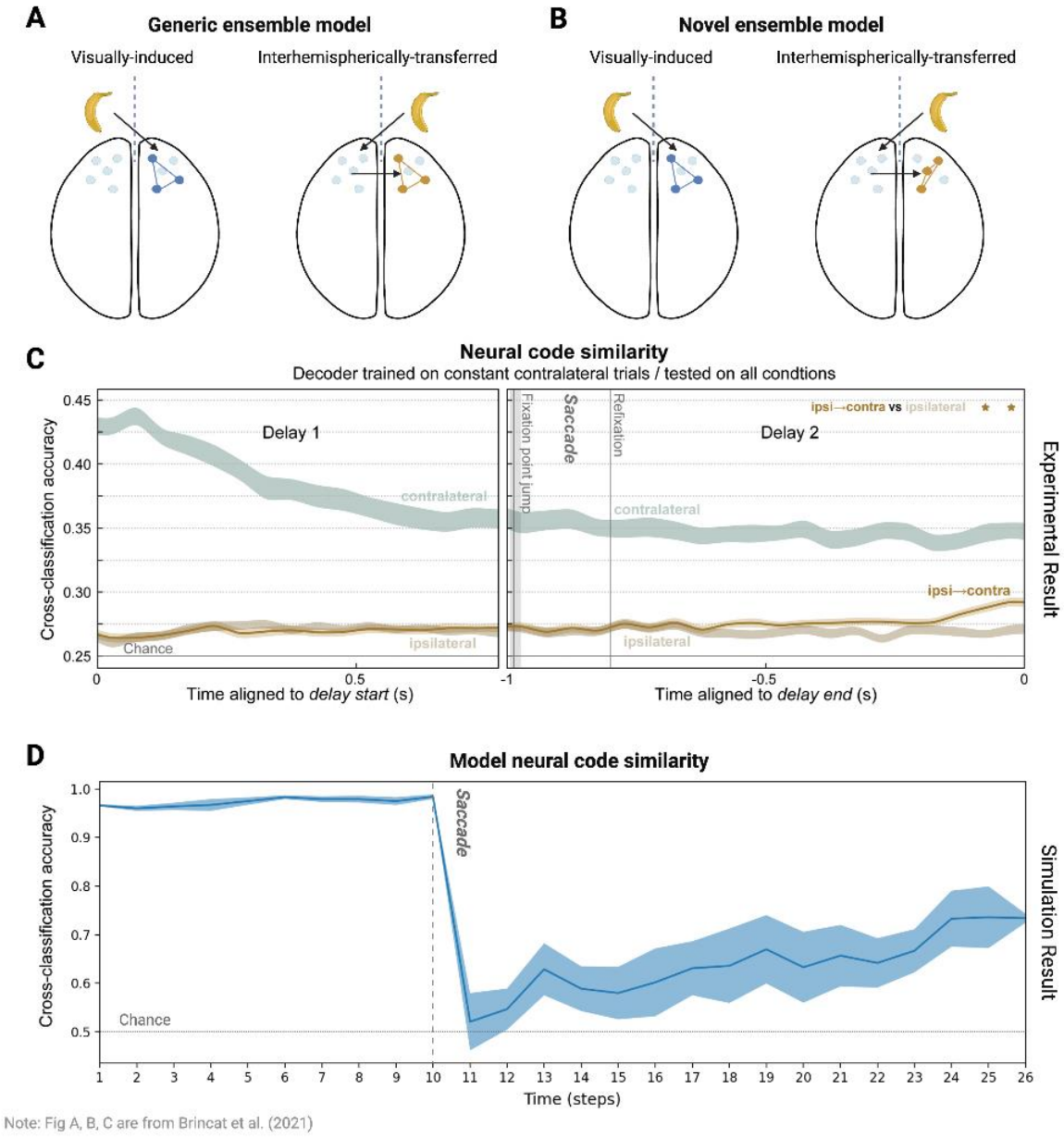
Support: NSF STC award CCF-1231216

Title: Synaptic Plasticity Explains the Creation and Convergence of Ensembles During Interhemispheric Transfer of Working Memory

Authors: *Y. XIE[1], A. RANGAMANI[1], E. K. MILLER[2], T. A. POGGIO[1];
[1]McGovern Inst. for Brain Res., [2]Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Working memory (WM) enables temporary information retention and volitional control in the brain during tasks. An intriguing feature of WM is its maintenance during interhemispheric transfer. Brincat et. al. (2021) recently demonstrated in physiological experiments the interhemispheric transfer of WM via a novel delayed match to sample task (Fig C). They found that during the transfer WM engages novel ensembles (Fig B) rather than generic ensembles (Fig A). These novel ensembles eventually converge towards generic ensembles, as evident by increased decoder accuracy towards the end of the trial in Fig C. This abstract uses a simple computational model with excitatory neurons, Hebbian synaptic plasticity, and K-winner-take-all inhibitory mechanisms to explain the creation of novel ensembles and their convergence to generic ensembles as observed in Brincat et. al. (2021). Our model incorporates 3 brain areas (stimulus, right hemisphere, and left hemisphere), each with N=1000 neurons, of which K=100 neurons are chosen to fire by a winner-take-all mechanism. Afferent and recurrent synaptic connections exist between areas, drawn independently at random with a density of p=5%. We measure the similarity between the ensembles created before and after WM transfer in Fig D by training a decoder to distinguish between two classes of stimuli. Post-transfer, decoder accuracy drops to random chance (50%±4%), indicating novel ensemble creation due to random synaptic connectivity. However, subsequent rhythmic coupling improves the accuracy to 70%±5%, indicating the novel ensembles converge towards generic ensembles. This is because information about the generic ensembles is stored through Hebbian plasticity in the recurrent synaptic weights of the area to which the memory is transferred. Our simulations thus establish that

Hebbian plasticity and random synaptic connections are sufficient to explain interhemispheric WM transfer without persistent neural spiking activity.



Reference: Brincat et. al. (2021). Interhemispheric transfer of working memories. Neuron, 109(6), 1055-1066.

Disclosures: **Y. Xie:** None. **A. Rangamani:** None. **E.K. Miller:** None. **T.A. Poggio:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.17/VV20

Topic: H.05. Working Memory

Support: NIMH: R01MH123686
NINDS: R01NS127785

Title: Diminished persistent activity in the posterior parietal cortex after repeated multimodal stress exposure in adolescence

Authors: *A. PRODDUTUR[1], D. J. RINDNER[2,3], G. LUR[4];
[2]Neurobio. and Behavior, [1]Univ. of California Irvine, Irvine, CA; [3]Neurobio. and Behavior, Univ. of California, Irvine, CA; [4]Univ. of California, Irvine, Irvine, CA

Abstract: Stress is known to impede numerous cognitive functions including working memory, a temporary memory storage that serves as a mental platform for ongoing activities and thus supports cognitive functions like planning and decision-making. The mechanisms underlying working memory is thought to be sustained action potential firing after a brief stimulus drive. Several studies reported that this persistent firing is mediated by an interconnected local microcircuit and intrinsic mechanisms such as HCN and calcium-dependent channels. The posterior parietal cortex (PPC) is an essential cognitive hub that plays a crucial role in attention, decision-making, and working memory. Our previous studies reported circuit-specific loss of input to the PPC and impaired visuo-spatial working memory in an adolescent mouse model of repeated multimodal stress (aRMS). However, how stress exposure alters the circuit-specific physiological mechanisms of working memory is not clearly understood. We used whole-cell patch-clamp electrophysiology to record sustained action potential firing in layer 5 pyramidal neurons of the PPC from mice exposed to aRMS for 1hr/day for 10 days and age-matched controls. Our initial evaluation of intrinsic properties in pyramidal cells showed no significant differences in the input resistance, SAG amplitude, resting membrane potential, and firing frequency between control and aRMS groups. However, the persistent firing duration in response to the increasing pulse duration of depolarizing current injections was significantly reduced in the aRMS group compared to controls. In the presence of AMPA and NMDA glutamate blockers, the persistent activity duration is diminished substantially in controls, suggesting that excitatory synaptic connections are essential in mediating persistent activity. These results suggest a role for excitatory circuit dysfunction in the stress-induced reduction of persistent activity in the PPC network of adolescent mice rather than cell-autonomous intrinsic channel mechanisms.

Disclosures: A. Proddutur: None. **D.J. Rindner:** None. **G. Lur:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.18/VV21

Topic: H.05. Working Memory

Support: IBS-R002-A1

Title: Laminar differences in working memory-related neural activity in mouse medial prefrontal cortex

Authors: *S. KO, J. BAE, M. JUNG;
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Abstract: It is unclear how neurons in different layers of the cerebral cortex work together to support working memory. To investigate this matter, we trained mice in two variations of a working memory task: a delayed match-to-sample task (task 1) and a delayed conditional response task (task 2). In task 1, the mice were presented with one of two lick ports as a sample cue. Following a delay, the mice were required to choose the lick port that matched the sample. In task 2, the mice were presented with a sample cue, followed by a delay (delay 1) and the presentation of one of two odors, indicating a matching or non-matching rule. After an additional delay (delay 2), the mice had to select the lick port that either matched or did not match the sample, based on the presented odor. We found that chemogenetic inhibition of pyramidal neurons in the superficial layer of the medial prefrontal cortex (mPFC) had little effect on task 1 performance. However, it significantly impaired performance in task 2. A preliminary analysis of physiological data also suggested that superficial-layer mPFC neurons conveyed stronger and more persistent match/non-match rule signals than deep-layer neurons in the second delay period. These results suggest that the mPFC superficial layer might play an important role in working memory tasks that require the integration of multiple neural signals.

Disclosures: S. Ko: None. **J. Bae:** None. **M. Jung:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.19/VV22

Topic: H.05. Working Memory

Support: DFG JA 1999/6-1

Title: Single neuron correlates of working memory maintenance and flexible updating in the human lateral prefrontal cortex

Authors: *H. CHEN[1,2], B. MEYER[1], J. GEMPT[3], S. N. JACOB[1];
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Neurosciences, Ludwig-Maximilians-Universität München, Munich, Germany;
[3]Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

Abstract: Working memory, the temporary storage and manipulation of task-relevant information, is a crucial cognitive function of the prefrontal cortex (PFC). It serves as the basis for cognitive flexibility, which allows us to make adaptive decisions in a changing environment. However, the neuronal mechanisms that govern the context-dependent retrieval of information from working memory remain unclear. To investigate the neuronal underpinnings of flexible working memory retrieval, we recorded large-scale spiking activity and local field potentials (LFP) in an individual with intracortical planar microelectrode arrays chronically implanted in the right middle frontal gyrus (MFG) and inferior frontal gyrus (IFG). Each array comprised 64 channels. Neuronal data acquisition proceeded in parallel to the patient performing a delayed match-to-number task. The task required the patient to memorize two sample quantities that were visually displayed either simultaneously or sequentially and then retrieve one of the samples indicated by a retro-cue for comparison with a subsequently presented test number. The numerical stimuli were presented in either non-symbolic notation (sets of dots, numerosities) or symbolic notation (digits, Arabic numerals) and controlled for low-level visual properties. We hypothesize to find populations of individual neurons that represent the two sample numbers. The cued number would then be selected through retro-cue modulation of activity in the population representing the respective sample number (left or right, first or second) or alternatively represented by a separate, distinct population without prior numerical coding in the trial. Behavioral analyses demonstrate that the patient's task performance was comparable to that of healthy subjects, exhibiting classic behavioral signatures of numerical cognition including the numerical size effect and the distance effect. Preliminary results further show well-isolated prefrontal single units that display task-related activity. Notably, we found neurons in both the MFG and IFG that were selective to non-symbolic and symbolic number in multiple trial epochs. Ongoing neuronal analyses will shed light on the neurodynamic mechanisms of flexible working memory retrieval and pave the way for future investigations into the roles of neuronal population dynamics in other human higher cognitive functions.

Disclosures: **H. Chen:** None. **B. Meyer:** None. **J. Gempt:** None. **S.N. Jacob:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.20/VV23

Topic: H.05. Working Memory

Support: Spanish Ministry of Economy and Competitiveness with the European Regional Development Fund (SAF2015- 70324-R)
Fundación La Caixa para Doctorados en España 17/18 (LCF/BQ/DE17/11600009)
Spanish Ministry MCIN/AEI/ 10.13039/501100011033

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Spanish Instituto de Salud Carlos III (PIE 16/00014)
Fundación privada Cellex

Title: Neural Circuit Mechanisms underlying Working Memory Errors

Authors: T. ONA-JODAR[1], G. PRAT-ORTEGA[3], E. CARRILLO[1], C. T. LI[4], J. DALMAU[5], *A. COMPTE[1], J. DE LA ROCHA[2];
[2](Q5856414G), [1]IDIBAPS, Barcelona, Spain; [3]Univ. of Pittsburgh, Dept. of Bioengineering, Pittsburgh, PA; [4]Inst. of Neurosci., Shanghai Inst. of Biol. Sciences, Chinese Acad. of Sci., Shanghai, China; [5]Catalan Inst. for Res. and Advanced Studies (ICREA), Barcelona, Spain

Abstract: Working memory (WM) is central for cognition and is impaired in many brain disorders. Electrophysiological evidence has identified persistent selective activity as the neural correlate of WM. In mice, persistent activity has been shown to underlie a basic form of WM: the internal maintenance of a prospective motor action. Theoretical work, on the other hand, has proposed attractor states as a potential network mechanism underlying persistent activity. The stability and capacity of attractor network models has been extensively studied and yet, direct comparisons of experimental data with the model dynamics during memory errors are still scarce. To investigate what makes WM fail, we used a two-alternative delayed-response task in which mice listen to a lateralized auditory stimulus and, after a variable delay (duration D=0-10 s), they lick the associated lateral port. Response accuracy decreased with delay showing that there were forgetting errors (average forgetting rate 0.01/s) . Mice also showed non-memory errors or lapses, which occurred independently of delay duration and which tended to repeat previous choices. We attempted to model this behavior by fitting an extension of the classical double-well attractor model that could show the same forgetting rate as well as the tendency to repeat previous choices. The double well model inevitably induced a dependence of the repeating bias on the delay length. Behavior in the task was better fitted by an alternative two-state hidden Markov Model that stochastically switches between (1) a task-engaged WM state where memory-guided responses are dictated by the dynamics of a double well attractor model, and (2) a task-disengaged state which elicits lapse responses with a strong repeating tendency. Population recordings in the anterolateral motor cortex (ALM) found distinct physiological dynamics underlying the two states of the behavioral model. Persistent selective activity encoded the impending choice during the mnemonic period in task-engaged correct trials but this code was significantly weaker in task-disengaged correct trials. Furthermore, task-disengaged phases during the session correlated with transient surges in 8 Hz spiking network synchrony. On the other hand, errors in task-engaged, but not task-disengaged, trials showed the reversal of choice encoding along long delays, the neural signature of a switch between the correct and the error choice attractor. Our results show that mice performance in a delayed-response task is limited by both lapses and forgetting errors, the latter possibly caused by fluctuation-driven switches between attractor memory states in area ALM.

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Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.21/VV24

Topic: H.05. Working Memory

Support: BRAINCITY, International Research Agenda Programme of the Foundation for Polish Science
NCN Grant: 2019/34/E/HS6/00257
Kosciuszko Foundation Exchange Program to the United States

Title: Attended and unattended working memory items: evidence from single-neuron recordings in humans

Authors: *K. PALUCH[1], M. MAGNUSKI[1], W. ŚREDNIAWA[1], D. IVANOVSKI[2], W. FORTUNA[3], K. SMARZEWSKA[3], M. SLUZEWSKA-NIEDZWIEDZ[3], P. TABAKOW[3], H. BABU[2], J. KAMINSKI[1];
[1]Nencki Inst., Warsaw, Poland; [2]SUNY Upstate Med. Univ., Syracuse, NY; [3]Wroclaw Med. Univ., Wroclaw, Poland

Abstract: Working memory (WM), which allows for temporary storage and manipulation of a small amount of information, is believed to work through sustained neuronal firing. This concept is backed by extensive animal and human research. However, in most of these studies, the information held in WM was not only remembered but also continuously attended to. To separate memory storage from attention focus recent EEG and fMRI studies used retro cues to shift attention between multiple items simultaneously held in WM. Interestingly, these studies found accurate recall of previously unattended WM content without detectable brain activity while it was temporarily outside the focus of attention. These results suggested that unattended WM items are coded in another, activity-silent mechanism, which does not require neuronal firing. To test this theory, we studied persistent activity of image selective cells when their preferred stimulus was held in memory and attended to as well as when it was outside the focus of attention. The study was conducted in patients with drug resistant epilepsy who had depth electrodes implanted in medial temporal lobe (MTL) for seizure monitoring. In line with previous noninvasive studies patients had lower accuracy when asked to recall items which previously were temporarily outside of attention focus. We replicated results of earlier single neuron studies, demonstrating sustained firing of MTL neurons for attended items held in WM. For the first time, we monitored neuronal activity when WM items were outside the focus of attention. Using machine learning (support vector machine) we were able to decode (from the delay period activity) if preferred item was held in WM while it was attended but not while it was unattended. Neuronal representation for attended WM item was stable in time, decoder trained on first delay period was successfully tested on data from the second delay period. Our preliminary results suggest that prominent part of persistent activity of MTL cells during delay period reflects focus of attention rather than WM memory storage.
This poster has been financially supported by: BRAINCITY project carried out within the International Research Agenda Programme of the Foundation for Polish Science; the Polish

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Disclosures: K. Paluch: None. **M. Magnuski:** None. **W. Średniawa:** None. **D. Ivanovski:** None. **W. Fortuna:** None. **K. Smarzewska:** None. **M. Sluzewska-Niedzwiedz:** None. **P. Tabakow:** None. **H. Babu:** None. **J. Kaminski:** None.

Poster

PSTR236. Central and Prefrontal Mechanisms I

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR236.22/VV25

Topic: H.05. Working Memory

Title: Prefrontal D1 dopamine-receptor neurons and delta resonance during working memory

Authors: *Y.-C. KIM[1], M. OYA[1], K. A. MORGAN[2], A. M. OPPMAN[1], A. ESPINOZA[1], N. S. NARAYANAN[1];
[1]Neurol., [2]Univ. of Iowa, Iowa City, IA

Abstract: The prefrontal cortex plays a key role in the maintenance of task-relevant information short tasks involving working memory. Single neurons in the prefrontal neurons exhibit memory-specific modulation. Data from humans, primates, and mice has shown that prefrontal neurons expressing D1-type dopamine receptors (D1DRs) are critical for working memory, flexibility, and timing, leading to the hypothesis that prefrontal D1 neurons directly encode cognitive processing. We tested this idea in a mouse-optimized version of delayed non-matching position task performed in operant chambers. The task was specifically designed to eliminate non-mnemonic strategies by requiring active responses during the delay period. We tested dopamine dependency of the task performance with the D1 dopamine receptor agonist SKF82958 and found that mouse working-memory performance was specifically with dopamine receptor agonism. In addition, we optogenetically inhibited prefrontal D1DR+ neurons, and found marked impairments. We hypothesized the medial frontal cortex D1DR+ neurons would exhibit task-specific activity during delayed non-matching task. We leveraged optogenetic tagging to identify D1DR+ neurons in the mouse medial frontal cortex while recording medial frontal neuronal ensembles. While we found distinct firing patterns of D1DR+ neurons in trial start and delay start, we did not find clear evidence that D1DR+ specifically maintained mnemonic information. However, we found massive low frequency ~4 Hz coherence of D1DR+ neurons with other prefrontal neurons and local-field potentials. These findings provide insight into prefrontal networks and have relevance for neuropsychiatric disorders such as ADHD, schizophrenia, and Parkinson's disease.

Disclosures: Y. Kim: None. **M. Oya:** None. **K.A. Morgan:** None. **A.M. Oppman:** None. **A. Espinoza:** None. **N.S. Narayanan:** None.

Poster

PSTR237. Human Behavior and Social Interaction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR237.01/VV26

Topic: H.06. Social Cognition

Support: the National Natural Science Foundation of China (62293550, 62293551, 61977008)

Title: The emergence of equality in the toddler's brain

Authors: *K. JIANG[1], Y. ZHAI[1], T. ZHANG[1], X. HE[1], Z. WANG[2], C. LU[1];
[1]Beijing Normal Univ., Beijing, China; [2]Capital Normal Univ., Beijing, China

Abstract: Previous studies have shown that the age of three is a pivotal period for the emergence of equality, during which children undergo a developmental shift in their fair distribution actions. However, the neurocognitive mechanisms behind the differentiation are not well understood. Here we showed that among a total of 82 3-year-old children, two groups of children could be identified based on their choice preference in a gift distribution task. While one group made equitable decisions depending on the level of inequality (i.e., pass group), the other adhered to a tendency to maximize their own benefits (i.e., preserved group). Computational modeling results indicated that the behavior of the pass group was driven by their aversion to inequity, whereas that of the preserved group was driven by self-interested objectives (Experiment 1). Additionally, these results were well replicated in an independent sample even when a partner (i.e., a puppet) was presented (Experiment 2). In Experiment 3, the neural underpinnings were investigated, showing that the left temporoparietal junction (lTPJ) and sensorimotor cortex were associated with the choice preference of the pass and preserved groups respectively. Additional resting-state-functional-connectivity analysis revealed a higher node degree of the lTPJ in the pass than in the preserved group, which is further positively correlated with children's cognitive ability. Finally, a significant correlation was found between children's cognitive ability and their choice preference when the level of inequality was high. Together, these findings revealed the neurocognitive mechanisms of the developmental differentiation among the 3-year-olds when equality is internalized, shedding new light on the emergence of social and cognitive abilities in the young brain.**Keywords**: Equality; Inequity aversion; Toddler; Computational modeling; fNIRS

Disclosures: **K. Jiang:** None. **Y. Zhai:** None. **T. Zhang:** None. **X. He:** None. **Z. Wang:** None. **C. Lu:** None.

Poster

PSTR237. Human Behavior and Social Interaction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR237.02/VV27

Topic: H.06. Social Cognition

Support: NIH K01 grant

Title: Brain Mechanism Underlying Clinicians Pain Perception.

Authors: *N. KHALILKHANI[1], T. VAROUDAKI[2], M. GIANOLA[3], E. A. R. LOSIN[4]; [1]Biobehavioral health, Pennsylvania state Univ., state college, PA; [2]Huck institute, Penn State, state college, PA; [3]Univ. of Miami, Dept. of Psychology, Coral Gables, FL; [4]Biobehavioural health, Pennsylvania state university, state college, PA

Abstract: Intro: Despite substantial evidence that patient demographics influence clinician pain assessment and treatment decisions, the sociocultural and neurobiological mechanisms underlying these treatment disparities remain largely unknown. Method: We used our novel virtual pain management paradigm and fMRI to address these open questions in N = 68 (34 f) medical students and residents. Each clinician saw 36 virtual patients (18 m) who identified as one of the three racial and ethnic groups most impacted by pain treatment disparities in the U.S.: non-Hispanic Black (N=12), Hispanic white (N= 12), non-Hispanic white (N=12). Each clinician-patient interaction consisted of 4 parts: 1) a written medical vignette , 2) 12 seconds of patient video of evoked pain meant to simulate the clinical exam, and 3-4) clinicians ratings the patient's pain and their likelihood of prescribing any analgesic. Result: Preliminary whole-brain GLM analyses revealed increased activity in brain regions associated with pain empathy (e.g., superior and middle temporal gyrus) when clinicians viewed patient pain videos, consistent with previous studies. Whole-brain comparisons while clinician's viewed patients from different demographic groups revealed more activity in pain empathy related regions when clinicians viewed female and Black or African American patients compared to male and non-Hispanic white patients. These same demographic groups were, on average, also rated to be in more pain and more likely to receive analgesic treatment. However, trends in the data suggest that when controlling for patients pain facial expression intensity at the single trial level (a planned follow-up analysis), this pattern may reverse such that white males may be rated in more pain and given more pain medication at the same level of pain facial expression intensity as we have observed in previous behavioral studies. Conclusion: Our findings provide preliminary evidence of brain mechanisms that may underly pain assessment and treatment biases related to patient demographics.

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Poster

PSTR237. Human Behavior and Social Interaction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR237.03/VV28

Topic: H.06. Social Cognition

Support: (JH) NIH grant 1R01MH119430-01
(MK) NIH grant F99NS129174

Title: Real-face-specific neural processing in lateral and dorsal parietal cortices are associated with alpha activity

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Abstract: Introduction. An emerging body of evidence suggests that viewing a real face activates distinct neural systems in lateral (LC) and dorsal parietal (dPC) cortices which are not activated by a simulated face[1,2,3]. How such differences emerge is unknown. We propose that, due to their rapidly changeable and information rich nature, real faces require constant processing which promotes cognitive readiness for tracking any facial changes. Such processing would present as significant peaks in activity in the LC and dPC regions of interest (ROIs) occurring throughout the duration of real but not simulated face view. We hypothesized that we would see such peaks in either the theta (4-8hz) or alpha (8-13hz) frequency bands.
Methods. To test these hypotheses, we collected scalp electroencephalography (EEG) from participants (n=20) while they engaged in 1.6-S face viewing epochs with another real human partner as well as with a robot as control, enabling us to establish real face specificity. We assessed baseline-normalized theta and alpha current flow in the ROIs using the Brainstorm sLORETA source localization function.
Results. ROI theta activity was comparable for both real and robot face viewing, suggesting that it is not specific to real faces. Conversely, ROI alpha activity (Fig. 1) when viewing a real human face (left), showed multiple peaks specific to the ROIs (black arrows, z-score>2.33; p<0.01). No such subsequent peaks occurred after the initial peak during robot face viewing (right).
Conclusions. These results suggest that real faces are processed in a temporally extended and specific manner for LC and dPC.

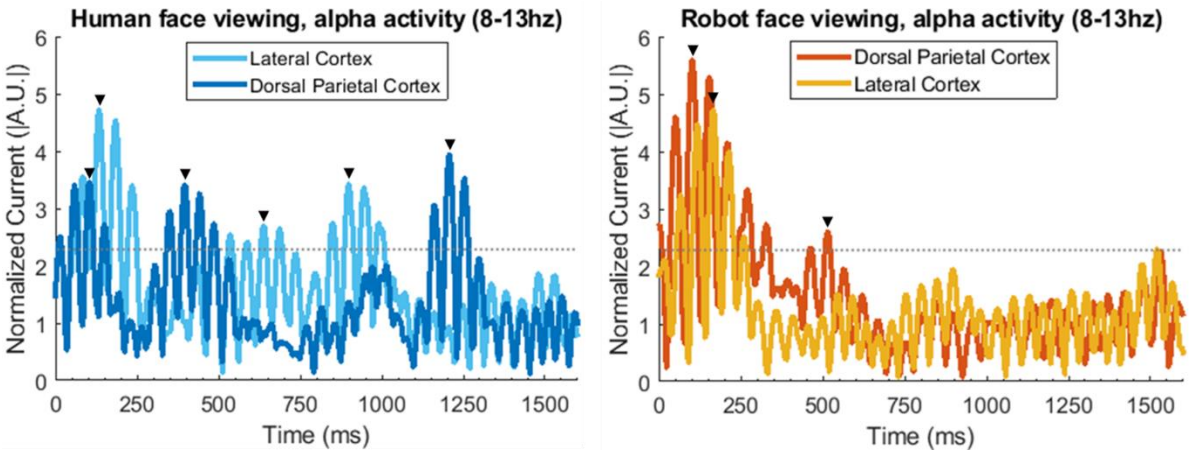

Figure 1. Alpha current flow in ROIs during human (left) and robot (right) face viewing. Dashed lines: p=0.01. Black arrows: significant peaks in the signal envelope. (left) During human face viewing, ROIs showed numerous significant alpha peaks. (right) During robot face

viewing, ROIs showed an initial alpha peak before returning to baseline.

[1]Noah, et al, 2020, Frontiers in Human Neuro.; [2]Kelley, et al., 2021, Frontiers in Robotics & A.I.; [3]Hirsch et al, 2022, Plos One.

Disclosures: **M. Kelley:** None. **M. Tiede:** None. **J.A. Noah:** None. **X. Zhang:** None. **J. Hirsch:** None.

Poster

PSTR237. Human Behavior and Social Interaction

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR237.04/VV29

Topic: H.06. Social Cognition

Support: the National Natural Science Foundation of China (62293551)

Title: Analysis and Evaluation of Collaborative Argumentation from Neuroscience Perspective

Authors: *Y. WANG[1], X. LI[1], Y. XIE[1], S. YANG[1], Y. ZHENG[1], C. LU[2];
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Abstract: Argumentation is a specific form of talk in the classroom that has received significant attention in educational studies. However, previous studies have not identified why some students are more successful than others from the cognitive neuroscience perspective. To address this gap, this study employed Functional near-infrared spectroscopy (fNIRS) to measure the natural collaborative argumentation of 62 pairs of college students (n=124). this study first assessed the quality of collaborative argumentation according to the number of elements such as claim, justification, rebuttal, and evidence. The collaborative argumentation was then categorized into high- and low-quality groups. The results showed that the high-quality group scored significantly higher than the low-quality group ($t = 5.9$, $p < 0.01$). Next, interbrain coupling was calculated for each group, revealing that participants with superior collaborative argumentation performance exhibited more frequent and higher-quality rebuttals, with the rebuttal stage demonstrating increased levels of interbrain coupling in the dorsolateral prefrontal cortex (DLPFC). Furthermore, this study coded different reasoning modes of collaborative argumentation, namely rational reasoning mode, emotional reasoning mode, and intuitive reasoning mode. It discovered that high-quality collaborative argumentation displayed a significantly higher proportion of rational and emotional reasoning modes compared to intuitive reasoning. Moreover, interbrain couplings are significantly higher in the emotional and rational reasoning modes than in the intuitive reasoning mode. However, no significant difference in interbrain coupling was observed between the emotional and rational reasoning modes. The neural data results suggest that rebuttal indeed serves as a valid indicator of collaborative argumentation quality. Additionally, the interbrain couplings corresponding to the rational and

emotional reasoning modes can moderate the occurrence of rebuttal in collaborative argumentation. This study represents an initial endeavor by educational researchers in the field of cognitive neuroscience. It contributes to the analysis and evaluation theories of collaborative argumentation from a cognitive neuroscience perspective, providing supplementary evidence for the conclusions drawn in previous educational research.

Disclosures: **Y. Wang:** None. **X. Li:** None. **Y. Xie:** None. **S. Yang:** None. **Y. Zheng:** None. **C. Lu:** None.

Poster

PSTR237. Human Behavior and Social Interaction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR237.05/VV30

Topic: H.06. Social Cognition

Title: Cognitive factors correlating with collective intelligence

Authors: *K. MOGI[1,2];
[1]Sony Comp Sci. Lab., Shinagawa-Ku, Japan; [2]Collective Intelligence Res. Lab., Univ. of Tokyo, Tokyo, Japan

Abstract: Collective intelligence is an important aspect of human cognition, from collaboration in the workplace to finding the appropriate price in the market. It has been suggested that there is a collective intelligence factor c, in analogy with the general intelligence factor g (Spearman 1904), and that several conditions correlate with cognitive intelligence, e.g., social sensitivity, turn taking, and gender ratio (Woolley et al. 2010). Advancements in artificial intelligence systems (OpenAI 2023) have opened new possibilities in facilitating and enhancing collective intelligence, by augmenting modes of communication, verbal and non-verbal, and embodied or otherwise. Here I investigate cognitive factors correlating with collective intelligence. Subjects were asked to report their perceived ease of making balanced turn taking in situations involving variable parameters constraining the exchange of information, including familiarity of people, gender ratio, embodiment conditions, and time pressure. Parameters affecting social sensitivity were analyzed based on the subjects' responses. The Chatham House Rule (CHR) states that "when a meeting, or part thereof, is held, participants are free to use the information received, but neither the identity nor the affiliation of the speaker(s), nor that of any other participant, may be revealed." CHR has been known to be effective in realizing tangible results of agreements in negotiations, especially in international politics, a cognitively loaded task domain. Subjects' perceived ease of communication in the original CHR and its variants were studied, with implications for achieving collective intelligence. The significance of anonymity in promoting free exchange of information towards collective intelligence was analyzed, with implications for augmentation. Based on the results, I discuss the possible neural mechanisms involved in communications related to collective intelligence. Turn taking are subserved by brain areas including the left inferior frontal cortex (Bögels and Levinson 2016). The supplementary motor

area, together with other cortical circuits, are involved in preparing the brain for action (Cunnington et al. 2005). The inferior fronto-occipital fasciculus might be essential in social cognition including the RME (reading the mind in the eyes) task (Ng et al. 2021). Finally, I discuss modes of interaction between the brain and environment facilitating collective intelligence, and possible ways to enhance it with artificial intelligence.

Disclosures: K. Mogi: None.

Poster

PSTR237. Human Behavior and Social Interaction

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Topic: H.06. Social Cognition

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NIMH R15 AREA MH099498
Diversity Supplement to Guadalupe Chim
NIMH R15 AREA MH112091
Women's Health Supplement
NSF RUI Award BCS

Title: The Role of Neighborhood Quality and Nervous System Development on Emotional Regulation in Latino Children

Authors: *A. A. SEGARRA, K. D'ANNA HERNANDEZ;
Psychology, Marquette Univ., Milwaukee, WI

Abstract: Introduction: Mexican American children make up the largest group of Latinos in the US due to high birth rates. These children are more likely to have more documentation of behavioral issues that do not address underlying influences on behavior, such as emotional regulation development. According to the fetal programming hypothesis, embryonic growth is influenced by the fetal environment, contingent on the mother's physical and mental health. Mexican American women experience more systemic stressors that relate to neighborhood quality. Living in stressful environments can be a risk factor for central nervous system (CNS) development that can be measured through a sympathetic response through heart rate variability (HRV). Low HRV is associated with poor CNS development. However, the connection between neighborhood stress and emotional regulation is unknown. We hypothesized that infants born to mothers who experienced stress from living in a low-quality neighborhood will have a low HRV and lack emotional regulation. Further, we hypothesized that low HRV moderates the relationship between neighborhood stress and emotional regulation. **Methods:** In the second trimester (n = 75) we used the Neighborhood Quality Evaluation Survey sense of safety subscale to measure the mother's perception of their neighborhood quality. At 6 months postpartum, mothers answered the Rothbart Infant Temperament Questionnaire (ITQ) to measure behavior

such as impulse regulation through the effortful control subscale. At the same time frame, we ran EKGs of the infants to measure their heart rate variability. **Results:** We observed no relationship between sense of safety and low HRV: $R^2 = 0.005$, B = 40.679, t = -0.0811, p = 0.562, and no relationship with effortful control and low HRV: R^2 0.002, B = -3.271, t = -0.018, p = 0.985. However, there was a positive correlation between sense of safety and effortful control: $R^2 = 0.058$, B = -0.120, t =3.310, p = 0.001. There was no evidence that HRV moderated the relationship between effortful control and sense of safety: $R^2 = 0.025$, B = -3.865, t = -0.0811, p = 0.421. **Discussion:** We found that mothers who reported safer neighborhoods had infants who scored higher on the effortful control subscale of the ITQ. Suggesting that maternal stress and physical environment shape infant emotional regulation. HRV is known to be affected by stress, but our data does not support that it influences later behavior. Researching disparities in behavioral development will help identify those at risk of later emotional regulation issues.

Disclosures: A.A. Segarra: None. **K. D'Anna Hernandez:** None.

Poster

PSTR237. Human Behavior and Social Interaction

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Program #/Poster #: PSTR237.07/VV32

Topic: H.06. Social Cognition

Support: JSPS KAKENHI Grant Number 23H00036

Title: Effective connectivity analysis of human consensus-making

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Abstract: Collective decision making in human society is based on a principle of a majority rule but also accompanies consensus-making among individuals with different interests and ideas. Making a collective decision is often more desirable than non-decision even if it results in requiring some individuals to compromise. This motivates humans to make concessions, and also requires coordinating conflicting interests to make a majority decision. Consensus making, if successful, is expected to link to distinct brain functions, such as social and non-social cognitions, and/or rational calculation and empathy. However, little is known about the mechanism through which distinct regions are connected and interacted during consensus-making. Our functional MRI study required participants make a majority decision in a small group and penalized them when a collective decision was failed to make. During consideration for voting decision, participants activated regions in the prefrontal cortex, anterior Cingular cortex, and the precuneus. In dynamic causal modeling analysis, effective connectivity across activated regions revealed distinct patterns when consensus was and was not made for collective decisions. Furthermore, the between-subject analysis found that strength of effective connectivity

from one region to another was distinct between individuals who were more and less successful in making a collective decision. Our novel findings imply that successful consensus making not only involves different neural processes but also their interaction.

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Poster

PSTR237. Human Behavior and Social Interaction

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Program #/Poster #: PSTR237.08/VV33

Topic: H.06. Social Cognition

Support: JSPS KAKENHI JP22K15635

Title: Proportions of fast and slow decision-making in relation to social distance and their neural mechanisms

Authors: *K. TSUJIMOTO, M. ABE;
Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan

Abstract: Humans have been shown to consider not only their own interests but also those of others. However, it is evident that altruistic behavior is not displayed equally toward all individuals. Research has demonstrated that individuals tend to exhibit more altruistic behavior as social distance decreases, while its proportion decreases as social distance increases. Moreover, fast and slow decisions regarding/in altruistic behavior differ in certain aspects. This study aims to investigate the proportions of fast and slow decision-making in relation to social distance as well as identify the neural mechanisms underlying these decisions.Eighteen participants underwent fMRI imaging (3.0-T MR scanner) while performing a social discounting task. Before the scanning session, the participants were asked to select individuals from their social environment and write down their names. One representative was selected for each of the following social distances: 1, 5, 10, and 20. The experiment also included social distance levels of 50 and 100, representing remote acquaintances or strangers, for which the participants were not required to indicate a name. Thus, a total of six social distances were considered. During the task, the participants were presented with the social distance number. They were then shown the amount of reward to be distributed and had to choose between two options: keeping the reward for themselves or sharing it with the individual represented by the given number.We calculated the social discounting rate, and within these proportions, the percentages of fast and slow decision-making were determined based on reaction times. Additionally, we identified the neural activity associated with fast and slow decision-making.The results of the task demonstrated that as social distance decreased, the proportion of prosocial allocation increased, while it decreased as social distance increased. Moreover, regardless of social distance, the proportion of slow decisions was significantly higher than that of fast decisions. In terms of neural activity, the temporo-parietal junction exhibited significantly higher activation during fast decision-making

compared with slow decision-making. Conversely, the ventromedial prefrontal cortex showed significantly higher activation during slow decision-making compared with fast decision-making.These findings suggest that the temporo-parietal junction is involved in the neural encoding of fast decision-making in altruistic behavior, while the ventromedial prefrontal cortex plays a significant role in the neural encoding of slow decision-making.

Disclosures: K. Tsujimoto: None. **M. Abe:** None.

Poster

PSTR237. Human Behavior and Social Interaction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR237.09/VV34

Topic: H.06. Social Cognition

Title: Analyzing Team Trust: Insights from Eye Data Analysis

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[2]Computer Sci. and Electrical Engin., UMBC, Baltimore, MD

Abstract: Trust is a relatively new area of inquiry in neuroscience. Trust has been linked to hormonal activity (e.g., oxytocin), eye behavior, pupil size, and specific brain regions like the default mode network. Among teams, trust is critical in achieving mission success, facilitating effective collaboration, and overcoming challenges. In this study, we investigated the prediction of human-human team trust by analyzing subjective, physiological, and behavioral data. The study involved multiple simulated Army missions that utilized novel technologies. Soldiers engaged in various missions within a simulated Next Generation Combat Vehicle environment, generating approximately 280 outputs from 14 crew stations (n=28) across 20 missions. Despite the challenge of assessing changes in trust during a mission, we enhanced a classification machine learning model using time-series eye data as features and trust questions as targets. The calculated eye features include pupil size, saccade and fixation. Participants completed the Team Trust Questionnaire where response options ranged from 1 (completely disagree) to 7(completely agree). Questions were divided into two classes, 0 and 1, based on scores being lower than 5 and equal to or higher than 5, respectively. Our binary classification approach provided valuable insights into participants' visual attention patterns, gaze fixation positions, and eye movement dynamics. This contributed to accurately predicting team trust levels reported on the trust questionnaire. Notably, the model demonstrated good performance with an F1 score of0.74 and a ROC AUC of 0.76 on the test data. Since the data was collected from repeated subjects, within-subject standardization was separately applied to the training and testing datasets. This approach ensured that individual-specific variations would not influence the overall analysis. Among the standardized calculated features, two key features, namely fixation in the y direction and mean pupil size, emerged as particularly vital in our study, demonstrating

their significance. The improved machine learning model serves as a robust tool for monitoring team trust during collaborative activities. However, it is important to study the generalizability of this model in assessing the human state, as task-specific physiological features can significantly influence team dynamics across different missions. By leveraging eye data analysis, we can gain insights that facilitate timely interventions and targeted strategies, optimize team performance, strengthen trust dynamics, and contribute to the growing literature on human-to-human trust in social neuroscience.

Disclosures: M. kucukosmanoglu: None. **S.M. Conklin:** None. **Q. Dang:** None. **G. Kargosha:** None. **J. Brooks:** None.

Poster

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Topic: H.06. Social Cognition

Support: National Center for Advancing Translational Science of the National Institutes of Health Award UL1TR003015/ KL2TR003016

Title: Examining the neurological and behavioral effects of musical theater training on people with disabilities

Authors: *N. TASNIM[1], D. GYAMFI[2], K. MAKANI[2], K. T. LATTIG[2], N. SINGH[3], L. B. ROCKWELL[4], D. F. ENGLISH[3], J. C. BASSO[2,3,5];
[1]Translational Biology, Medicine, and Hlth., [2]Human Nutrition, Foods, and Exercise, [3]Sch. of Neurosci., Virginia Tech., Blacksburg, VA; [4]Blacksburg High Sch., Blacksburg, VA; [5]Ctr. for Transformative Res. on Hlth. Behaviors, Virginia Tech. Carilion, Roanoke, VA

Abstract: Creating inclusive performing arts practices is important for marginalized groups, especially within the disability community. Contact with the performing arts in disabled individuals supports social and economic inclusion and promotes empowerment. However, minimal research to date has studied the effects of performing arts training on the brain and behavior for this population. STEP VA (Fredericksburg, VA) is a non-profit organization that engages the disability community through creative expression and sensory exploration. We collaborated with STEP VA to study the behavioral and neurological effects of a musical theater training program on disabled adults and children (N = 14, ages: 9-30 [median: 17], 10 male and 4 female). Participants' disabilities included Autism Spectrum Disorder (N = 11), intellectual disability (N = 2), and chromosomal abnormality (N=1). The training lasted 4 months with rehearsals 2 hours/week, along with 1 weekend rehearsal per month. The program ended with a performance of *You're a Good Man, Charlie Brown*. Self-report questionnaires (QuestionPro, Austin, TX) were administered before and after the musical training experience and were compared with Wilcoxon signed-rank tests. Participants also completed cognitive tasks

(Millisecond, Seattle, WA) and electroencephalography (EEG) testing at the end of the program. They wore a 32-channel wet electrode mobile EEG cap (Brain Products GmbH, Germany) and had their brain activity recorded (500 Hz) during interactive performance art practices, such as dancing, singing, and acting. Participants (N = 7) completed the Piers-Harris Self-Concept Scale (3rd Ed.) and reported a statistically significant ($z = -2.236$, $p = 0.025$) improvement in freedom from anxiety. Participants also completed the Revised Children's Anxiety and Depression Screening and reported a statistically significant decrease in overall anxiety ($z = -2.375$, $p = 0.018$) and total internalizing ($z = -2.366$, $p = 0.018$), which was driven by a decrease in social phobia ($z = -2.120$, $p = 0.034$) and separation anxiety (trend, $z = -1.897$, $p = 0.058$). Emotional regulation tested by the Emotion Regulation Strategies for Artistic Creative Activities Scale was significantly related (Spearman correlation) to overall quality of life ($r = 0.795$, $p = 0.010$), self-concept ($r = 0.701$, $p = 0.035$), and social connectivity ($r = 0.826$, $p = 0.006$). Overall, findings indicate that the performing arts may be a novel way to help improve mental health, especially in the realm of anxiety. Future analyses will focus on the relationship of these behavioral effects to neural activity, with a focus on intra- and inter-brain synchrony.

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Poster

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Topic: H.06. Social Cognition

Support: NSF IUCRC BRAIN #2137255
NEA Research Lab at Rice U
IUCRC BRAIN at Tec de Monterrey

Title: Understanding inter-brain synchrony and functional connectivity during dance performance.

Authors: ***M. A. PACHECO RAMÍREZ**[1], D. HUBER[2], A. J. AGUILAR-HERRERA[1], M. A. RAMIREZ MORENO[1], A. BRANDT[4], A. NOBLE[5], D. NOBLE[5], J. L. CONTRERAS-VIDAL[3]; [1]Biomed. Engin., Inst. Tecnologico y de Estudios Superiores de Monterrey, Monterrey, Mexico; [3]Electrical and Computer Engineering, IUCRC BRAIN, [2]Univ. Of Houston, Houston, TX; [4]Rice Univ., HOUSTON, TX; [5]Sam Houston State Univ., Houston, TX

Abstract: Dance encompasses a creative process that involves various cognitive-motor functions such as learning, planning, and the execution of expressive movement sequences. These elements are developed and perfected through continuous practice and refinement in a social context. Understanding the neural basis and mechanisms underlying the learning and production of expressive movements in ecological settings is an area of social neuroscience that has received

limited scientific attention. In this study, dance, music and technology were integrated in a 28-min research performance (*LiveWire*) with professional dancers and directed by a choreographer and a music composer. This unique art-science collaboration deployed hyperscanning and mobile brain-body imaging (MoBI) methods in seven rehearsals and three public performances over a period of several months. The performance drew inspiration from brain science and it was organized into five distinct movements, each representing different neuroscience concepts. Data acquisition included simultaneous recording of 28 ch. scalp electroencephalography (EEG), 4 ch. electrooculography (EOG), head motion, and video recording data of two dancers. EEG data was denoised by removing physiological and non-physiological artifacts using a pipeline based on adaptive H-infinity, ASR, and Independent Component (IC) Analysis. Dipoles from both dancers were estimated using the sources from the ICs, clustered and only shared dipole sources across the two dancers were retained; centroids were estimated to locate them to corresponding Brodmann areas (BA). These areas were projected onto the MNI template for visualization. Dancers exhibited shared recruitment of brain areas associated with visual processing (BA17, BA 18), cognition (BA 31), motor learning and planning (BA 6), kinesthesia (BA 7), and visuospatial processing (BA 5) - all of which play essential roles in dance. Intra- and inter-brain synchrony, estimated using bispectrum, fluctuated in accordance with the choreographic movements, with higher values observed during interactive movements and lower values when one dancer ceased dancing. Functional connectivity analysis showed a progressive consolidation of influence from visual areas of Dancer 1 to the motor area of Dancer 2 throughout the performances. This suggests an enhanced communication and coordination between the visuomotor networks, contributing to a more synchronized and harmonious performance.These findings have implications for social neuroscience and creativity.

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Poster

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National Science Foundation IUCRC BRAIN #2137255
Zurich University of the Arts (ZHdK)
IUCRC BRAIN at Tec de Monterrey

Title: Shared neural dynamics of actor-actor dyads during an acted scene

Authors: *Y. E. LIMA CARMONA[1], A. J. AGUILAR HERRERA[1], E. A. DELGADO JIMÉNEZ[1], M. A. RAMÍREZ MORENO[1], M. F. HENDRY[2], J. G. CRUZ-GARZA[3], J. L.

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Abstract: Cognitive neuroscience has gained increasing importance over the past few decades due to the use of ecologically valid research and mobile brain-body imaging (MoBI) techniques, which provides researchers a unique opportunity to examine the neural underpinnings of complex social behaviors in real-world settings. Acting involves the engagement of social and cognitive processes, offering valuable insights into the intricate functional brain network dynamics as well as the relations between the individual actor and actress. This study presents the evaluation of shared neural dynamics and inter-brain activation patterns of acting student dyads to investigate the neural mechanisms underlying shared cognitive, emotional and physical interactions during an acted scene. For this study, we used MoBI to capture physiological and behavioral data. Data included 28 ch, scalp electroencephalography (EEG), 4 ch. electrooculography (EOG), body and head movement, electrodermal activity (EDA), as well as video and audio recordings. All recordings were synchronized offline using event markers. Data acquisition occurred during two rehearsal sessions involving three dyads, culminating in a live public performance. To ensure the integrity and quality of the EEG signals, a robust denoising pipeline was implemented. This pipeline incorporated a combination of visual inspection and sophisticated algorithms such as adaptive filtering (H-inf), Artifact Subspace Reconstruction (ASR) and Independent Component Analysis (ICA) for removal of physiological and non-physiological artifacts from the EEG data. The Dipfit toolbox was used to identify dipole source localizations of task-related ICs, followed by clustering of such ICs across dyads. Brodmann areas were mapped using the MNI template. Data were segmented into two states: No-Gaze/Gaze, depending on whether actors did/did not engage in direct eye contact. Analysis of the live performance resulted in five clusters of common ICs (Brodmann Areas 4, 6, 10, 18 and 21), and across all dyads. Estimation of inter-brain synchrony (normalized bispectrum) during transition between these two states, revealed an incremental trend of neural synchronization during moments of gaze. This suggests that direct eye contact between actors fostered increased neural coherence and coordination, potentially facilitating enhanced interpersonal communication and emotional connection. These findings contribute to the understanding of the neural basis of acting, such as the complex interpersonal cognitive, emotional, and physical tasks required by this art form.

Disclosures: Y.E. Lima Carmona: None. **A.J. Aguilar Herrera:** None. **E.A. Delgado Jiménez:** None. **M.A. Ramírez Moreno:** None. **M.F. Hendry:** None. **J.G. Cruz-Garza:** None. **J.L. Contreras-Vidal:** None.

Poster

PSTR237. Human Behavior and Social Interaction

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR237.13/VV38

Topic: H.06. Social Cognition

Support: German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), SFB 1528 - Cognition of Interaction, project A01

Title: Confidence over competence: Perceptual decision-making during dyadic interactions in a continuous perceptual report

Authors: *F. SCHNEIDER, A. CALAPAI, A. GAIL, I. KAGAN, S. TREUE;
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Abstract: Perceptual decision-making is a dynamic process in which individuals collect, evaluate, and integrate sensory evidence to produce adequate behavior. Perceptual confidence plays a key role in this process. However, the majority of perceptual decision-making paradigms do not provide a readout of confidence. We developed a continuous perceptual report (CPR) paradigm to measure perceptual confidence in real-time. With this approach we assessed (i) the influence of sensory evidence strength on individuals' decision-making and (ii) how real-time access to perceptual responses of a partner influences decision-making. We collected psychophysical data from human participants in two conditions: alone (*solo)* and together with a partner (*dyadic*). Dyadic conditions could be real, with two participants playing simultaneously; or simulated, with a participant (who is led to believe to be playing with another participant) playing alongside a reliably accurate computer agent. In the CPR task, a random-dot pattern (RDP), changing frequently in motion direction and coherence level within a stationary circular aperture, is predictive of the location of briefly appearing reward targets. Subjects control a cursor with a joystick and are instructed to 'collect' targets by aligning the cursor to the perceived motion direction. The cursor size is coupled to the joystick's eccentricity, such that higher eccentricity - indicative of higher confidence - results in a smaller cursor. When a target is collected both accuracy and confidence are taken into account to determine the reward score. This results in peri-decision wagering, as accurate and confident responses yield high scores; less accurate and low confidence responses, or accurate and low confident responses, both yield low scores; and inaccurate responses are always misses. While in the solo condition, subjects see only their own cursor, in dyadic settings both cursors and scores are visible to both participants at all times. We find that the strength of the sensory evidence modulates accuracy, confidence, and correlation between visual motion and joystick dynamics. In dyadic compared to solo conditions, most subjects show higher perceptual confidence but unchanged accuracy. When playing a reliably accurate computer player, we observe gains in both accuracy and confidence. Overall scores improve when playing the computer player, and decrease when playing another human. These results suggest that when two individuals have access to each other's real-time assessments of shared sensory information, a partner's assumed competence and indicated confidence modulate decision-making.

Disclosures: F. Schneider: None. **A. Calapai:** None. **A. Gail:** None. **I. Kagan:** None. **S. Treue:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.01/VV39

Topic: H.08. Learning and Memory

Title: Hierarchical replay of spontaneous bursting activity in the anterior temporal lobe reflects different information layers in an episodic memory

Authors: *J. ZHANG[1,2], S. INATI[2], K. ZAGHLOUL[2], T. BEHRENS[1];
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Abstract: Replay of internally generated sequences of neuronal activity observed in rodents and humans is believed to encode past and future experiences in spatial and non-spatial contexts. In humans, magnetoencephalography has revealed that replay encodes reverse paths of visual stimuli and abstract task structure. Moreover, spontaneous bursts of spiking units in the anterior temporal lobe (ATL), which encode stimuli-specific semantic information, also replay in conserved temporal sequences during successful memory retrieval. To unify these two forms of replay in humans, we proposed the existence of a hierarchical 'sequence of sequences' replay structure in the single unit bursting activity. This structure entails that burst sequences encoding stimuli-specific semantic information not only replay to reflect individual stimuli but also replay in the order in which such stimuli were presented. To investigate this, we recorded single unit data from participants who completed a visual stimuli task involving remembering and later recalling sequences of four categorically distinct images presented in randomized order. Among participants with bursts significantly encoding stimuli-specific category information, we found evidence of hierarchical replay during the rest period prior to successful recall. Specifically, seven sessions had significant encoding of category information in the average spike rate of individual bursts and four sessions had significant encoding of category information in the burst sequences. Sessions with strong burst rate encoding displayed significant backwards replay of pairwise bursts that reflected the true stimuli presentation order. Sessions with strong burst sequence encoding displayed near significant backwards replay. In both cases, significant replay occurred across bursts that were approximately 300ms apart. These findings indicate that replay of internal neuronal sequences in single unit activity of the ATL occurs within individual bursts and across bursts, reflecting different hierarchical layers of information in an episodic memory.

Disclosures: **J. Zhang:** None. **S. Inati:** None. **K. Zaghloul:** None. **T. Behrens:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.02/VV40

Topic: H.08. Learning and Memory

Title: A neural substrate for encoding the probability of sensory inputs

Authors: *T. DALAL, R. HADDAD;
Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: Estimating the statistics of the events occurring around us is a crucial feature for survival. However, how the brain achieves this is poorly understood. We devised a 'Go/No-go' odor-discrimination task in which mice could utilize a preceding odor cue to estimate the probability of the Go odor. Recording the neural activity in the anterior piriform cortex (aPC) during task-performance, we found two non-overlapping neuronal subpopulations that encode the odor probability differentially. The first neural population ramp its firing rate from the cue onset up to the predicted odor onset. This change in firing rate was proportional to the probability of the predicted odor. The second subpopulation encoded the stimulus prediction error: strongly responding to the Go odor when it was less probable, and weaker when it was more probable. Reversing the probability contingencies remap the activity of these neuronal subpopulations to reflect the new probabilities. To explore the circuit underling such probability estimation we bilaterally silenced the orbitofrontal cortex (OFC) during task performance using inhibitory DREADDs. We found that mice learned to discriminate between the Go/No-go odors, but failed to learn the cue probability-contingencies. These results demonstrate that the primary olfactory cortex encodes the probability of an odor event, and further suggest a novel top-down OFC-aPC projection that facilitates it.

Disclosures: **T. Dalal:** None. **R. Haddad:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.03/VV41

Topic: H.08. Learning and Memory

Support: NIH/NINDS Grant 2RF1NS023945-28

Title: Basal forebrain projections to the retrosplenial and cingulate cortex in rats

Authors: *H. KONDO, L. ZABORSZKY;
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Abstract: Our previous studies (Kondo and Zaborszky, 2016; Gielow and Zaborszky, 2017; Chavez and Zaborszky, 2017) have shown that different cortical regions receive cholinergic/noncholinergic projections from distinct portions of the basal forebrain (BF). In the present study, we examined the distribution of cholinergic and noncholinergic neurons in BF that project to the retrosplenial (RSC) and cingulate (Cg) cortex in rats, two association cortical

regions, not investigated in detail in previous studies. We injected the retrograde tracers Fast Blue (FB) and Fluoro-Gold (FG) in RSC and Cg and brain sections were processed for choline acetyltransferase immunostaining. We found that RSC and Cg receive similar but some differential projections from BF. RSC and Cg receive cholinergic and noncholinergic projections mainly from the vertical limb of the diagonal band (VDB) and horizontal limb of the diagonal band (HDB) and neurons projecting to these cortical regions intermingle in BF. In addition, Cg receives input from the rostral but not caudal part of the globus pallidus (GP) whereas RSC receives weaker input from the rostral GP than Cg. RSC receives stronger input from the rostral BF (VDB) than Cg. In HDB, BF neurons projecting to these areas are present in the medial part of HDB. We found some double labeled noncholinergic and cholinergic neurons that project to both cortical regions but these neurons were few. Compared to our previous results in other cortical regions, the distribution of BF neurons projecting to RSC and Cg is different from that to other cortical regions. The perirhinal, postrhinal, and auditory cortex receives input mainly from the caudal BF including the caudal GP. The somatosensory and motor cortex receives more input from GP and no or weak input from the rostral BF. These results suggest that RSC and Cg receive similar but some differential projections from BF and that the projection pattern from BF to these cortical regions is different from that to other cortical regions.

Disclosures: H. Kondo: None. **L. Zaborszky:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.04/VV42

Topic: H.08. Learning and Memory

Support: OU Vice President for Research and Partnerships

Title: Iron deficiency results in changes to brain activity that persist over learning

Authors: *M. J. WENGER[1], M. P. DUNGAN[2], L. A. DE STEFANO[1], S. E. RHOTEN[2]; [1]Psychology Cell. and Behavioral Neurobio., [2]Psychology, Univ. of Oklahoma, Norman, OK

Abstract: We previously (SfN Connectome, 2021) presented data suggesting that iron deficiency produces marked changes in brain dynamics. We here sought to determine whether those changes were attenuated over the course of learning. A total of 42 healthy female participants (19-27 y), 22 iron deficient non-anemic (IDNA) women and 20 iron sufficient (IS) women, completed two category learning tasks. The stimuli were grayscale Gabor patches that varied in spatial frequency and orientation. Two category structures were learned by each participant. The first (rule-based, RB, a declarative memory task) was designed to require that participants combine the information from each dimension in a way that could be described by an easily verbalizable rule. The second (information integration, II, a procedural memory task) could not be easily described by a rule and had to be learned by stimulus-response association.

Concurrent EEG was recorded during the learning of both categories. Both the IS and IDNA participants successfully learned the tasks, with performance differences disappearing after approximately 800 trials. Accuracy for both groups increased reliably as a function of practice, and accuracy for the IDNA participants was lower than that for the IS participants but only initially. There was a reliable decrease in RTs as a function of practice for both groups. Overall, RTs for IDNA participants were longer than those for IS participants. In addition, lower initial accuracies and longer initial RTs were observed for the ID women relative to the IS women, only on the RB task. To determine whether learning changed the differences in brain activity between IDNA and IS women, we focused on the first 800 trials, and divided those into two sets of 400 trials. The first 500 ms of EEG data on each trial were divided into 20 ms bins, and an average for each electrode for each time bin was obtained. We used logistic regression with leave-one-out cross-validation and stepwise model selection to determine the smallest set of electrodes needed to classify the activity with respect to the iron status (IDNA, IS) of the participant. For both tasks, classification accuracy (area under the curve, AUC) exceeded 0.90 as early as 250 ms using fewer than 10 electrodes. Although there was some evidence for left lateralization in the II task, the electrodes used were primarily central and parietal. Learning (early vs. late) did not appear to alter classification performance. This suggests that the effects of iron deficiency on brain activity persist over the course of learning, even to the point at which there were no differences in behavior.

Disclosures: M.J. Wenger: None. **M.P. Dungan:** None. **L.A. De Stefano:** None. **S.E. Rhoten:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.05/VV43

Topic: H.08. Learning and Memory

Support: 1 R01 MH113238

Title: Neural correlates of reappraisal and distraction during the regulation of negative memories in an adult lifespan sample

Authors: *J. GRANER, L. FAUL, J. M. DIEHL, D. MADDEN, M. J. SMOSKI, K. S. LABAR;
Duke Univ., Durham, NC

Abstract: Cognitive reappraisal and attentional distraction constitute two core strategies for regulating emotions according to Gross's process model. Prior studies have implicated distributed cortical networks that are engaged when individuals volitionally recruit these strategies to down-regulate negative affect. This research has largely focused on young adults who regulated simple laboratory stimuli, with few direct fMRI comparisons between these

strategies. Here we expanded the typical age range of participants, compared reappraisal and distraction within subjects, used ecologically-valid autobiographical memories as the regulatory targets, and implemented distraction by redirecting participants' thoughts toward positive experiences. Sixty-two healthy adults aged 35-75 yrs were recruited to generate cue words for negative and neutral autobiographical memories. Participants were trained to reappraise, distract, or let their emotions flow naturally in response to the personalized negative memory prompts (with flow only for neutral memories). In the scanner, participants rated their current affective valence following implementation of each strategy. Strategy-specific contrasts were derived from whole-brain fMRI data using univariate analyses implemented in FSL. Results largely replicated prior work in younger adults, with distributed patterns of activity for reappraisal, relative to flow, in bilateral occipital cortex and primarily left-sided frontal, temporal, and parietal cortices, as well as in the right cerebellum and cingulate cortex. Distraction, relative to flow, engaged bilateral lateral prefrontal, medial parietal, cingulate, occipital and retrosplenial regions, as well as left cerebellum. Direct comparisons between the two active regulatory strategies yielded similar results as the strategy-specific maps. Common areas of activation across both strategies were found in occipital and frontocingulate cortices. Behavioral ratings confirmed the efficacy of the experimental manipulation and revealed a negative impact of age on reappraisal success. Reappraisal success was correlated with greater engagement of visual cortical processing. These findings validate and extend knowledge regarding the neural mechanisms of emotion regulation across the adult lifespan for autobiographical events.

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Poster

PSTR238. Cortical Networks

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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Topic: H.08. Learning and Memory

Support: National Research Foundation of Korea (NRF) grant funded by the Korean government (0684-20230001 to S.J.K)
National Research Foundation of Korea's Brain Korea 21 FOUR Program (A0432-20230100)

Title: Temporal dynamics of Purkinje cell excitability drives rapid cerebellar systems consolidation

Authors: *J. SEO[1,2], Y. KIM[1,2], Y.-S. LEE[1,2,3], S. KIM[1,2,3];
[1]Dept. of Physiol., [2]Dept. of Biomed. Sci., [3]Memory Network Med. Res. Center, Neurosci. Res. Institute, Wide River Inst. of Imm, Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Initially encoded motor memory in the cerebellar cortex becomes mature through systems consolidation. Despite evidence for the involvement of intrinsic excitability of Purkinje cell (PC-IE) in the consolidation, the underlying neural mechanism awaits elucidation. Here, we demonstrate a specific spatiotemporal profile of PC-IE during the consolidation. An optogenetic enhancement of PC-IE robustly impaired memory consolidation that is only effective within the 90 min post-learning period, which we defined as an essential time window for long-term storage. Accordingly, PC-IE becomes depressed after motor learning, but past that critical time point, the depression is diminished. Furthermore, abnormally increased PC-IE not only disrupts the formation of long-term memory but also abolishes the intrinsic plasticity of flocculus-targeting neurons (FTNs), a post circuitry of floccular PCs, in medial vestibular nuclei (MVN). This finding demonstrates that PC-IE is a plausible neural substrate for coupling two interdependent regions, the cortex and nuclei, which underlies cerebellar systems consolidation. Collectively, these results suggest that the precise timing and distinctive plasticity of PC-IE determine systems consolidation.

Disclosures: **J. Seo:** None. **Y. Kim:** None. **Y. Lee:** None. **S. Kim:** None.

Poster

PSTR238. Cortical Networks

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Program #/Poster #: PSTR238.07/VV45

Topic: H.08. Learning and Memory

Support: NIH Grant 5R01NS106611-05
NIH Grant 5U01NS113198-04

Title: Theta phase synchrony underlies improved memory retrieval

Authors: *A. RAO[1], M. J. KAHANA[2];
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Abstract: Phase synchrony effects in the theta band are a robust electrophysiological correlate of free recall performance. Previous studies have found that macroscale theta synchrony underlies improved encoding of list items, and that this effect resides in synchronous hubs distributed throughout the brain. However, the character of the theta synchrony effect underlying retrieval is not well understood. Here, we analyzed free recall data from patients implanted with intracranial electrodes for epilepsy monitoring (n = 218). Each session consisted of trials of sequentially presented word lists that the subject subsequently attempted to recall. For each subject, we computed the difference between theta (3-8 Hz) phase locking across the 1 s period preceding correct recall of an item, and phase locking before a matched period of silence, for each pair of electrodes, also controlling for spurious effects by comparing phase locking difference values against a null distribution. Functional theta synchrony networks were then averaged across subjects to produce a general theta synchrony network map for memory retrieval. We report that

a robust general theta synchrony effect, spanning all region-region pairs, theta frequencies, and time epochs, underlies retrieval (p = .002). Moreover, we find 28 regions that serve as significant hubs of theta positive synchrony (p < .05, FDR-corrected), while no region was a significant hub of negative synchrony. Among the most significant hubs were regions heavily implicated in memory processes, such as the MTG, EC, and HPC, but the hubs were overall distributed throughout the brain. Moreover, the time epochs and frequencies at which the theta synchrony effect was most robust varied considerably from hub to hub, although in the whole-brain network, it remained concentrated to 3-4 Hz and the period immediately preceding retrieval. We also report no correlation between node strength and power across brain regions (r = 0.172, p = 0.417). Future work will probe further the relationship between these findings of theta synchrony and the modulation of power in theta and other frequency bands to understand dependencies between the two dimensions of electrophysiological correlates of memory. Moreover, we will further compare the functional networks of memory encoding and retrieval to understand whether the retrieval network recapitulates encoding effects, or whether it represents a distinct pattern of functional connectivity.

Disclosures: **A. Rao:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **M.J. Kahana:** None.

Poster

PSTR238. Cortical Networks

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Program #/Poster #: PSTR238.08/VV46

Topic: H.08. Learning and Memory

Support: NSFC Grant 31827803

Title: Opposing roles of projections from lOFC and AIC to BLA in learning and memory

Authors: *J. ZHANG[1,3], C. QI[4], C. T. LI[2];
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Abstract: How cortical inputs from various regions are integrated in subcortical areas to control behavior is an essential question in systems neuroscience. Here we addressed the question focusing on cortical inputs to the basolateral amygdala (BLA), which is critical for working memory and reward learning. BLA receives inputs from the orbitofrontal cortex (lOFC) and agranular insular cortex (AIC), yet it is not clear how the projections from these two regions to BLA contribute to learning and memory. Anterograde tracing showed that the BLA neurons receiving projections from lOFC exhibited more anterior and dorsal distribution than those from AIC. We then trained head-fixed mice to perform olfactory-based working-memory task. Optogenetically suppression of delay-period activity of the lOFC-BLA projections in the

learning phase improved task performance, whereas suppression that of the AIC-BLA projection impaired task performance. To examine neuronal encoding of learning and memory related information by these two pathways, we conducted two-photon calcium imaging of BLA neurons receiving projections from either lOFC or AIC. We found stronger encoding of working-memory related information in the BLA neurons receiving AIC projections than that receiving lOFC projections. Thus, the projections from the lOFC and AIC to BLA exert opposing roles in learning of working-memory task, through distinct neuronal populations.

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Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

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Program #/Poster #: PSTR238.09/VV47

Topic: H.08. Learning and Memory

Title: Serotonergic cortical dynamics associated with trace fear extinction: retrosplenial cell types expressing 5HT2c receptors as a target of psilocybin

Authors: *S. A. ROGERS[1], S. WISSER[2], K. CZARNECKI[3], E. A. HELLER[4], G. CORDER[1]; [1]Psychiatry, [2]Neurosci. Grad. Group, [3]Pharmacol., [4]Dept. of Systems Pharmacol. and Translational Therapeut., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The psychedelic psilocybin has demonstrated efficacy in the treatment of a wide array of psychiatric disorders that display high comorbidity and share the common phenotype of cognitive inflexibility, such as depression and PTSD. Psilocybin is hypothesized to enhance cognitive flexibility via cortical 5HT2a/cRs, but the role of cortical 5HT2cRs is poorly understood. In rodents, dynamics in the retrosplenial cortex (RSC), comprised of a unique subpopulation of 5HT2cR+ neurons, are necessary for cognitive flexibility tasks including the extinction of trace fear conditioning (TFC). Psilocybin has been found to enhance TFC extinction. We hypothesize that psilocybin targets 5HT2cR+ RSC neurons to enhance trace fear extinction by enhancing neural dynamics associated with cognitive flexibility.
We first used RNAscope to replicate findings that the RSC expresses a large and unique subpopulation of putative excitatory 5HT2c+ neurons. We then confirmed that 1mg/kg i.p. psilocybin administered during the first extinction session of a five day TFC paradigm enhanced extinction 1, 2, and 30 days later. Chemogenetic inhibition of 5HT2cR+ RSC neurons during the first extinction session impaired extinction the subsequent two days, suggesting 5HT2cR+ RSC neurons are necessary for TFC extinction. To establish the neural dynamics associated with underlying TFC extinction in the RSC, we employed one-photon single-cell calcium imaging to track the changes of stimulus- and trace-evoked neural activity. To control for context-, motion-, and position-related neural activity, we compared one group of TFC mice to another group of pseudo-TFC mice that were never shocked. dPCA revealed that the contribution of freezing decision alone to neural activity was significantly reduced in shocked mice compared to non-

shocked mice; instead, the contribution of learning phase and phase/decision interaction became significantly more important. The contribution of phase/decision interaction significantly predicts freezing and the rate of extinction in shocked but not non-shocked mice, suggesting that this component of neural activity is associated with the execution of TFC extinction. Overall, we identified a putative excitatory 5HT2c+ population in the RSC necessary for TFC extinction, a behavior requiring cognitive flexibility and that the 5HT2a/cR agonist psilocybin accelerates. Having identified neural dynamics in the RSC associated with TFC extinction, our next steps will be to test the hypothesis that psilocybin accelerates these neural dynamics in a manner dependent on RSC 5HT2cR activation and that 5HT2c+ neurons are sufficient for driving these dynamics themselves.

Disclosures: S.A. Rogers: None. **S. Wisser:** None. **K. Czarnecki:** None. **E.A. Heller:** None. **G. Corder:** None.

Poster

PSTR238. Cortical Networks

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Topic: H.08. Learning and Memory

Support: CIHR Grant 156070
NSERC Discovery Grant 402642

Title: Dynamics in rodent perirhinal cortical activity during configural oddity discrimination task

Authors: S. S. DHAWAN, A. LEE, ***R. ITO**;
Univ. of Toronto, Toronto, ON, Canada

Abstract: The perirhinal cortex (PRC) has been suggested to represent complex conjunctions of object features, and is recruited across a range of cognitive processes including perception and memory. For example, the rodent PRC has been implicated in the discrimination of simultaneously presented objects that share overlapping features (i.e., high feature ambiguity) but not objects that can be differentiated on the basis of a single feature (i.e., low feature ambiguity). Notably, this evidence, to date, is derived from studies using lesion approaches that typically produce damage encroaching on other parts of the medial temporal lobe, and direct PRC involvement during configural object discrimination using real-time in vivo recording has yet to be demonstrated. The current study, therefore, employed in vivo optogenetics and fibre photometry to examine PRC activity in Long Evans rats during an oddity discrimination task, in which the rats were simultaneously presented with 2 copies of one object pair (e.g., AB1 & AB2), 2 copies of another object pair (e.g, CD1 & CD2), and 1 ‘odd’ object pair that possessed high feature ambiguity with the other presented object pairs (e.g., AD), and their odd object preference compared to a low feature ambiguity control condition in which the ‘odd’ object pair

was a novel object pair (e.g., EF1, EF2, GH1, GH2, IJ). As expected, optogenetic inhibition of PRC (n=18) led to impairments in oddity preference during the high, but not low, feature ambiguity condition. Fiber photometry recordings from PRC (n=19, n=8 females) revealed that animals exhibited greater magnitudes of calcium activity (e.g., peak amplitude) for longer periods of time (e.g., AUC) when exploring the configural odd object-pair compared with a control object-pair. Furthermore, this increase selectively occurred during the second half of the test session, after animals were able to gather information about the object stimuli. This effect also coincided with a significant decrease in calcium activity when exploring familiar objects, which is consistent with repetition suppression of PRC neural activity during object recognition memory. Our findings provide further support for a role for the PRC in representing conjunctions of object features.

Disclosures: S.S. Dhawan: None. **A. Lee:** None. **R. Ito:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

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Program #/Poster #: PSTR238.11/VV49

Topic: H.08. Learning and Memory

Title: Investigating the neurological substrates of creative thinking in engineering design

Authors: *F. MIKAMI, K. KOIZUMI, K. UEDA, M. NAKAO;
the Univ. of Tokyo, Tokyo, Japan

Abstract: Creative thinking is unique to human beings and is fundamental in several activities. It can be divided into two main phases: divergent and convergent thinking. The former is the first step, elaborating many ideas without constraints, and the latter applies the ideas to the constraints of the problem at hand. These two different phases are complicated and intertwined; thus, it is difficult to separate them. Recent studies have revealed the large-scale brain networks involved in each phase, and how the corresponding brain areas work during creative thinking. In addition, the brain areas activated during engineering design have been detected (Hay, 2019). However, the separate divergent and convergent thinking phases in the same design tasks have not been investigated in detail. In this study, we focused on both phases in engineering design applications and compared the results to standard widespread creativity tasks, namely alternate uses task (AUT) and remote associates task (RAT). For this research, we employed 5 of 20 creative tasks as engineering design challenges. These activities required ideas to solve everyday life problems, such as "lighting towns and cities at night has negative environmental impacts (e.g., fossil fuel depletion, light pollution, and disruption to wildlife). Generate concepts for products that may improve the environmental impacts of lighting urban areas." (Hay, 2019). To disentangle divergent and convergent thinking, we segregated the thinking time into limited periods. In the divergent thinking step, a participant looped five times for each design task of the routine, thinking about one idea for 10 seconds and then writing it down. We analyzed the EEG data for

each 10-second period of thinking time as divergent thinking. In the convergent thinking step, a participant examined all the ideas generated in the former phase, evaluated and summarized them for 30 seconds, and sketched the best idea for 2 minutes. We analyzed the EEG data for these 30 seconds as convergent thinking. For comparison, we also conducted an experiment with AUT and Japanese RAT (Terai, 2013) in a similar way. We evaluated the scores of the answers, analyzed the EEG data, and
compared the active brain areas during high score phases and low score phases. Furthermore, we compared the results to determine the differences between divergent and convergent thinking during an engineering design test, as well as between design and standard creativity tests.

Disclosures: **F. Mikami:** None. **K. Koizumi:** None. **K. Ueda:** None. **M. Nakao:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.12/VV50

Topic: H.08. Learning and Memory

Support: STI2030-Major Projects

Title: Memory-like representation of trial outcome during sensorimotor associative learning in primate posterior parietal cortex

Authors: *Z. LIU, Z. JIANG, L. SHI, Y. ZHOU;
Peking Univ., Beijing, China

Abstract: Memory-like representation of trial outcome during sensorimotor associative learning inprimate posterior parietal cortexZiang Liu[1], Zhuangyi Jiang[1], Li Shi[1], Yang Zhou[1]*1School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Peking-TsinghuaCenter for Life Sciences, Peking University, Beijing, 100871, ChinaCorresponding author e-mail address: yangzhou1@pku.edu.cn***Abstract**Interacting with environment usually results in reward or punishment. During associativelearning (AL), such outcome information is crucial for establishing new sensorimotorassociations by strengthening correct associations and modifying incorrect ones.Although the neural representations of trial outcome have been identified in frontalcortex and several subcortical areas, the neural mechanisms underlying monitoring thebehavioral outcome during long-term AL remines elusive. Meanwhile, whether and howposterior parietal cortex (PPC), which is another important cortical node for manysensorimotor functions, is involved in monitoring trial outcome to guide AL have not beenexplicitly studied. Here, using two photon calcium imaging technique, we recorded theactivity of more than 20000 neurons in 7a, a PPC subregion, from two monkeys whenthey were learning new image-saccade associations. We found that about half of the 7aneurons significantly represented the behavior outcome in the current trial and\orprevious trials. This outcome representation did not just reflect receiving\missing rewardas it changed with the levels

of punishment after incorrect response, and dramaticallydecreased in the task requiring no AL. Importantly, tracking the activity of the samepopulations of neurons through the long-term learning course, we found that theneuronal representation of trial outcome in 7a largely reorganized when monkeysswitched from exploiting a well-learned associations to learning new image-saccadeassociations, and then gradually evolved in a predictable manner during learning the newassociations across different learning days. These results indicate that primate PPC mightplay a substantial role in the long-term AL through evaluating behavioral outcome toguide future behavior, extending beyond its widely recognized role in sensorimotorprocessing.

Disclosures: Z. liu: None. **Z. jiang:** None. **L. shi:** None. **Y. zhou:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.13/VV51

Topic: H.08. Learning and Memory

Support: NIH DC019124
NIH MH083809

Title: Neuronal Responses in the Anterior Olfactory Nucleus During a Complex Odor Memory Task

Authors: *W.-Y. WU[1], J. FANG[1], T. A. CLELAND[1], C. LINSTER[2], D. M. SMITH[1];
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Abstract: The anterior olfactory nucleus (AON) is an important but understudied component of the olfactory sensory processing stream. It is interconnected with the olfactory bulb and the piriform cortex, and it receives input from the hippocampus, amygdala and a variety of other forebrain structures (Quintela et al, 2022, *Neuroforum*), making it a likely gateway through which spatial and contextual information might influence olfactory perception (Aqrabawi & Kim, 2018, *Nat Comm*). However, unlike the more well-studied olfactory bulb and piriform cortex, no studies have documented the neurophysiological responses of AON neurons in awake behaving subjects performing complex olfactory memory tasks. Here, we trained rats to dig for a buried treat in cups of odorized digging medium and recorded AON neuronal responses using custom microdrives containing 24 independently movable tetrodes. Eighteen pure odorants served as cues, and the rats were trained on two different lists of 12 odors in different contexts (black or white boxes). Within each odor list, half of the odors were rewarded and the other half were not. The lists were constructed such that half of the odors were common to both lists, but their predictive value was reversed. For each trial, we placed a single cup containing one of the odor cues into the box and the rat was allowed to approach the cup and dig or turn away from the cup in order to initiate the next trial. Errors were recorded if the rat dug in a non-rewarded cup or failed to dig in a rewarded cup. Recording sessions consisted of 144 trials, half from list 1 and

half from list 2. Thus, the rats had to remember whether each odor was rewarded or not under the rules of the current odor list and context conditions. Rats learned to perform this task at a high level (90% correct, on average) and AON neurons exhibited robust responses to the odor cues (n=297 neurons recorded thus far). Approximately 39% of AON neurons responded to at least one of the odor cues with an increased firing rate, and more than half of them (53%) responded to more than one odor. Additionally, ~30% of AON neurons exhibited an 'off' response, with reduced firing at the time of odor sampling. Many neurons exhibited 'on' responses to some odor cues and 'off' responses to others. The response patterns to the various cues were often complex, with some neurons sensitive to the valence of the odor cues and others exhibiting context-dependent responses. These results indicate that AON neurons are strongly engaged during odor sampling and they exhibit a complex array of responses that likely play a key role in olfactory memory processes.

Disclosures: W. Wu: None. **J. Fang:** None. **T.A. Cleland:** None. **C. Linster:** None. **D.M. Smith:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.14/VV52

Topic: H.08. Learning and Memory

Support: NIH Grant MH083809

Title: Time Cells in the Retrosplenial Cortex

Authors: *D. SUBRAMANIAN, D. M. SMITH;
Dept. of Psychology, Cornell Univ., Ithaca, NY

Abstract: The retrosplenial cortex (RSC) is a key component of the brain's memory system, with anatomical connections to the hippocampus, anterior thalamus, and entorhinal cortex. This circuit has been implicated in episodic memory and many of these structures have been shown to encode temporal information, which is critical for episodic memory. For example, hippocampal time cells, which reliably fire during a specific segment of time during a delay period, are thought to play a role in encoding temporal information (Eichenbaum, 2013, *TiCS*). Although the RSC lesions have been associated with a specific deficit in temporal memory processes (Bowers et al, 1988, *Brain and Cog*), time cells have not been observed there. In the present study, we examined delay-related firing patterns of RSC neurons (granular b subregion) in two behavioral tasks from previously published studies, a blocked alternation plus maze task (Smith et al, 2006, *J Neurosci*) and a light-cued T-maze task (Vedder et al, 2017, *Cer Cortex*). For the plus maze task, rats (n=10) were required to approach the east arm of the maze for reward during the first block of 15 trials and then switch to the west arm for the second block of 15 trials. The east and west trials were not cued, so the rat had to remember where the rewarded arm was for each trial.

In contrast, for the light-cued T-maze task, the reward location was explicitly cued with a bright flashing light at the start of each trial and the rats (n=5) simply had to approach the light for reward, so there was no requirement to hold a memory during the intertrial delay. We found that time cells were readily observed in the plus maze task, with ~31% of neurons (56 out of 180) showing reliable time fields. Most of these neurons (~70%) exhibited clear epochs of elevated activity which covered the entire duration of the delay, similar to those seen in the hippocampus, although the firing fields were broader and baseline firing rates were higher. Interestingly, we also found that RSC neurons can exhibit inhibitory time fields, epochs where firing rates are reliably lower than baseline, often near zero (~30% of neurons). Similar to the hippocampus (Gill et al, 2011, *Hippocampus*), RSC time cells differentiated the east and west trials in the plus maze task. Time cells were also observed in the light-cued T-maze. However, they were less prevalent (11%, 22 of 203 neurons) and they did not differentiate left and right trials as well as they did in the plus maze, suggesting that RSC time cells are sensitive to the memory demands of the task. Overall, these results suggest that temporal coding may be a prominent feature of RSC firing patterns, consistent with an RSC role in episodic memory.

Disclosures: D. Subramanian: None. **D.M. Smith:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.15/VV53

Topic: H.08. Learning and Memory

Support: NIH MH083809
Cornell A&S New Frontiers Grant

Title: Medial prefrontal cortical neurons differentiate match and non-match cues in a continuous olfactory match-to-sample task

Authors: *H. TURKER[1], D. A. BULKIN[2], D. SMITH[1];
[1]Cornell Univ., Ithaca, NY; [2]MathWorks, Natick, MA

Abstract: Ongoing goal-directed behavior involves the encoding of task-relevant information, retrieving said information when needed to make a decision, executing the appropriate behavior, and then discarding the information when it is no longer useful, to prevent interference. The prefrontal cortex is thought to be involved in all those steps by supporting working memory, decision-making, cognitive control of memory retrieval, and behavioral control. We examined the neural underpinnings of those processes by recording from the medial prefrontal cortex (mPFC) while rats performed a continuous match-to-sample (CMTS) task which depends on the mPFC (Peters & Smith, 2020, Behav Neurosci). Sessions consisted of 96 trials, in which the rats were presented with a cup containing one of 12 odors mixed into digging medium. They learned to dig for a buried reward when the cup contained the same odor as the previous trial (Match),

but to refrain from digging on unrewarded trials where the current odor was different (Non-Match). Trials began when the cup was placed into the box and the rat approached the cup, investigated the odor and either dug for a treat or turned away to initiate the next trial. Errors were recorded if a rat dug on Non-Match or refrained from digging on Match trials. Thus, the CMTS requires repeated comparison of the current odor to a constantly changing memory representation of the previous odor, while ignoring interfering effects of odor memories from trials further back. Nevertheless, rats performed well (82% correct). We recorded 859 mPFC neurons from 50 sessions with four rats. Many exhibited striking responses when the rat arrived at the cup: they were not sensitive to the sensory qualities of the odor cues, but they clearly differentiated between Match and Non-Match conditions, regardless of the specific odor. At the population level, a classifier trained on firing rates in the first 2 seconds following cup arrival successfully differentiated Match from Non-Match trials 80% of the time. Other neurons had firing patterns time-locked to digging. We were able to disentangle neural Match signals, which elicit a digging response, from the execution of the response itself, meaning mPFC activity is not simply a reflection of the behavioral responses. Still other responses were time-locked to turning away from the cup, which may be related to the decision to withhold digging and initiate the next trial. In sum, mPFC activity reflected the task structure, including Match/Non-Match comparison and the decision to dig or turn away. Thus, our results support the idea that the mPFC plays a role in working memory, resisting the effects of proactive interference, and behavioral control.

Disclosures: H. Turker: None. **D.A. Bulkin:** None. **D. Smith:** None.

Poster

PSTR238. Cortical Networks

Location: WCC Halls A-C

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Program #/Poster #: PSTR238.16/VV54

Topic: H.08. Learning and Memory

Support: NIH MH083809
A&S New Frontiers Grant to D. Smith

Title: Differing Roles of the Prelimbic and Infralimbic Cortices in a High-Interference Olfactory Memory Task.

Authors: *D. J. JUN, R. SHANNON, L. GENAO, A. MOK, D. M. SMITH;
Dept. of Psychology, Cornell Univ., Ithaca, NY

Abstract: The medial prefrontal cortex (mPFC) plays a key role in memory and behavioral flexibility. Previous work in our laboratory and others has indicated that the mPFC is particularly important for resolving mnemonic interference (Peters et al., 2020, *Behav Neurosci*). Other studies have indicated that the prelimbic (PL) and infralimbic (IL) subregions of the mPFC may have different functions, with separate roles in drug seeking, avoidance, and fear conditioning (Gourley & Taylor, 2016, *Nat Neurosci*). One hypothesis suggested by these findings is that the

PL and IL are involved in promoting and suppressing behavioral responses and memory. In the present study, we used inhibitory DREADDs (pAAV-hSyn-hM4D(Gi)-mCherry) to examine the differing roles of the PL and IL cortices in an olfactory memory task designed to induce high levels of interference. Rats were first trained on one set of eight odor discrimination problems (List A), in which they learned to dig for a buried treat in one cup of odorized digging medium and avoid digging in the other cup with a different, non-rewarded odor. After reaching asymptotic performance on List A, the rats were trained on a second set of discrimination problems (List B), in which each pair contained a conflicting odor (i.e. List A odors with reversed reinforcement contingencies) and a novel odor. After reaching asymptote on List B, the rats were given three days of testing involving a mid-session switch from one list to the other. Prior to each test session, control and DREADD rats were given i.p. injections of clozapine-N-oxide (CNO). We found the control rats performed significantly better on the most recently learned list (List B) across all 3 days of testing, suggesting that the learning of List B resulted in the suppression of potentially interfering items from List A. PL-inhibited rats performed similarly, suggesting that the loss of PL does not disrupt this function. In contrast, IL inhibited rats did not perform better on List B. Instead, they performed significantly better on the first list of the day, regardless of whether it was List A or List B. This result suggests that the loss of IL disrupts the memory control processes whereby new learning produces an enduring suppression of previously acquired memories and renders subjects abnormally sensitive to recent experience. Our findings are consistent with the general idea that the PL and IL play distinct roles in memory retrieval.

Disclosures: D.J. Jun: None. **R. Shannon:** None. **L. Genao:** None. **A. Mok:** None. **D.M. Smith:** None.

Poster

PSTR238. Cortical Networks

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR238.17/VV55

Topic: H.08. Learning and Memory

Support: NIH Grant MH083809

Title: Hippocampal remapping is unaffected by chemogenetic inactivation of the anterior thalamus or the retrosplenial cortex.

Authors: *Y.-Y. YANG, T. G. JOHNSON, J. J. HO, D. M. SMITH;
Cornell Univ., Ithaca, NY

Abstract: The anterior thalamus (AT), hippocampus (HPC) and retrosplenial cortex (RSC) are interconnected components of a neural circuit that is critical for spatial learning and memory. Damage to either the AT or RSC leads to memory deficits similar to HPC lesions in both human patients and animal models. However, the specific contributions of the AT and RSC are not fully

understood. Moreover, although disrupting communication within this circuit (e.g. fornix lesions) produces severe deficits (Warburton et al., 2000, *Eur J Neurosci*), it is not clear which direction information flows during various cognitive operations. Previous studies suggest that AT lesions have modest effects on HPC spatial representations (Calton et al., 2003, *J Neurosci*) and RSC inactivation has been found to produce spontaneous remapping in the HPC (Cooper & Mizumori, 2001, *J Neurosci*), but we do not know whether AT and RSC input are important for the kind of HPC remapping typically observed in response to changes in the environmental context. To examine this, we recorded the neural firing patterns of CA1 neurons in rats with inhibitory DREADD receptors (hM4Di) expressed in the AT (N=4) or RSC (N=6) while they foraged in two highly distinctive contexts in an ABAB sequence. Neither AT nor RSC inactivation blocked remapping in the HPC. In both cases, within-context spatial correlations were significantly greater than between-context correlations (AT, $F(1, 1205) = 2529.37$, $p < 0.0001$; RSC, $F(1, 356) = 198.03$, $p < 0.0001$), and these values did not differ from control sessions in most cases. However, AT inactivation did produce a small, but significant reduction in the spatial correlation scores for repeated visits to the same context ($p = 0.027$), as indicated by Tukey posthoc tests following a significant interaction of the inactivation and context conditions ($F(1, 1205) = 3.839$, $p = 0.050$). These findings suggested that HPC representations may be slightly less stable without AT input, although the effect size was small (Cohen's d = 0.163). RSC inactivation produced no discernable effects on remapping in the HPC. These results were surprising given that, in separate experiments, DREADD inactivation of the AT impaired T-maze alternation and produced a sex-dependent impairment in the Morris Water Maze. Overall, these results suggest that inputs from the AT and RSC are not necessary for CA1 remapping and the spatial memory impairments seen after AT or RSC damage are not likely due to disruption of HPC representations. Instead, these impairments may be the result of disrupted output from the HPC to the AT and RSC, and the underlying mechanisms deserve further study.

Disclosures: **Y. Yang:** None. **T.G. Johnson:** None. **J.J. Ho:** None. **D.M. Smith:** None.

Poster

PSTR239. Intrinsic Hippocampal Circuits

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR239.01/VV56

Topic: H.09. Spatial Navigation

Support: ERC Grant 948716

Title: All-optical electrophysiology reveals behavior-dependent dynamics of excitation and inhibition in the hippocampus

Authors: *Q. YANG, S. BAROR-SEBBAN, R. KIPPER, Y. MELAMED, Y. ADAM;
The Edmond and Lily Safra Ctr. for Brain Sci., Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The dynamics of excitation and inhibition (E/I) in hippocampal circuits are central to cognitive functions. Voltage imaging using genetically encoded voltage indicators (GEVIs), allows optical intracellular recordings of both the spiking output and subthreshold inputs. Combining GEVIs with optically-orthogonal optogenetic actuators enables all-optical control and readout of neuronal activity. Here, we employ this all-optical electrophysiology approach to investigate the dynamics of excitation and inhibition of identified cell types in the CA1 area during different behavioral states, specifically quiet versus forced locomotion. We found that both vasoactive intestinal polypeptide (VIP) INs and pyramidal neurons exhibit higher spontaneous firing rates during quiet periods, while somatostatin (SST) INs increase their firing rates during walking. By adjusting light intensities in optogenetic stimulation, we evaluated neuronal excitability and discovered that SST and pyramidal neurons were significantly less excitable during walking, indicating state-dependent gain control of the circuit. Theta oscillations, a hallmark of hippocampal activity during exploratory behaviors, were present in the intracellular activity of SST and VIP INs during walking but were weak in pyramidal neurons. To identify whether intracellular theta oscillations are driven by inhibitory or excitatory inputs, we used tonic optogenetic depolarization, which is expected to amplify inhibitory inputs and weaken the excitatory drive. We found that all cell types displayed strong theta oscillations during walking upon prolonged depolarization, an effect that was particularly strong in pyramidal neurons, suggesting that hippocampal theta activity is largely driven by inhibitory inputs. Re-imaging the same cells 2 weeks later showed similar results, suggesting high stability of these network properties. Overall, our study offers novel insights into the state-dependent regulation of hippocampal neuronal activity, E-I balance, and modulation of theta oscillations. These in-depth electrophysiological properties serve as a rich foundation for computational modeling of networks of excitatory and inhibitory neurons, further deepening our understanding of hippocampal function.

Disclosures: Q. Yang: None. **S. Baror-Sebban:** None. **R. Kipper:** None. **Y. Melamed:** None. **Y. Adam:** None.

Poster

PSTR239. Intrinsic Hippocampal Circuits

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR239.02/VV57

Topic: H.09. Spatial Navigation

Support: ERC consolidated Grant, IN-Fo-trace-DG

Title: Dynamics of dentate gyrus granule cell activity during combinatorial modification of space.

Authors: *C. ELGUETA[1], Q. LI[1], H.-L. CHEN[2], M. BARTOS[1];
[1]Phisiology I, Freiburg Univ., Freiburg Im Breisgau, Germany; [2]Freiburg Univ., Phisiology I, Germany

Abstract: Theoretical and experimental data support the hypothesis that the Dentate Gyrus (DG) is important for context discrimination. The small number and low activity of granule cells (GCs) engaged in a spatial representation contribute to the sparsification of the rich multimodal input arriving from the entorhinal cortex and the orthogonalization of overlapping information. The DG can efficiently distinguish different spatial contexts, either by the activity of different GCs or by changes in their firing rate. However, it is currently unclear which features of a spatial experience induce GC remapping, how newly formed representations change over time and how they might relate to past experience. To investigate this, we recorded Ca^{2+} signals from mature GCs in freely moving Thy1-GCaMP mice implanted with microprisms and used head-mounted miniscopes during the presentation of three different contextual conditions. First, animals explored two very different novel environments during eight consecutive days. In these conditions, GC activity was sparse and showed high spatially selectivity from the first day of recording. Afterwards, the two contexts were merged into a larger arena by removing a separating wall. Interestingly, this generated an abrupt and progressive increase in the number of recruited GCs during eight consecutive recording days and an increase in the mean network activity. Intriguingly, the representation of the combined environment included both, GCs that were previously active in one of the separated rooms, and a high number of cells selective for the merged arena. Therefore, a combined environment is represented in the DG as more than the sum of its individual components. Interestingly, performing the same experiment in reverse order, by presenting animals first to the merge environment and afterwards to the separated contexts, produced similar effects, suggesting that it is the novelty of this experience which induces the increase in active granule cells and not the geometry of explored space.

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Poster

PSTR239. Intrinsic Hippocampal Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR239.03/VV58

Topic: H.09. Spatial Navigation

Support: Australian Research Council's Discovery Projects funding scheme (Project DP220101166)
Melbourne Research Scholarship
RMH Neuroscience Foundation

Title: Neural Networks' Distance from Criticality During a Cognitive Task in a Psychopharmacological Mouse Model of Alzheimer's Disease

Authors: *F. HABIBOLLAHI SAATLOU[1], D. SUN[2], A. N. BURKITT[4], **C. FRENCH**[3]; [1]Univ. of Melbourne, Melbourne, Australia; [2]Univ. of Melbourne, Melborune, Australia; [3]Med., Univ. of Melbourne, Melbourne, Australia; [4]Dept. of Biomed. Engin., The Univ. of Melbourne, Melbourne, Australia

Abstract: Dynamical systems may transition between ordered and disordered states and “criticality”, where the input is neither strongly damped nor excessively amplified, occurs when the system is at the borderline between these states. It has been hypothesized that impairments in brain function such as dementia or epilepsy could be related to suboptimal critical behavior and that deviation from criticality may be a potential biomarker for cognition-related neurological and psychiatric impairments. 15-minute episodes of widefield calcium imaging from several hundred hippocampal CA1 neurons using Miniscopes in 8 freely-behaving mice were used to study criticality measures during rest, novel object recognition (NOR) task, and NOR following the amnestic drug scopolamine. A constrained non-negative matrix factorization algorithm extracted neuronal spatial footprints and temporal traces. Neural avalanches were identified by thresholding the temporal calcium transients of ensemble activity. Four independent criticality measures were calculated: power-law distribution, deviation from criticality (DCC), shape collapse error (SCe), and branching ratio (BR). At rest, the hippocampal ensembles displayed some features of a critical system (power law expansion < 2 orders of magnitude, DCC=1.115±0.182, SCe=0.263±0.035, BR=0.918±0.012 (mean±SE)). However, during the cognitive task of NOR, the network activity shifted significantly closer to criticality (power law expansion >= 2 orders of magnitude, DCC=0.298±0.076, SCe=0.047±0.014, BR=0.976±0.014). The dynamics shifted away from criticality when scopolamine was administered, resulting in memory and task impairment (power law expansion < 2 orders of magnitude, DCC=1.356±0.416, SCe=0.267±0.038, BR=0.832±0.160). Our results show that changing from inactivity to a cognitively engaged state shifts the hippocampal neural network towards criticality, decreasing DCC and SCe while increasing BR. The significance of all pairwise differences was established using a post-hoc Tukey’s test. Additionally scopolamine-induced functional deficits shift the network away from criticality. In contrast to previous studies, we find that hippocampal ensembles move closer to criticality when successfully processing increased cognitive load during the object recognition task. Our findings suggest that cognitive tasks utilize the computational advantages of critical network behavior, such as maximization of dynamical range, information content, and transmission. Increased distance from criticality due to scopolamine that functionally mimics Alzheimer's pathology is consistent with this hypothesis.

Disclosures: **F. Habibollahi Saatlou:** A. Employment/Salary (full or part-time):; University of Melbourne, Cortical Labs. **D. Sun:** A. Employment/Salary (full or part-time):; University of Melbourne. **A.N. Burkitt:** A. Employment/Salary (full or part-time):; University of Melbourne. **C. French:** A. Employment/Salary (full or part-time):; University of Melbourne.

Poster

PSTR239. Intrinsic Hippocampal Circuits

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR239.04/VV59

Topic: H.09. Spatial Navigation

Support: AMED PRIME 21gm6510003h0001
JST PRESTO JPMJPR1882

JSPS KAKENHI 20K06878
JSPS KAKENHI 21H05831
JSPS KAKENHI 20H03356
JST SPRING JPMJSP2139

Title: Distinct manifold encoding of diverse navigational information in the subiculum and hippocampus

Authors: *S. NAKAI[1,2], T. KITANISHI[2,3,4], K. MIZUSEKI[1,2];
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Abstract: The subiculum (SUB) plays a vital role in spatial navigation and encodes navigational information in a unique manner compared to the hippocampal CA1 area. However, the geometry of the subicular population activity remains unknown. In this study, we examined the neural population activity recorded extracellularly from the CA1 and SUB of 11 rats engaged in T-maze and open-field tasks, in which the firing activities of dozens of neurons were measured simultaneously using 256-channel silicon probes. The population activity in both areas formed low-dimensional neural manifolds homeomorphic to the external space. The neural manifold conveyed information about the rats' position, speed, and future path, with the SUB displaying higher decoding accuracy than the CA1. The neural manifolds exhibited common geometry across different rats and regions in the CA1 and SUB, as well as between tasks in the SUB. During post-task ripples in slow-wave sleep, the population activity in the SUB more frequently represented reward locations/events compared to the CA1. Thus, CA1 and SUB encode information in distinct ways within neural manifolds that underlie navigational information processing during wakefulness and sleep.

Disclosures: S. Nakai: None. **T. Kitanishi:** None. **K. Mizuseki:** None.

Poster

PSTR239. Intrinsic Hippocampal Circuits

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR239.05/VV60

Topic: H.09. Spatial Navigation

Support: Simons Foundation SCGB

Title: Neural representations in the hippocampus change during flexible navigation

Authors: *P. D. RICH[1], S. THIBERGE[2], B. B. SCOTT[4], C. GUO[5], D. G. TERVO[6], C. D. BRODY[7], A. Y. KARPOVA[8], N. D. DAW[3], D. W. TANK[3];
[1]Princeton Neurosci. Inst., princeton, NJ; [3]Princeton Univ., [2]Princeton Univ., Princeton, NJ; [4]Psychological & Brain Sci., Boston Univ., Boston, MA; [5]Janelia, Ashburn, VA; [6]Janelia Res.

Campus, Washington DC, VA; [7]Princeton Neurosci. Inst., HHMI / Princeton Univ., Princeton, NJ; [8]HHMI/ Janelia Res. Campus, Ashburn, VA

Abstract: Neural representations in the hippocampus change their responses over days and hours. Such temporal coding is thought to support memory storage and recall by providing a distinct pattern of activity that encodes individual episodes. How such a dynamic representation may support ongoing decision making is poorly understood, as such studies are usually performed in simple behavioral tasks that do not require recall of previous episodes to perform. We trained rats to perform a dynamic navigation task that required the continual learning of a new routes to various goals.
Animals were presented with an odor cue which indicated the availability of reward at a certain location in the maze. In blocks of trials, the configuration of the maze was changed so that a new route would need to be taken to get to some goals. Animals showed incremental learning of the new routes after a block change, and were able to complete 10s of blocks in sessions of 100s of trials.
We recorded cellular activity in dorsal CA1 during the odor cue presentation period while the animals were voluntarily head-fixed underneath a two-photon microscope. Voluntary head fixation allowed us to isolate the contribution of the activity in the hippocampus to cognitive processes by eliminating any positional or movement confounds.
Neural activity during the head-fixation and odor presentation showed clear odor encoding, but was invariant to the (subsequent) left right-choice and the configuration of the maze. Moreover, the neural representation changed throughout the 4 hour sessions, exhibiting representational drift at the single cell and ensemble level. We found that the degree of drift was reduced for fixed-route odors compared to odors that had dynamic routes that had to be re-learned. This difference might reflect the difference in task demands for the different odors; flexible behavior may be enabled by a more flexible code. Alternatively, since fixed-route odors were presented less frequently, the difference could reflect a familiarity-dependent change, implicating representational drift as part of an ongoing mnemonic process.

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Poster

PSTR239. Intrinsic Hippocampal Circuits

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR239.06/VV61

Topic: H.09. Spatial Navigation

Title: Animals learn to efficiently navigate complex and dynamic environments in virtual reality

Authors: *K. KAJIKAWA, W. SUN, M. MICHAELOS, R. GATTONI, N. SPRUSTON; Janelia Res. Campus, Ashburn, VA

Abstract: Through learning, animals build internal models of their environment, called cognitive maps. These maps, which are used to guide adaptive behavior, are influenced by sensory cues, motor actions, and the valence of associated outcomes. However, the relative contributions of these factors to cognitive maps and behavior are poorly understood.
In this study, we trained mice on a linear two-alternative choice (L2AC) task in a virtual reality (VR) environment. In this task, reward position is determined by distance from an initial indicator cue, as well as sensory cues that are positioned at the two possible reward locations. To determine the contributions of position and sensory cues to the cognitive map, we decoupled these features in a variety of ways using probe trials introduced occasionally in well trained mice. We found that behavioral performance was impaired on initial probe trials, but animals rapidly adapted and adjusted their behavior adaptively during additional probe trials of the same kind. Our results suggest that both position (VR distance traveled) and visual information are used in L2AC task, and mice could flexibly adjust behavior to increase reward collection.
During the behavioral tasks, we also performed large-scale 2-photon calcium imaging of thousands of neurons in area CA1 of the hippocampus. We found that the CA1 population activity was influenced in a complex way by position, visual cues, and rewards. These results suggest that animals use multi-sensory information to form cognitive maps and establish behavioral policies. The ability to record from a large number of neurons in a VR environment provides a platform for developing and testing hypotheses on neural circuit mechanisms responsible for cognitive flexibility.

Disclosures: K. Kajikawa: None. **W. Sun:** None. **M. Michaelos:** None. **R. Gattoni:** None. **N. Spruston:** None.

Poster

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Topic: H.09. Spatial Navigation

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JST SPRING, Grant Number JPMJSP2129

Title: Vta-hippocampal dopaminergic input plays a crucial role in quickly adapting spatial goals.

Authors: *Y. TAMATSU[1], H. AZECHI[2], K. IDE[2], S. TAKAHASHI[2];
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Abstract: The search for food, territory, and other rewards in animals is a fundamental behavior. The location of that reward is not always the same and can change. Therefore, animals need to adapt quickly to the location of their targets in the environment. Dopamine, a neuromodulator present in the midbrain, triggers such reward-related behaviors. However, the specific role of

dopamine neurons in the hippocampus, a brain structure deeply involved in spatial navigation, remains unclear. In this study, we examined the effects of dopamine input from the ventral tegmental area (VTA) to the hippocampus on spatial goal persistence and adaptation. Using a circular maze with varying reward locations, we compared non-deficient and VTA dopaminergic lesioned mice and found that in the absence of VTA dopaminergic neurons, spatial memory persistence was reduced when the reward location was unchanged. On the other hand, when the reward position was changed, there was no significant difference in adaptation to the reward position, although scores were lower compared to controls. In addition, a Cre-inducible viral construct encoding ChrimsonR (ChrimsonR - tdTomato) fused with enhanced red fluorescent protein was injected into the VTA of DAT-IRES-Cre mice. Optical fibers were implanted just above the pyramidal cell layer of the dorsal hippocampal CA1 of these mice. Specific stimulation of VTA dopaminergic axons within the dorsal hippocampus during task performance in these mice improved the consistency and specificity of target adaptation, regardless of timing or location of stimulation. These findings provide new insight into the contribution of intrahippocampal VTA dopaminergic inputs to goal adaptation in spatial navigation, in addition to their contribution to sustained spatial memory.

Disclosures: **Y. Tamatsu:** None. **H. Azechi:** None. **K. Ide:** None. **S. Takahashi:** None.

Poster

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Title: Double dissociation between the spatial and reward information gradients along the dorsoventral axis of the hippocampus in goal-directed navigation

Authors: *S.-W. JIN, I. LEE;
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Abstract: It is widely known that the ventral hippocampus (vHP) is densely innervated with the areas that process reward information. However, it is largely unknown how single cells in the vHP represent the reward information and what separates the area from the rest of the hippocampus. To test this, we simultaneously recorded single units along the longitudinal axis of the hippocampus [n=570 in the dorsal HP (dHP), n=1058 in the intermediate HP (iHP), and

n=106 in the vHP] using a 24-tetrode carrying hyperdrive. Rats performed a spatial alternation task on a V-shaped track in which they shuttled between two adjacent arms to obtain the reward at the end of each arm (180 trials/day). We found some cells showed their peak firing during reward consumption and their firing rates during that period were significantly greater than those during running the track. We named these cells “reward-on cells" to differentiate them from the “reward cells” reported previously (Gauthier and Tank, 2018) to fire maximally as animals approached the reward. The proportion of reward-on cells increased almost linearly along the dorsoventral axis; that is, 18% (n=101/570) in dHP, 26% (n=278/1058) in iHP, and 39% (n=41/106) in vHP (r^2=0.96, p<0.01; Linear regression). The strength of firing modulation by reward was further measured by the rate modulation index (RMI) between the firing rates during reward consumption and track running. We found that RMI gradually increased along the dorsoventral axis (r^2=0.09, p<0.001). The proportion of reward-on cells discharging at both reward locations increased gradually along the dorsoventral axis; 3% (n=17/570) in dHP, 15% (n=154/1058) in iHP, 32% (n=34/106) in vHP (r^2=0.96, p<0.01). In contrast, the spatial information score as well as the proportion of place cells gradually decreased from the dHP to vHP (p-values<0.001). This double dissociation between the spatial and reward information gradients along the dorsoventral axis in the HP should provide a different perspective on the functional organization scheme of the hippocampal networks compared to the purely spatial view especially with respect to the roles of the hippocampus in goal-directed navigation in space (Kjelstrup et al., 2008).

Disclosures: **S. Jin:** None. **I. Lee:** None.

Poster

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Topic: H.09. Spatial Navigation

Support: Fundación La Caixa (LCF/PR/HR21/52410030; DeepCode)

Title: Imaging and neural population strategies to investigate sublayer coordination of CA1 hippocampal representations

Authors: *J. QUINTANILLA[1], J. ESPARZA[1], E. CID[1], C. FORTUNATO[2], P. E. JERCOG[1], J. A. GALLEGO[2], L. M. DE LA PRIDA[1];
[1]Inst. Cajal CSIC, Madrid, Spain; [2]Imperial Col., London, United Kingdom

Abstract: The hippocampus plays a major role in spatial navigation and memory. Recent data suggest different cell-type-specific subcircuits are instrumental in orchestrating these functions. However, the difficulty to sample from different cell types simultaneously in freely moving animals is limiting progress. Here, we implement single- and dual-color miniscope-based calcium imaging of genetically identified neurons to evaluate hippocampal representations in

behaving mice running in an automated multi-track maze. We began by imaging multicellular GCaMP7 signals from the entire CA1 pyramidal layer, while mice learned to alternate for water reward in the linear track. Using population analyses, we observed that pyramidal cell activity was constrained to a neural manifold where both continuous (e.g. position) and discrete (direction) task-specific variables were jointly mapped. To tease apart the roles of superficial and deep CA1 pyramidal cells in these representations, we next applied complementary dual-color imaging strategies. Thus, we imaged all pyramidal cells using GCaMP7 while tagging superficial or deep cells with the mCherry reporter expressed in transgenic cell-type-specific Cre lines. To record activity from both layers in real time, we combined transgenic and viral injections to express green (GCaMP7) and red (jRGECO) calcium indicators in different populations. Using these strategies, we found that activity from deep and superficial CA1 pyramidal cells mapped differently onto the manifold when local cues were added to the linear track. Finally, we added cognitive complexity to the behavior by exposing mice to working and reference memory-guided dual choice tasks in the multi-track maze. The geometry of neural manifolds changed to reflect the behavioral option space with complementary contribution from deep and superficial CA1 sublayers. Our results reveal promising strategies for unbiased inference of internal hippocampal representations, and offer new insights into sublayer organization dynamics.

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Poster

PSTR239. Intrinsic Hippocampal Circuits

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Program #/Poster #: PSTR239.10/WW1

Topic: H.09. Spatial Navigation

Title: Neural activity in the hippocampus in response to the partner in prairie voles

Authors: *J. LIU[1], Y. IKEGAYA[1,2], N. MATSUMOTO[1,2];
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Abstract: The tendency of animals to interact with others and to live in groups is called sociality. Animals show sociality toward novel individuals, mates (partners), and offspring. Sociality toward novel individuals has been extensively studied in the field of neuroscience. On the other hand, sociality with specific members of the opposite sex, such as spouses, has not been well studied. In particular, it is still unclear how the abstract concept of sociality with one's spouse is represented in the brain. In this study, we used prairie voles, a naturally monogamous rodent, to investigate the neural activity corresponding to socialization with a partner. Previous research has investigated the hippocampal CA1 area as the brain region that represents abstract concepts and personal identities. Therefore, we hypothesized that partner representations also take place in the hippocampus.Silicon probes were chronically implanted into the CA1 area of

the hippocampus of male prairie voles to record the neural activity of CA1 neurons during interaction with either partner or non-partner females. In this paradigm, the males huddled with their partner females significantly earlier than their non-partners. We found that some CA1 neurons exhibited significantly higher firing rates in the partner session than the non-partner session. These results suggest that information about specific partners may be represented in the hippocampus.

Disclosures: **J. Liu:** None. **Y. Ikegaya:** None. **N. Matsumoto:** None.

Poster

PSTR239. Intrinsic Hippocampal Circuits

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Program #/Poster #: PSTR239.11/WW2

Topic: H.09. Spatial Navigation

Title: Depletion of the gut microbiome with oral antibiotics disrupts spatial cognition, neural dynamics, brain metabolism, and the blood-brain barrier

Authors: *J. M. GLYNN[1,2], J. CARRIóN[1], J. J. STROHL[1], C. BAGNALL-MOREAU[1], P. HUERTA[1,2,3];
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Abstract: While antibiotics have been tremendously beneficial in combating infection, there has been a concerning rise in the consumption of oral antibiotics worldwide. Ingestion of oral antibiotics is known to substantially alter the composition of the gut microbiome. Since the gut microbiome is increasingly being recognized as playing an important role in brain health and disease, it is imperative to understand the effects of oral antibiotic consumption on the gut-brain axis and cognition. We hypothesize that administration of antibiotics to deplete the gut microbiome will disrupt spatial cognition as well as place cell dynamics, brain metabolism, and blood-brain barrier (BBB) permeability. Male C57BL/6J mice (2-4 months) were given drinking water containing broad-spectrum antibiotics (ABX). Additional cohorts were supplemented with butyrate, a key microbiome-derived metabolite, either alone (BA) or with antibiotics (ABXBA). Spatial cognition was assessed using the object-place memory (OPM) and clockmaze tasks (PMC6568215), at 2-3 weeks of treatment. Mice underwent positron emission tomography using [^{18}F]-fluorodeoxyglucose (FDG) and [^{11}C]-aminoisobutyric acid (AIB) to assess brain metabolism and BBB permeability, respectively, at 4 weeks of treatment. BBB permeability was further evaluated using immunohistochemistry for tight junction proteins. Finally, mice were implanted with tetrode arrays to perform electrophysiological recordings of place cells in CA1 of the hippocampus. Our results show that, compared to controls, ABX mice have impairment in the OPM and clockmaze tasks, demonstrating poor spatial cognition. In addition, ABX mice have reduced FDG uptake in the entorhinal cortex, subiculum, and CA1, as well as increased

uptake of AIB in the entorhinal cortex. Furthermore, the area of the entorhinal cortex occupied by occludin, a tight junction protein, was decreased in ABX mice. Analysis of neural recordings reveals that ABX place cells have larger field sizes, reduced spatial information, and altered remapping. Remarkably, ABXBA mice display normal spatial cognition, BBB integrity, and place cell dynamics compared to BA mice. We conclude that depletion of the gut microbiome with antibiotics leads to disruptions within the hippocampus, subiculum, and entorhinal cortex, which are reflected by disrupted spatial cognition, lower metabolism, leakier BBB, and aberrant place cell properties. Coadministration of butyrate preserves spatial cognition, BBB integrity, and place cell dynamics in microbiome-depleted mice, pointing to potential therapeutic approaches.

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Poster

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Title: Hippocampal sequences span experience relative to rewards

Authors: *M. SOSA[1], M. H. PLITT[2], L. M. GIOCOMO[1];
[1]Neurobio., Stanford Univ., Stanford, CA; [2]Univ. of California, Berkeley, Berkeley, CA

Abstract: Hippocampal place cells are known to fire in sequences that span a spatial experience and ‘remap’, or change their preferred firing locations, across different spatial environments. This sequential firing is common to multiple modalities beyond space, suggesting that hippocampal activity can flexibly anchor to the most behaviorally relevant or salient aspects of experience, guided by the demands of the task. As reward is a highly salient event and a critical feature of many rodent tasks, we hypothesized that reward can likewise anchor broad sequences of hippocampal activity relative to reward. This hypothesis is supported by prior work demonstrating that reward is overrepresented by the population place field map, and by the observation of hippocampal cells that fire precisely at reward locations even when the reward is moved. If reward is indeed an anchor for hippocampal activity spanning the experience,

however, then moving the reward within a constant environment should also induce significant remapping at locations far away from the reward. To test this hypothesis, we performed two-photon imaging of calcium activity in hippocampal area CA1 as mice navigated virtual linear environments with multiple changing hidden reward locations. We tracked the activity of the same neuronal population across days to understand how individual neurons are dynamically reallocated to represent updated reward information within and across environments. We found that the hippocampus constructs and preserves broad sequences of activity relative to reward locations in a subpopulation of neurons. When the reward moves, individual cells remap to the same relative position with respect to reward, including cells with firing fields distant from reward. The density of these sequences increases with task experience, as does the overrepresentation of reward at the population level. In parallel, mice show rapid learning with each new reward update. These results suggest that the hippocampus builds a generalized representation of the task anchored to reward in parallel to its spatial map, providing insight into how hippocampal ensembles may flexibly encode the most behaviorally relevant aspects of experience.

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Poster

PSTR239. Intrinsic Hippocampal Circuits

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Title: Isolating the role of AMPA receptor trafficking in hippocampal place code plasticity

Authors: *M. PLITT[1], K. KAGANOVSKY[2], T. C. SUDHOF[1], L. M. GIOCOMO[2];
[2]Stanford Univ., [1]Stanford Univ., Stanford, CA

Abstract: Long-term potentiation (LTP) is a key plasticity mechanism underlying the formation and maintenance of hippocampal place codes. Neurons in the CA1 region of the hippocampus often express LTP by inserting GluA1-positive AMPA receptors into the postsynaptic density. However, controversy persists regarding whether various forms of LTP require dedicated molecular machinery for activity-dependent AMPA receptor exocytosis. To investigate the contribution of this pathway to in vivo plasticity we performed conditional genetic deletion of a necessary component of the postsynaptic membrane fusion machinery, Syntaxin3 (Stx3),

specifically in CA1 neurons of mice. Previous experiments show that this manipulation prevents activity-dependent insertion of AMPA receptors without affecting basal synaptic integration or homeostatic AMPA receptor trafficking. Surprisingly, we find that many hippocampal-dependent behaviors are not affected by CA1 Stx3 deletion. However, mice lacking CA1 Stx3 do not express typical novel environment preferences. By imaging calcium activity of CA1 neurons during a novel environment virtual reality behavior, we find that Stx3 is not required to form stable neural representations of context and space. This result is accounted for by a computational model in which stable CA1 codes are inherited from upstream CA3 inputs without LTP. This model also predicts differences in place field properties between mice with and without Stx3 such as place field width and the number of place fields per cell. Expanding on this hypothesis, Stx3 is necessary for endowing population codes with the properties that are unique to CA1 but not present in CA3. First, Stx3 is necessary for the experience dependent backward shift of place fields in novel environments. Second, Stx3 is essential for the increased population activity in novel environments. Third, Stx3 is required for the overrepresentation of reward locations and for the strengthening of reward location memory. In addition, CA1 Stx3 deletion prevents offline consolidation of hippocampal sequences during replay-like events. Collectively, these results suggest that activity-dependent AMPA receptor trafficking is not necessary for recomputing spatial codes that are already present in upstream regions. Spatial and contextual information can be inherited from upstream regions or learned through Stx3-independent plasticity. Instead, Stx3-dependent postsynaptic membrane fusion is central to forming and consolidating hippocampal representations of reward and novelty.

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Poster

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Title: From Molecules to Behavior: Understanding How Aging Impacts Entorhinal-Based Navigation

Authors: *C. HERBER[1], L. M. GIOCOMO[2];
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Abstract: Aging causes the gradual decline of specific cognitive abilities, like spatial memory, reducing quality of life. However, the neurobiological mechanisms underlying aging-mediated cognitive decline remain unclear, limiting the development of therapies that extend the brain's healthspan. Here, we aim to enrich our mechanistic understanding of neural aging by simultaneously characterizing and then correlating molecular, cellular, and network changes in a well-studied brain region. Across species, medial entorhinal cortex (MEC) facilitates goal-directed navigation, which is important for mobility and independence. Neurons in MEC represent self-motion cues like speed and head direction, environmental landmark locations, and maps of space within their electrical firing patterns, helping us integrate our location in space as we move. In young and aged mice (n = 8, 8) navigating an unchanging virtual reality (VR) environment, we identified a significant, age-dependent degradation in the firing patterns of spatially-tuned MEC neurons recorded *in vivo* at high density with Neuropixels 1.0 silicon probes. In addition, we observed that the short-term stability of MEC spatial cell firing is significantly compromised in aged mice. Next, we interrogated the flexibility of MEC spatial coding dynamics during a spatial memory VR task that challenged young, middle-aged, and aged mice (n = 9, 10, 10) to acquire and alternate between context-associated hidden rewards. Aged mice demonstrate significantly impaired reward alternation performance and deficits in coherent MEC spatial cell remapping to context. Ongoing analyses will explore the relationship of these neural and behavioral findings and assess the contributions of changes in interneuron firing and theta rhythm properties to instability and inflexibility in aged MEC spatial coding. Finally, in these same mice, we will characterize single neuron transcriptomic changes in MEC neurons to identify candidate genes underlying the observed changes in MEC circuit function and spatial memory during aging.

Disclosures: C. Herber: None. **L.M. Giocomo:** None.

Poster

PSTR239. Intrinsic Hippocampal Circuits

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Title: The subiculum encodes environmental geometry

Authors: *Y. SUN[1], D. A. NITZ[2], X. XU[3], L. M. GIOCOMO[1];
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Abstract: To successfully navigate, animals need to understand the geometry of their current environment. Crucial to the reconstruction of the geometric layout of natural environments are concave and convex features, such as corners and protrusions. However, the neural substrates that could underlie the perception of concavity and convexity in the environment remain elusive. Here, using longitudinal calcium imaging in freely behaving mice, we show that the dorsal subiculum contains neurons that encode corners across environmental geometries in an allocentric reference frame. Corner cells tuned their activity to reflect the geometric properties of corners, including corner angles, wall height and the degree of wall intersection. A separate population of subicular neurons encoded convex corners of both larger environments and discrete objects. Both concave and convex corner cells were non-overlapping with the population of subicular neurons that encoded environmental boundaries. Furthermore, concave and convex corner cells generalized their activity such that they responded to concave and convex curvatures within an environment. Together, our findings suggest that the subiculum contains the geometric information needed to re-construct the shape and layout of naturalistic spatial environments.

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Poster

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Title: Unveiling the structure of the grid cell code in novel environments

Authors: *J. WEN, B. SORSCHER, S. GANGULI, L. GIOCOMO;
Stanford Univ., Stanford, CA

Abstract: Animals face a near constant challenge of navigating novel environments to find food, shelter, or mates. In mammals, neurons in the medial entorhinal cortex create a map-like representation of the external environment. An essential component of this system are grid cells, neurons that are active at hexagonal spatial positions and as a population, can map the animal's position in the world. However, it remains unclear how the grid cell map can adapt to novel environmental features on a rapid, behaviorally relevant time scale. Here, by recording tens of thousands of neurons during navigation of virtual environments, we found grid cell activity was consistent with a fixed relationship between landmark input and the grid cell network. A computational model based on this fixed network accurately predicted grid spatial patterns of novel landmark arrangements. Finally, a medial entorhinal cortex dependent task revealed that while grid cell firing patterns remain fixed to landmarks, behavior can adapt to changes in landmark location, via a downstream region implementing behavioral time scale synaptic plasticity rules. Our results show that entorhinal cortex grid cells can rapidly generate maps for novel environments, but at the cost of perfect mapping between distance traveled in neural space and real space. Nevertheless, imperfections can be corrected by behaviorally relevant synaptic plasticity rules, enabling accurate navigation in rapidly changing environments.

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Poster

PSTR239. Intrinsic Hippocampal Circuits

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Program #/Poster #: PSTR239.17/WW8

Topic: H.09. Spatial Navigation

Title: Topographical organization of object coding in the human medical temporal lobe

Authors: *Y. WANG[1], R. CAO[1], X. LI[2], M. E. RAICHLE[1], S. WANG[1,3];
[1]Univ. of Washington, St. Louis, Saint Louis, MO; [2]West Virginia Univ., [3]West Virginia Univ., Morgantown, WV

Abstract: The human medial temporal lobe (MTL) plays a crucial role in memory-related behaviors. A hallmark for the MTL to support this function is by forming a highly sparse code of individuals and objects at the level of single neurons. However, the mechanism by which the brain transforms information about visual stimuli from the distributed representations of features in the higher visual cortex to the sparse representations of semantics in the MTL remains unknown. Additionally, due to the limited spatial coverage of human single-neuron recordings, a detailed delineation of this visual processing pathway is missing. To address this fundamental question, our study employed an established high-resolution 7T fMRI dataset that covers the entire human MTL while presenting a diverse range of natural scene stimuli. We investigated three distinct forms of neural coding of objects in different subregions of the MTL, including the perirhinal cortex (PRC), entorhinal cortex (ERC), parahippocampal cortex (PHC), hippocampus, and amygdala. First, we examined the semantic coding model to analyze how each subregion of

the MTL encoded visual categories. Our findings revealed that all five subregions of the MTL exhibited a significantly above-chance number of category-selective voxels and the category selectivity and sparseness increased along the processing pathway in the MTL. Moreover, each subregion displayed a distinct distribution of preferred object categories. Second, we examined the axis feature coding model using linear regression with deep neural network (DNN) features. Notably, the PHC and PRC demonstrated a substantial percentage of voxels exhibiting axis coding. Third, we examined the region feature coding model (i.e., receptive field in the high-dimensional feature space) and observed a significant number of voxels in the PHC and PRC. By visualizing the underlying visual features, we discovered that objects within a given tuning region shared a combination of similar features. Lastly, we applied representational similarity analysis (RSA) between MTL subregions and DNN units representing different levels of visual features. We found a gradient correspondence between DNN layers and different MTL subregions, suggesting that different subregions of the MTL contribute to different stages of information processing. Together, our study provides valuable insights into the coding mechanisms within the MTL, shedding light on how the brain translates visual information from the higher visual cortex to the sparse semantic representations crucial for memory-related functions.

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Poster

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Title: Dynamics of entorhinal reactivations over learning

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Abstract: Our brains represent how events unfold over time and space through neural firing sequences. When an animal is immobile, neurons in the hippocampus and medial entorhinal cortex (MEC) sequentially fire to represent time-compressed trajectories of the animal's path through space (reactivations). In hippocampus, these reactivations can represent task-relevant locations and are required for learning. However, we know little about the content or structure of reactivations in MEC, and how this may adapt to support learning. We recorded from superficial MEC in mice learning two variations of a spatial delayed-match-to-sample task. During movement, position could be accurately decoded from the MEC population, and MEC cells overrepresented rewarded locations. During immobility, the MEC population reactivated representations of either local or nonlocal single locations, rather than trajectories. MEC was less likely to represent the track's decision point during immobility as task performance improved. In ongoing work, we are further characterizing these reactivations and comparing them to MEC activity co-occurring with CA1 sharp-wave ripples. We are also examining how putative parvalbumin-expressing and somatostatin-expressing inhibitory neurons are differentially recruited to reactivations and how their modulation of monosynaptically connected partners changes strength during reactivations. Our findings will uncover how MEC reactivations during immobility could support consolidation and adaptation of representations over learning.

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Poster

PSTR239. Intrinsic Hippocampal Circuits

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Title: Rapid induction of place fields in retrosplenial cortex via holographic stimulation during spatial learning in virtual reality environments

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Abstract: Animals rapidly learn to navigate in novel environments. The formation of new spatial representations requires the integration of sensory cues with self-motion information. The

retrosplenial cortex (RSC) has been shown to encode both visual landmarks and information about self-motion and plays an important role in registering allocentric and egocentric coordinate frames. However, the processes that govern the formation of novel spatial representations in RSC are not well understood. Here, we study the dynamics of position encoding in RSC by inducing place fields using two-photon holographic optogenetic stimulation in virtual reality (VR) environments. We use a newly developed transgenic approach to co-express the indicator GCaMP6m and the soma-targeted opsin ChRmine in excitatory neurons. This approach leads to stable long-term read and write all-optical access throughout cortex. Using two-photon imaging in head-fixed mice, we find that spatially organized firing emerges rapidly in RSC when mice explore novel environments. The spatial representations stabilize within the first few days of experience, with neurons possessing one or multiple place fields. To study the dynamics of new place field emergence and how activity in individual neurons influences population level representations, we probed if place fields can be generated artificially by activating groups of neurons at different positions in the VR. We demonstrate that repeated targeted stimulation can induce new place fields in RSC neurons. The induction of new place fields at the stimulation-coupled locations occurred rapidly within a single session and persisted across days. Interestingly, the capacity to induce new place fields was highly dependent on the novelty of an environment. While optogenetic stimulation led to new, long-lasting place fields when mice explored a novel environment, the same optogenetic stimulation protocol had only little lasting influence on the activity of targeted neurons in a familiar environment. We observed similar effects in primary visual cortex, where targeted optogenetic activation biased activity towards stimulation-paired locations in novel environments, but not in familiar environments. This contrasts with our observations in hippocampal CA1, where stimulation can induce new place fields even in familiar environments, consistent with previous studies. In ongoing work, we are investigating possible mechanisms gating the rapid plasticity in RSC. Together, our data suggest a rapid emergence of spatially organized activity in RSC and V1 in novel environments. Once established, these representations are stable and robust to perturbations.

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Poster

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Title: Hippocampal coding in dynamic environment - organization of neuronal ensembles in presence of a moving robot

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Abstract: Dynamic organization of activity within hippocampal networks is gaining attention as a key component of understanding hippocampal function and its pathologies. Different concepts used to describe network activity, such as attractor networks, cell assemblies, manifolds, all share the view that activity of neurons is mutually organized with functional significance. Here we set to study, how this organization is affected by complexity of environmental situation and cognitive task. Activity in ensembles of dorsal hippocampal CA1 units was studied in situations with different “behavioral complexity”. Rats were either collecting food pellets on an empty circular arena, or they were interacting with a stationary or moving robot in the same environment. First, we assessed correlations of activity between cell-pairs. We observed that correlations of firing rate binned at one second intervals were preserved across experimental conditions, regardless of presence or absence of the robot or robot movements. In a parallel study we showed that firing rate maps of these place cells very often differed between different conditions. This observation indicates that change in firing rate maps (change in spatial representations) may not require changes in mutual organization in neuronal firing reflected in cell-pair correlations. We next used Fruchterman-Reingold force-directed algorithm to assess structure of correlations within ensembles. We observed that neurons did not organize into several coactive subgroups of cell, but they typically formed a group of coactive neurons with a few outlier cells that did not participate in correlated activity. Activity in the entire ensembles during short time intervals was characterized by momentary ensemble rate vectors. Activity across different time intervals was compared by vector correlations, which revealed larger variability in activity between sessions compared to within session variability. Isomap dimensionality reduction method was used to visualize structure of state space of neuronal activity patterns. Activity often clustered into distinct states that were identified using k-means clustering algorithm. Typically between 3 and 7 states were identified, and their number did not differ in different behavioral conditions with and without a robot. The frequency of transitions, which reflects the stability of states, did not differ between different behavioral conditions. Overall, the basic characteristics of network level dynamics were not dramatically affected by complexity of behavioral conditions the rat was exposed to.

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Poster

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Topic: H.09. Spatial Navigation

Support: Czech Science Foundation Grant 22-16717S

Title: Hippocampal coding in dynamic environment - spatial organization of neuronal firing in presence of a moving robot

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Abstract: Hippocampal representation of space in the form of place cell firing is best characterized and understood when a rat moves in two-dimensional empty spaces. In real life, animals are often facing environments with multiple moving objects, such as pray, predators, conspecifics. Whether and how such dynamic, multidimensional spatial situations are reflected in hippocampal discharge was focus of this study. We recorded 334 complex spike cells and 96 theta cells from CA1 region of the hippocampus while rats interacted with a moving robot. Experiment was organized into four 10-minute sessions. A session with stationary robot and a session with moving robot were flanked by two sessions without the robot. Rats of a "trained" group were trained to avoid a circular area located either on the left or right side of the robot. The avoidance behavior was reinforced by mild electric foot shock. Rats in the "untrained" group were not trained for robot avoidance.On average, parameters that characterize spatial organization of place cell firing (rate map coherence, information content) were not affected by presence of stationary or moving robot, compared to conditions without robot in either group of rats. However, when stability of place cell maps was followed across the four recording sessions, some cell's spatial maps remained stable, while other cells' maps changed - remapped. The proportion of stable cells was higher in untrained rats (49% of cells were stable) and lower in trained rats, where 3% of cells were stable. Cells that remapped and those that did not remap did not differ in any of the basic parameters such as firing rate, rate map coherence or information content. The cells in trained rats that "remapped" often discharged in two firing fields, one dominant in conditions with the robot, and the other without robot. This may indicate a cell toggling between two maps within the same sessions. Overdispersion in place cell's firing was higher in trained, compared to untrained rats.In sessions with moving robot, representations of different spatial relationships were analyzed separately: rat's position in the room, rat's position relative to robot, robot's position relative to the rat, and robot's position in the room. The activity was clearly best organized according to the position of the rat in the room. We observed that even in an environment inhabited by a significant moving object, CA1 place cells dominantly represented position of a rat in the experimental room, and the spatial organization of cells' firing was not affected much by robot's presence. Training rats to avoid particular part of the robot made, paradoxically, the representations less stable.

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Poster

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Title: activity of hippocampal and cortical neurons in quinpirole-induced model of obsessive-compulsive disorder in rats

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Abstract: Obsessive-compulsive disorder (OCD) is a disease characterized by recurring behavioral patterns (compulsions) which are carried out in order to relieve intrusive thoughts (obsessions). The neuronal mechanisms giving rise to the pathology are, however, still poorly understood. Apart from the canonical Cortico-Striato-Thalamo-Cortical loop, long thought to be implicated in the formation of the disease, the attention of neurobiologists has recently shifted toward the Anterior Cingulate Cortex (ACC) and the hippocampus as brain regions involved in OCD pathophysiology. We recorded single unit activity from ACC and hippocampus of freely-moving rats treated chronically with D2/D3 agonist quinpirole. The animals explored a square arena populated with three inanimate objects. Both quinpirole-treated group and saline-treated control group preferred to spend more time in some parts of the arena over others. The quinpirole-treated group, however, differed in how often the rats returned to different parts of the arena: quinpirole-treated rats visited parts of the arena containing an object significantly more than controls, suggesting OCD-like stereotypical checking behavior. Units recorded from both quinpirole-treated and control animals showed comparable firing rates. In both groups, units from the hippocampus displayed more coherent spatial representations and carried more spatial information than units in the ACC. According to the attractor hypothesis of neuropsychiatric diseases, some symptoms of OCD are caused by neuronal activity being “too stable” and therefore preventing neuronal networks from switching from one state (attractor) to another. We assessed the stability of single cell activity pattern in time by calculating autocorrelation function of an individual unit's spike train. Hippocampal units from quinpirole-treated rats displayed significantly greater stability than hippocampal units from controls. The stability of autocorrelation functions of ACC units was similar for both groups. We continue to analyze neuronal ensembles using dimensionality reduction and machine learning approaches. To summarize, quinpirole-treated rats developed stereotypical checking behavior, measured by their repeated visits to preferred, rat-specific objects in the arena. The observed enhanced stability of autocorrelation functions of hippocampal units from quinpirole-treated rats suggests, in accordance with the attractor hypothesis, a pathological increase in stability of hippocampal attractors.

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Poster

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Topic: H.09. Spatial Navigation

Support: NIH Grant R01NS104917
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Title: Plasticity and representational drift of a cognitive map

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Abstract: Context representation is achieved via the interplay between externally-driven and internally-generated activities of hippocampal neuronal ensembles in the form of a cognitive map. The stability of context representation for a given environment is believed to be due to a stronger contribution from the external stimuli, while representational drift is contributed primarily by changes in the internally-driven patterns of activity in relation to an unchanged environment. The primary driver for the hippocampal representational drift for an unchanged environment is currently unknown. We hypothesized that plasticity of neuronal networks achieved during the unaccounted activity in-between consecutive recording epochs could partly account for the observed representational drift. To explore this possibility, we imposed unexpected detours in a familiar maze context that adult rats were exploring for food rewards followed by a reversal to the original context configuration while neuronal ensemble activity was being recorded from the dorsal CA1 area. We found that neuronal activity on the detour portions of the maze and its plasticity could explain part of the representational drift observed between the pre- and post-detour sessions at single neuron and neuronal ensemble levels. These findings indicate that internally-generated activity patterns can explain, in part, the representational drift observed during re-exploration of otherwise unchanged environments.

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Poster

PSTR239. Intrinsic Hippocampal Circuits

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Title: Euclidean geometry sculpts the development and dynamics of hippocampal sequential assemblies

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Abstract: We experience various features of Euclidean space throughout our lives including geometric linearity, 90-degree corners and flat floors. Whether these experiences impact how our hippocampus represents space and forms memories of these spatial experiences is not known. We reared rats from birth inside non-Euclidean contexts - spherical home cages - or Euclidean contexts - cuboidal home cages together with the dam and the rest of the litter. We tested the influence of these different early-life experiences on depicting space by hippocampal neuronal ensembles as animals navigated multiple novel Euclidean linear spaces over days and slept. Sphere-rearing did not induce stress or restrict locomotion across development but altered the nature of movement patterns in early life. While our sphere-reared rats lacked experience with Euclidean geometry, that did not prevent rapid emergence of time-compressed place cell sequences expressed as preplay and replay during sleep and awake-rest and theta sequences during run depicting their first Euclidean linear trajectory when tested at postnatal day 24. However, sleep preplay and replay occurred less frequently in the sphere-reared rats. On the other hand, early-life experience with non-Euclidean, curved geometry resulted in more similar mapping of linear environment ends and corners by neuronal ensembles, suggestive of warped representations of linear tracks, likely due to higher proportions of hippocampal neurons exhibiting lower tuning or symmetric coding of Euclidean linear space. In addition, sphere-rearing resulted in an impairment in the ability to form unique representations for multiple linear environments (i.e., pattern separation), partly due to reduced preconfigured network repertoires during sleep. Experience over 3-4 days with linear, orthogonally oriented Euclidean environments reversed these deficits in the sphere-reared rats. Thus, early-life experience with Euclidian geometry is crucial for development of a rich hippocampal repertoire of preconfigured neuronal patterns, which would support unique neuronal representations of multiple future linear environments.

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Poster

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Title: Hippocampal and prefrontal mechanisms underlying schema-based learning

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Abstract: Most rapid learning during adulthood builds on prior knowledge, such as the case of assimilation of new information into existing mental schemas. Medial prefrontal cortex (mPFC) and the hippocampus have both been shown to be critical for schema-based learning in the rat, but the underlying neural mechanisms have remained poorly understood.
To better understand the neural mechanisms by which mPFC and the hippocampus support schema-based learning, we designed a complex task where adult rats learned 6 different flavor-place associations over the course of several weeks. After the animals learned these associations, we surgically implanted electrodes targeting both mPFC and the CA1 area of the hippocampus. After post-surgical retraining, animals were presented with 3 new flavor-place associations replacing 3 old associations, which were assimilated into new memories within one day. By recording multiple single units (neuronal activity) at millisecond timescale simultaneously from mPFC and the hippocampus, we aim at understanding the underlying neural processes that support the rapid assimilation of novel associations.
To this end, we recorded single units simultaneously from mPFC and the hippocampus while rats performed the baseline tasks and during assimilation of new associations into pre-existing schemas. We are invstigating several neural correlates of the behavioral task at the single cell and neuronal ensemble levels that could be instrumental for the rapid learning of new associations.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

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Topic: H.09. Spatial Navigation

Title: Neural signal modulation by facing direction

Authors: Y. KASSIF[1], **S. MAIDENBAUM**[2];
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Abstract: Understanding directions is crucial in our daily lives as humans, and is required for navigating and understanding our surroundings. While several representations of directions have been found in animal models, their generalization to humans remains limited. Here we search for a non-invasive reflection of our neural representation of direction, building upon recent work such as the finding of hexadirecitonal modulation in the human brain. We found a robust directional modulation of neural signals by direction across several imaging modalities,

including ECoG, fMRI and EEG recordings. We demonstrate the empirical existence of neural representations of direction in these modalities under different conditions including mobile vs. stationary, virtual reality vs. real world and in different types of environments. Finally, we attempt to bridge the gap between these modalities and between human results and the extensive literature in animal models by suggesting the ways these signals might reflect between them and what may be underlying them. This non-invasive representation for direction that can be recorded under naturalistic conditions has great potential for the basic science of spatial cognition and neuroscience and could have significant ramifications for the early diagnosis of patients with spatial disorders.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

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Topic: H.09. Spatial Navigation

Support: NIH Grant R01EY022350

Title: Separate neural mechanisms for integrating views into places in scene-selective cortex

Authors: *L. HAN, R. A. EPSTEIN;
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Abstract: People rely on representations of places to navigate the world. To learn a new place, the visual system must integrate discrete views into a unified representation. There are at least two ways that this might be done: First, by integrating across the panorama of views obtained when standing at a single location; second, by integrating across different views of the same landmark obtained when standing in different locations. Guided by previous work (Berens et al., 2021; Marchette et al., 2015; Robertson et al., 2016), we hypothesized that these two viewpoint-integration processes would be associated with different neuroanatomical substrates in scene-selective cortex. To test this hypothesis, we familiarized participants (N = 24) with a route through a virtual city containing 24 storefronts that were controlled for their low-level visual similarities. The storefronts were locally associated in pairs. These could either be storefronts on different buildings that were directly across from each other on the same street (same-panorama condition), or storefronts on different sides of the same building visible from different streets (same-landmark condition). These associations were learned through multiple viewings of a movie sequence that strictly controlled the time that each item was viewed and the interval between them. After learning, participants were scanned with fMRI while viewing the storefronts and performing a judgment of relative direction task. Multivoxel pattern analyses in the scene-selective retrosplenial complex (RSC) and parahippocampal place area (PPA) showed an interaction between type of association (same panorama, same landmark) and brain region,

whereby RSC showed a significant association for same-panorama but not same-landmark storefront pairs, while PPA showed a significant association for same-landmark but not same-panorama storefront pairs. These results support the existence of separate neural mechanisms for integrating across views to represent places as either the location of the observer (same panorama) or the location observed (same landmark).

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

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Title: Human single-neuron recordings show grid-like neural activity during emotional processing

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Abstract: When animals and humans move through space, grid cells in the entorhinal cortex activate in a triangular pattern at multiple locations. This could help the navigating subject to form a map of the spatial environment. Theoretical accounts suggest that grid cells may also map non-physical, conceptual spaces to support a variety of complex behaviors (Bellmund et al., Science, 2018; Behrens et al., Neuron, 2018). Here, we investigated whether grid cells might also be involved in the processing of emotional information. Using intracranial recordings in epilepsy patients (n = 14) encoding emotional images, we demonstrate that neurons in the human medial temporal lobe exhibit grid-like neural activity in a two-dimensional feature space spanned by emotional valence and arousal. These neurons were particularly prevalent in the entorhinal cortex, were different from cells representing either valence or arousal, and exhibited theta-phase locking. These results are consistent with a more general role of grid-like activity for human cognition, and may indicate that cognitive maps are relevant beyond spatial navigation for complex functions like emotion processing.

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Poster

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Title: The relationship between spatial navigation abilities and white matter structural integrity in midlife adults

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Abstract: Spatial navigation is a highly complex behavior that remains critical for survival across many species. Despite its importance, there are large individual differences in navigation ability. One question is the extent to which these individual differences are reflected in differences in brain structure. While this relationship has been examined to some extent in young adults, the relationship between brain structure and individual performance in navigation tasks has not been fully tested in the earliest stages of aging, during midlife. To address this question, we used diffusion spectrum imaging (DSI) to examine the relationship between white matter structural integrity in midlife participants (ages 45-55) and navigational abilities in three tasks. 1) Wayfinding abilities were assessed using a virtual maze learning task in which participants freely explored a maze environment with objects located throughout, and then were assessed on their knowledge of the object locations. 2) Navigation strategy was assessed using the dual solution paradigm (DSP) in which participants were guided on a route through a virtual maze environment and were assessed on the number of shortcuts taken to reach target objects. Finally, 3) path integration abilities were assessed using the loop closure task, in which participants were guided to walk in a loop in a virtual desert environment and indicated when they thought they had returned to the starting location. We hypothesized that performance would be associated with mean diffusivity and quantitative anisotropy in the fornix, a major output tract of the hippocampus. Preliminary results indicate that, surprisingly, structural integrity in white matter tracts within the medial temporal lobe and prefrontal cortex often corresponded to worse navigation performance in wayfinding and path integration abilities. However, the relationship between white matter and performance differed between men and women. These results suggest that white matter structure corresponds to spatial navigation performance in midlife, but that this relationship may be influenced by sex. These results provide us with a greater understanding of the changes in brain and behavior that occur during early aging.

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Poster

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Title: Conjunctive Encoding in Human Place and Time Cells and Their Relation to Spatial Memory

Authors: *T. DONOGHUE[1], S. MAESTA PEREIRA[1], S. E. QASIM[3], A. PATEL[2], H. AZAB[4], E. H. SMITH[6], R. MATHURA[4], J. MYERS[5], A. ANAND[5], J. ADKINSON[5], T. DAVIS[7], B. SHOFTY[6], Z. KURTH-NELSON[8], H. G. REY[9], J. D. ROLSTON[10], T. E. BEHRENS[11], M. M. BOTVINICK[8], S. A. SHETH[5], J. JACOBS[1];
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Abstract: To navigate a dynamic world, the brain must be able to keep track of the ‘when’ and ‘where’ of actions in the world, as well as be able to integrate these information streams together to plan future actions. Work in animal models has demonstrated that single-neurons, especially in the medial temporal lobe (MTL), can encode place and time, as well as conjunctive encoding of such information - providing a potential mechanism for encoding this information as required for navigating complex environments. In this work, we extend this work into the human brain, examining single-unit responses collected from the MTL and surrounding regions of neurosurgical patients with implanted microwires. Patients completed a computer-based virtual navigation task, including navigation decisions (alternating directions across subsequent trials at a choice point), as well as a spatial memory component in which subjects learn and then recall specified locations. In this data, we first replicate that we find single neurons in the human brain that encode place or time, with firing rates relating to subjects’ virtual spatial position or elapsed time during stationary periods. We then looked for conjunctive encodings (or ‘splitter cells’), finding novel results whereby single neurons in the human brain encode a conjunctive representation of multiple features, including for current location + future turn direction and current time + future turn direction. We further analyzed this data by examining the propensity for conjunctive encodings across the different regions we recorded, finding a non-homogenous

distribution of conjunctive encodings across the MTL and surrounding areas. Finally, we analyzed the relationship between conjunctive encodings, neural responses to the chest-encounter events, and behavioral performance on the memory component of the task (computed as the distance error between response and true locations), finding that single-neuron activity related to behavioral performance on the task. Overall, our results serve to further establish conjunctive encoding in the human brain, as well as qualifying anatomical regions and relationships to behavior, altogether contributing to the scientific understanding of how we navigate through space and time.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.06/WW22

Topic: H.09. Spatial Navigation

Support: NSF CAREER

Title: Distributed neural fields of human spatial memory representations are traveling waves

Authors: *A. DAS[1], J. JACOBS[2];
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Abstract: Spatial memory is thought to involve widespread cortical and sub-cortical brain areas in rodents, nonhuman primates, and humans alike. Extensive research on the neurophysiology of spatial memory in rodents has revealed the presence of hippocampal place cells, which increase their firing rate when an animal passes through a specific location in the environment. There is now substantial evidence that the hippocampus does not function in isolation and that its feedforward and feedback interactions with the prefrontal and parietal cortices are essential for encoding and recall of spatial information via cognitive and strategic control over memory formation processes. However, how these different brain areas simultaneously interact with each other for spatial memory formation and processing remains unclear. Here, we propose that – "traveling waves" – a dynamic phenomenon of brain oscillations, that propagate progressively across the cortex to coordinate neural activity across widespread regions in space and time, can explain complex behaviors for spatial memory processing as well as specific memory representations, in the human brain. We used human electrocorticographic recordings across spatial episodic and working memory domains to understand the distributed spatiotemporal organization of neural fields for formation and processing of memory in the neocortex.

Participants were drug-resistant epilepsy patients who performed either a Treasure-Hunt spatial navigation and memory task or a Sternberg working memory task and were implanted with subdural grid electrodes. Using innovative multivariate approaches to analyze these field potential recordings, we first show that neuronal oscillations in the theta and beta frequency bands are widespread, consistent with recent findings in human iEEG studies which have shown prominent roles for theta and beta oscillations for coordinating feedforward and feedback information flow, respectively. We next show that the spatial distribution of the relative phase of these oscillations can distinguish broad cognitive states such as encoding, navigation, and retrieval as well as specific cognitive states such as English letters. Furthermore, we show that the phase shifts across these electrodes are organized as traveling waves. We also show that, in additional to planar traveling waves, these relative phases are organized as complex patterns such as circular/spiral and source/sink traveling waves. The methods that we describe here provide a general approach to distinguish different cognitive representations related to human behavior from distributed neural fields.

Disclosures: **A. Das:** None. **J. Jacobs:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.07/WW23

Topic: H.09. Spatial Navigation

Support: University of Arizona: Accelerate for Success Grant

Title: Deconfounding path familiarity, efficiency, and shortcut behavior in human spatial navigation

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Abstract: Navigation is an important function people employ daily that is relevant for survival. However, getting lost often causes stress and some studies have suggested that stress may impair navigation decisions. One explanation is that stress selectively impairs shortcut taking by decreasing the connectivity between the hippocampus and the prefrontal cortex. In contrast, stress may leave the ability to travel well-learned paths intact. However, it is unknown whether this decrease in connectivity is the result of a diminished ability to plan successful shortcuts or whether it represents a change in strategy away from risky shortcuts toward safe familiar paths. Many paradigms showing navigation stress effects have employed small-scale environments with limited path options. Therefore, the proportion of shortcuts vs. familiar path taking could be affected by city size and the limited number of path choices (e.g., peripheral vs. central paths).

These paradigms also often confound path familiarity with path efficiency such that the most efficient path was always novel. Thus, familiarity could influence path choice under stress rather than efficiency. In the current study, we aimed to determine shortcut and familiar path taking frequency under non-stressful conditions and understand how path familiarity interacts with path efficiency by controlling for one while testing the other. Participants learned two routes varying in familiarity through a large city in desktop virtual reality both by passively watching a video and actively using arrow keys to find 4 stores. Routes were repeated 4 and 8 times in one condition and 6 and 12 times in another. Then, participants were given a navigation test with 24 trials instructing them to find one store starting from another store. They could take learned paths, novel peripheral paths, novel central paths, or a combination of the three. Participants were able to reproduce the learned route even after only 4 repetitions. They used a combination of shortcuts and familiar paths during the navigation test. Participants tended to favor a more efficient central path rather than the peripheral path even after controlling for route repetition, suggesting that path efficiency may be more important than path familiarity. We also observed some individual differences in navigation strategies, suggesting that individual participants may have balanced familiarity and efficiency differently. Our findings help to establish baseline measures of shortcut behavior when controlling for familiarity under non-stressful conditions in a more ecological design which can be employed to understand the effects of stress on navigation.

Disclosures: **A.L. Muller:** None. **A. Wissinger:** None. **E. Robinson:** None. **N. Toosizadeh:** None. **K. Hazeli:** None. **A.D. Ekstrom:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.08/WW24

Topic: H.09. Spatial Navigation

Support: University of Arizona GPSC ReaP Grant

Title: Neural Correlates of Mismatch Detection and Integration of Visual and Body-based Cues in Virtual Reality

Authors: *Y. HUANG, Y. K. DU, R. C. WILSON, A. D. EKSTROM;
Dept. of Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Spatial navigation, which is essential for daily functions, involves integrating visual, proprioceptive, and vestibular cues. While the idea of a spatial representation (e.g., cognitive map) remains central to most modern theories of spatial navigation, understanding the role of multi-modal sensory integration in human spatial navigation has been relatively neglected. Yet, detecting mismatches between visual (landmark) and vestibular (path integration) cues is critical to staying oriented and may be affected by age and age-related neurodegenerative diseases like

Alzheimer's. Here, we employed mobile, wireless scalp EEG to better understand how humans integrate sensory and vestibular cues during simple rotations. The aim was to identify EEG signatures of mismatch detection and integration of multi-sensory inputs. Nine participants (five females, aged 18-25, all healthy with no history of traumatic brain injury or motion sickness, and have normal or corrected-to-normal vision) encoded different angular distance in a virtually rendered rectangular room by turning; they immediately reproduced these rotations in the darkness with brief visual feedback. The feedback was either aligned or misaligned with the actual room coordinates experienced during encoding. This task allowed us to examine how mismatch and integration of visual and body-based cues influence spatial navigation, with our previous results suggesting that participants tended to integrate visual feedback with lower angular offsets and ignore visual feedback with higher angular offset. Here, we performed a preliminary event-related potential (ERP) analysis to determine the neural correlates of visual-vestibular mismatch detection and integration at the onset of visual feedback during reproduction. For trials with larger visual offsets, we observed a deflection in ERP signatures around 100ms (P100 component) in the occipital region, with a corrected p-value < 0.001. For smaller offsets, we observed a more negative deflection around 200ms at the frontal midline, akin to Feedback-Related Negativity (FRN), which is part of the N2 component, with a corrected p-value < 0.001. These findings offer insights into the neural correlates of mismatch detection and integration of multi-sensory inputs in spatial navigation. Together, these findings can help to contribute to our understanding of the interplay between visual and body-based cues in an ecologically valid setting and mechanisms that affect orientation, which may be altered in disease like Alzheimer's.

Disclosures: Y. Huang: None. **Y.K. Du:** None. **R.C. Wilson:** None. **A.D. Ekstrom:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.09/WW25

Topic: H.09. Spatial Navigation

Title: Examining the connection between rodent grid cells and grid-like representations in humans

Authors: *T. NAVARRO SCHRÖDER, N. ALMOG;
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Abstract: One of the most intriguing and researched neural firing patterns in neuroscience is the hexagonal tessellation of two dimensional space created by grid cell firing. Several years following the discovery of this phenomenon in rats, human fMRI recordings revealed higher BOLD activity during free navigation in virtual reality environments along a hexagonal set of directions. This effect became known as the 'grid-cell-like representations' in humans, or the Hexadirectional Signal. Because both grid cell firing and the hexadirectional signal are observed

in homologue regions of the cortex and are both 6-fold symmetric in nature, it is easy to assume that they are causally related. While several hypotheses connecting the two have been posited, the way in which a single-neuron, 2D spatial firing pattern results in population-level angular tuning has never been thoroughly examined. In this work we build a probabilistic model of a population of grid cells firing modulated by the hemodynamic response to see if the hexadirectional signal emerges. We parameterize this model to simulate several grid cell configurations that have been previously hypothesized to cause this phenomenon. Preliminary results from the model show that averaged grid cell firing alone, regardless of population configuration, does not produce the hexadirectional signal. However, the variance of firing rates does show detectable hexadirectional modulation in certain scenarios. The most reliable explanation for the hexadirectional signal results from the firing of conjunctive grid/head direction cells tuned to a homogeneous set of grid angles. In addition to modeling, we use datasets of single-cell recordings of rodent grid cells to extrapolate population activity of the same form we model and perform a parallel analysis. The results of this study have sizable implications for how future studies interpret the hexadirectional signal and for the methodology of how spatially modulated brain activity is detected in fMRI recordings.

Disclosures: **T. Navarro Schröder:** None. **N. Almog:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.10/WW26

Topic: H.09. Spatial Navigation

Support: NIH/NEI RO1 EY024056

Title: Unsuspected large-scale brain reorganization in spatial navigation learning: Profound interhemispheric asymmetry of occipitotemporal clusters in the blind

Authors: *L. LIKOVA[1], Z. ZHOU[2], M. LIANG[2], C. TYLER[2];
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Abstract: The visual motion complex (hMT+), long thought to be a well-understood visual-processing territory, has challenged our traditional model over recent years with sensitivity in visual tasks beyond motion, such as to images of static body parts, to handedness or tool-usage effects, etc. How is this extended visual functionality reorganized without vision? Methods: Totally blind subjects underwent our Cognitive-Kinesthetic Memory-Drawing Training for spatial navigation, using raised-line tactile maps. The whole brain was scanned before and after the training in a Siemens 3T Prisma scanner while the maps were (i) haptically explored with left hand and memorized (30 s); after 20 s rest, the maps were (ii) drawn-from-haptic-memory (30 s) with the opposite hand, using a stylus. Results: Both non-visual tasks strongly activated hMT+

despite the lack of any visual motion. Moreover, our paradigm revealed for an unsuspected interhemispheric functional asymmetry in the occipitotemporal cortex. The left-hand haptic memory encoding task activated the right-hemisphere hMT+ only, but failed to do so in its ipsilateral hemisphere. In contrast, the right-hand drawing-from-haptic-memory task strongly activated hMT+ bilaterally. Further post-training analyses revealed for the first time a large-scale massive brain reorganization in the blind forming previously unknown clusters of occipitotemporal areas around hMT+, expressing the same left/right asymmetry as hMT+ per se. On the left, these areas were immediately dorsal (TPOJ 1-3) and anterior (along FST & MTG) to hMT+; the cluster on the right involved hMT+, FST, LOd. The profoundly asymmetric pattern of hMT+ thus spread across neighboring visual areas with well-known functional distinctions in the sighted, turning them into large homogeneous clusters with asymmetric responses in the blind. The pre/post training network organization was investigated by Granger Causal connectivity analysis (GCCA) between the cluster ROIs, motor, somatosensory and memory areas. Conclusions: The multidimensional results from this study on spatial navigation expand our knowledge of non-visual hMT+ functionality and have strong implications for the functional architecture of spatial navigation and its reorganization with learning.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.11/WW27

Topic: H.09. Spatial Navigation

Support: NSF Grant 1815506

Title: The role of visual feedback on leading and trailing leg behavior when stepping over obstacles in virtual reality

Authors: *A. PADILLA, A. PEER, K. PICKETT, K. PONTO, A. H. MASON; Kinesiology, Univ. of Wisconsin, Madison, Madison, WI

Abstract: Previous literature has shown that when individuals step over obstacles in natural environments, the horizontal distances of the trailing leg (toe-distance to obstacle) is larger when the lower limbs are occluded. The primary aims of this study were to assess whether similar results are found in virtual environments and to determine how the absence of visual information influences the heel position of the leading limb (heel distance after obstacle is cleared). We were also interested in quantifying the relationship between toe and heel position. **Method:** Young adults (n=23) were asked to walk across a 20m Zeno gait mat under three visual conditions: (i) overground (OG)-natural environment/normal vision (ii) Basketball Goggles (BG)- natural environment/occluded lower body; (iii) Virtual Reality (VR)- immersive virtual environment/no representation of lower body. On some trials, participants stepped over an obstacle positioned at

25%, 50%, or 75% of walkway. A 3x3 repeated measures ANOVA was used to examine the main effects of visual condition and obstacle location on toe distance and heel distance from the obstacle. In addition, Pearson Correlations were used to assess the linear relationship between toe distance and heel distance across visual conditions and distances. **Results:** There was a main effect of visual condition on toe distance ($F_{2, 21}$=13.641, p<.001) and heel distance ($F_{2, 44}$=3.243, p=0.049). Post-hoc analysis indicated that toe distance was greater in the VR condition (18.303±1.499) than the BG (14.894±0.789) and OG (12.572±.957) condition. For heel distance, the BG condition (19.945±1.107) was greater than the VR condition (17.754±1.427). Pearson Correlations revealed that there were no significant relationships between heel and toe distance for the BG and OG conditions but there were moderate negative correlations for VR25 (r(21)=[-0571], p=0<0.01), VR50 (r(21)=[-0.656], p<0.01) and VR75 (r(21)=[-0.638], p<0.01). **Conclusion:** The results of the current study suggest that visual condition affected toe and heel distance when stepping over an obstacle. Furthermore, in the VR50% and VR75% condition, an inverse relationship was found where toe distance increased and heal distance decreased. In contrast, participants tended to increase their heel distance and decrease their toe distance in the VR25% condition. The results suggest that visual feedback about the lower limbs and location of obstacles affect locomotor patterns differentially in natural and virtual environments. Designers of VR systems must keep these factors in mind when designing applications for rehabilitation, training and assessment.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.12/WW28

Topic: H.09. Spatial Navigation

Support: NSF Graduate Research Fellowship Program (GRFP)

Title: Reference frame utilization as a potential marker of aging-related deficits in spatial navigation

Authors: Y. BASSIL[1], A. KANUKOLANU[2], E. CUI[1], P. MAXIM[4], T. I. BROWN[3], M. R. BORICH[1];
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Abstract: With advancing age, older adults (OAs) report impaired *spatial navigation ability*, one of the earliest indicators of aging-related cognitive decline and neurodegenerative pathologies. Specifically, OAs demonstrate deficits in utilizing allocentric (world-centered) spatial information and often rely on egocentric (body-centered) cues during navigation, resulting in

reference frame (RF) bias with aging. While traditional navigational assessments have characterized aging effects on RF bias, RF utilization during complex, naturalistic, real-world-like navigation in humans remains unclear. This study aimed to characterize interactions between RF bias utilizing a classic computerized Y-Maze task with novel measures of navigational ability in a naturalistic, virtual reality (VR) environment ("NavCity"). We also introduce a City-Like Allocentric Representation Assessment ("CMARA") to quantify formation of allocentric representations (i.e., "cognitive map") after completing NavCity. Our central hypothesis is that OAs with egocentric bias will exhibit the largest deficits in naturalistic navigation and "cognitive map" formation, compared to OAs who primarily utilize allocentric RFs or younger adults (YAs).

To test this hypothesis, YAs (N = 12; 18-35 years old) and OAs (N = 12; 60+ years old) completed 3 repetitions of NavCity, the accompanying CMARA, and the Y-Maze. Independent t-tests and nonparametric chi-squared tests evaluated group differences.

Compared to YAs, OAs demonstrated increased mean completion time and distance traveled in NavCity (both $p < .01$). However, the rate of improvement across NavCity repetitions was similar between groups ($p > .05$). OAs exhibited lower CMARA scores than YAs, reflecting decreased allocentric RF utilization ($p < .001$). NavCity outcomes and CMARA scores were strongly correlated across groups ($p < .001$). Additionally, OAs exhibited greater prevalence of egocentric RF utilization on the Y-Maze task, compared to YAs ($\chi 2 = 14.96$, $p < .001$).

Findings suggest that RF bias characterization may serve as a marker of aging-related navigational deficits. Immediate next steps will identify underlying neural mechanisms of aging-related navigational impairments to support preclinical markers and prehabilitative interventions for cognitive decline.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.13/WW29

Topic: H.09. Spatial Navigation

Support: MRC PhD Studentship MR/N013867/1

Title: Tracking eye movements in a spatial memory task for early Alzheimer's diagnosis

Authors: *L. EMRICH-MILLS, A. CASTEGNARO, D. CHAN, J. KING, N. BURGESS; Univ. Col. London, London, United Kingdom

Abstract: Early detection of Alzheimer's disease is potentially crucial for better prognosis and more effective treatment. Spatial cognition testing is a promising approach for identifying early behavioural impairments, and immersive virtual reality is a suitable medium for administering

relevant behavioural tasks. With modern head-mounted virtual reality displays equipped with integrated eye tracking, combining insights from eye tracking results in Alzheimer's research with spatial memory testing has become feasible. In this study, we developed a novel virtual reality spatial memory task based on a classical viewpoint-shifting paradigm from experimental psychology. The aim of the study was to estimate the accuracy of this task, including eye movement metrics, in discriminating participants at the highest risk of Alzheimer's disease from age-matched controls. We are currently recruiting participants with Mild Cognitive Impairment who have undergone biomarker testing for early signs of Alzheimer's disease neuropathology. Those with biomarkers (MCI+) can be compared to those without (MCI-) and Healthy Older adults without cognitive impairment (HO). Healthy younger (HY) participants were also included to examine age-related differences. Early results suggest that group differences can be observed in how and where participants view spatial stimuli (objects on a table), independent of performance on the memory task. Specifically, time spent fixating on unmoved objects and the surrounding table significantly differs between groups. Spatial and temporal dynamics of viewing behaviour shows significant time-dependent differences in fixated object over time between groups. A cross-validated, regularised logistic regression pipeline was built to test accuracy of predicting MCI+ participants using task features. Indicative early results yield 81-92% classification accuracy of MCI+ participants based on a combination of eye-movement and behavioural features. Although data collection is still underway, this provides tentative evidence for a multi-modal spatial eye-tracking task as a promising addition to future diagnosis of Alzheimer's disease.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

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Program #/Poster #: PSTR240.14/WW30

Topic: H.09. Spatial Navigation

Support: HORIZON 2020 MSCA-ITN-2014-ETN (955590)

Title: Virtual Reality-based Assessment of Locomotion and Navigation in Glaucoma

Authors: *S. ANDAC[1], F. H. STOLLE[1], Y. HU[2], M. BERNARD[3], H. THIEME[1], K. AL-NOSAIRY[1], T. WOLBERS[3,4], E. BARTH[2], M. B. HOFFMANN[1,4];
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Abstract: Purpose Navigation is of vital importance in daily activities as a foundation of effective locomotion. A crucial cognitive function within navigation is path integration (PI), which uses cues such as optic flow and body-based feedback with environmental cues. The process allows for determining one's own position and thus facilitates orientation. Impairments in path integration, such as those resulting from reduced access to visual information, can significantly impact both locomotion and our overall independence.
Methods The study involved 14 glaucoma and 15 age-matched controls, who underwent ophthalmological examination such as visual acuity (logMAR), visual field (MD), and OCT (pRFNL). The objective was the assessment of the performance of both groups in a path integration (PI) task under different lighting conditions, specifically daylight and dawn, using immersive virtual reality (VR). In the task, participants followed a path pointed by three checkpoints and finally indicated the starting position of the path. The main measures were (i) duration of travel time, (ii) duration of the pointing task, and (iii) distance error between indicated and actual starting positions. Moreover, we used the acquired data on movement behavior along the path for the analysis of the locomotion behavior with machine-learning-based classification (SVM).
Results Compared to controls, in glaucoma travel and pointing duration were longer (by 8.2±1.7s; p=0.002 and by 5.3±1.6s; p=0.016), while there was no significant difference in performance accuracy between the two groups. In the daylight condition, for both groups, the performance accuracy improved (p=0.007) at the expense of increased travel and pointing duration (by 0.9±0.4s; p=0.03, and by 3.4±0.7s; p=0.03). Moreover, we examined the association between performance accuracy and visual impairments. Independent of lighting conditions, we observed a correlation between MD of visual field defects with travel time (dawn: R^2=.43, p<0.001; daylight: R^2=0.5, p<0.001) and pointing task duration (dawn: R^2=.38, p<0.001; daylight: R^2=.27, p=0.005) while there was a correlation between logMAR and performance accuracy. With our classifier as a preliminary diagnostic tool, we achieved an accuracy of 70% in predicting the group of an individual based on their movement behavior in the PI task.
Conclusions Our results suggest that the impact of glaucoma on the duration of completing navigation tasks varies depending on the progression stage of the disease. People with severe visual impairment need more time to complete such tasks. Longer durations in glaucoma appear to reflect specific changes in their locomotion behavior.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.15/WW31

Topic: H.09. Spatial Navigation

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AAIC grant SG-21-815077
FONDEF ID19I10302
BNI Project ACE210007

Title: One month of moderate-intensity physical exercise improves performance in a virtual spatial navigation task in Older adults

Authors: *M. GUTIÉRREZ[1], V. UBERUAGA[1], C. ROJAS[1], M. AZÓCAR[1], J. MORE[1], C. DELGADO[2], J. VALDÉS[1], P. MALDONADO[1];
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Abstract: Physical exercise has neuroprotective and positive effects on several cognitive abilities, such as memory. One current hypothesis about how exercise promotes memory improvement is the muscle-brain hypothesis. This idea proposes an endocrine role for the muscles, which releases humoral factors (myokines) that travel through the bloodstream to the brain, promoting neuroplasticity. However, the effects of exercise on spatial memory have never been studied in the Chilean population. Therefore, we propose that one month of moderate-intensity physical exercise increases the plasmatic levels of myokines and spatial memory in older adults. This study aims to determine the relationship between exercise-induced myokines levels and spatial memory performance in exercised versus sedentary older Chileans. For this purpose, we used a three-stage virtual-navigation task based on the Oasis Maze task adapted for the older population. Measures were made at baseline and after one month. After the inclusion criteria, 27 subjects (14 exercised, 13 control) were included. The exercise participants' spatial memory analysis showed improved latency in half of the exercised subjects, but this improvement was not detected in the sedentary group. In addition, the plasmatic myokine levels at baseline and post-exercise intervention were preliminary measured and correlated intrasubject with the spatial memory task. The results of this study contribute to understanding the role of physical exercise in cognition, a basis for future therapeutic applications for the prevention of neurodegenerative pathologies such as dementia.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.16/WW32

Topic: H.09. Spatial Navigation

Title: Mobile EEG provides evidence that alpha-band oscillations support processing of egocentric directional information during spatial memory judgments

Authors: *A. K. BONIN, S. YANG, D. J. HUFFMAN;
Dept. of Psychology, Colby Col., Waterville, ME

Abstract: The loss of spatial memory has devastating effects on an individual's ability to live an independent life. Prominent theories suggest that spatial memory can consist of egocentric knowledge (i.e., the location of landmarks relative to oneself) or allocentric knowledge (i.e., map-like knowledge of the location of landmarks relative to each other) that rely on distinct neural mechanisms. Additionally, these theories typically decompose spatial memory into different features. For example, egocentric knowledge relies on one's facing direction, and it is critical in helping individuals stay oriented within their environment. Classic studies have shown that egocentric directional knowledge is impaired in patients with damage to the parietal cortex (e.g., Bisiach and Luzzatti, 1978), and these findings have been supported by fMRI research (e.g., Schindler and Bartels, 2013). In addition, prior work has shown that alpha oscillations carry information about spatial attention for locations on a 2D computer screen (e.g., Foster et al., 2017). Recent theories have also suggested that body-based cues (e.g., head and body rotations) play a fundamental role in spatial memory (e.g., Taube et al., 2013), thus mobile EEG affords a novel opportunity to study spatial memory as navigators are embedded within the spatial environment. We aimed to connect these disparate lines of research and used mobile EEG to investigate whether alpha oscillations support egocentric memory of landmarks in a 3D space. Participants first learned the locations of 8 objects that were evenly spaced around them in a circle. Then, during each trial, participants saw a heading cue (which object they should face), a pointing cue (which object they should point to with a joystick), a 1500 ms delay with a fixation cross (planning and maintenance phase), and a response cue. We found that participants performed the task significantly better than chance (based on angular error) and had fast reaction times, which suggests that participants performed well on the task and that they used the delay period to prepare and maintain their responses, thus substantiating our later EEG analysis of the delay period. Time-frequency-based classification analysis revealed above-chance accuracy around 1.0-2.0 seconds post-stimulus onset. Critically, these analyses indicated that alpha-band oscillations were related to egocentric coding, as average classification accuracy for a classifier trained on frequencies between 8-12 Hz was significantly above chance 1.0-2.0 seconds post-stimulus onset. Our results indicate that alpha oscillations and control of spatial attention may support egocentric directional memory.

Disclosures: A.K. Bonin: None. **S. Yang:** None. **D.J. Huffman:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.17/WW33

Topic: H.09. Spatial Navigation

Support: NIH RO1MH123713

Title: Neural representations of cognitive maps facilitate flexible decision making across contexts

Authors: *S. C. SWEIGART[1], N. A. NGUYEN[1], C. RANGANATH[1], S. A. PARK[2], E. D. BOORMAN, 95618[1];
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Abstract: Cognitive maps, mental representations of relationships between items in an environment or task, are utilized by the brain to monitor both spatial and non-spatial connections. These maps allow it enables generalization and flexible decision-making in different task contexts. Past research has implicated the entorhinal cortex (ERC), hippocampus (HPC), and medial prefrontal cortex (mPFC) in the maintenance of cognitive maps. However, although cognitive maps offer conceptual flexibility, it remains uncertain how and to what extent they possess representational flexibility. To investigate this, we conducted an fMRI experiment where participants employed a previously learned 2D abstract space to solve a novel task that demanded cognitive map flexibility in changing context. Analysis revealed that the mPFC and HC/EC exhibited univariate effects related to context-dependent rank differences, indicating the flexible utilization of task-relevant relational values for decision-making. Moreover, employing whole-brain representational similarity analysis (RSA) on wine rank differences, we identified an abstraction hierarchy. Specifically, the EC exhibited a generalized code, the HC displayed an axis-specific code, and the mPFC tracked solely contextually-relevant information. Additionally, independent variance in BOLD patterns were explained by both context-invariant 2D Euclidean coding and by these context-dependent 1D rank map coding, suggesting a transformation from the former to the latter. These findings suggest that the brain employs multiple regions to track cognitive map relationships at different levels of abstraction and incorporates contextually-relevant information to calculate decision variables in accordance with task requirements.

Disclosures: S.C. Sweigart: None. **N.A. Nguyen:** None. **C. Ranganath:** None. **S.A. Park:** None. **E.D. Boorman:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.18/WW34

Topic: H.09. Spatial Navigation

Support: NIH Grant R01HD099165-02S1
NIH Grant 1R21HD098509-01

Title: Paradigms for assessing individual differences in navigation: Do they converge?

Authors: *J. L. LADER, K. V. NGUYEN, N. S. NEWCOMBE;
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Abstract: Navigating environments is necessary for everyday life; however, there are substantial individual differences in navigational skill. Although various paradigms have emerged to measure such variation, few studies look at internal reliability or at cross-paradigm relations. Thus, it is unclear whether we can assess human navigation as a single dimension (e.g., building 'cognitive maps') or whether there are several relevant dimensions, and if skill(s) are uniform across environments (e.g., grid vs non-grid). In this study, we use a within-subjects design in which participants encode environmental features in one real-world environment (RWE) and two virtual environments (VE). The first VE, Virtual SILCton, is a validated paradigm that measures integration of 8 landmarks along two routes. Participants learn each main route and two connecting routes to later make onsite pointing judgments within and between the route landmarks. The second VE and the RWE were designed similarly with a gridded layout and encoding of 16 object locations. Participants complete judgment of relative direction pointing, map building, and object location tasks for all paradigms. We also give participants measures of spatial understanding, self-reported navigation skill, and small-scale spatial skills. The current sample size is 50 adults (18-31 years), and the projected sample size is 70. We will analyze the within-paradigm correlations to evaluate internal consistency and across-paradigm relations to gauge reliability and possible real-world validity. We expect that performance will be consistent within paradigms but performance may vary across paradigms, e.g., the real world and virtual experiences, or the environments with and without grids. We will run a principal component analysis to assess dimensionality and understand the role of environmental differences.

Disclosures: J.L. Lader: None. **K.V. Nguyen:** None. **N.S. Newcombe:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

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Program #/Poster #: PSTR240.19/WW35

Topic: H.09. Spatial Navigation

Support: Ford URP Grant
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ONR N000142012002
NEI R01-EY031455
NSF GRFP DGE 1752814 & DGE 1106400

Title: Active, naturalistic navigation is subserved by three functionally distinct networks in the human brain

Authors: *T. ZHANG, E. X. MESCHKE, J. L. GALLANT;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Navigation is a complex and dynamic task that requires multiple cognitive processes, including perceiving the world, planning a route, representing a cognitive map, and planning

motor actions. However, most human neuroimaging studies so far have used constrained stimuli and passive tasks that do not reflect the demands of real-world, active navigation. Thus, previous experiments likely did not engage the full navigation system of the brain, and therefore they did not fully map the representation of navigation-related information. To more fully characterize the cortical networks that support naturalistic navigation, we developed an active navigation paradigm for fMRI. We built a 2×3 km virtual city populated by dynamic pedestrian and vehicular traffic. Prior to scanning, participants learned to navigate the virtual world. We then recorded BOLD activity while participants (3 male, 3 female, age 25-32) used an MR-compatible steering wheel and pedals set to perform a taxi driver task in this virtual world (110 min of data for P1, 180 min of data each for P2-P6). Voxelwise modeling was performed with 24,990 stimulus- and task-related features across 34 navigation-related feature spaces. These feature spaces ranged from low-level features such as visual motion energy to high-level features such as tracking progress along a planned route. Functional brain networks mediating naturalistic navigation were then recovered by applying a new method called model connectivity (MC) to the model weights. MC identified 15 functional brain networks from this task. Navigation-related features are predominantly found in three closely-related networks, while other features, such as low-level vision or motor actions, are predominantly found in the other 12 networks. The most posterior of the three navigation networks includes the known navigation ROIs of PPA, OPA, and RSC, and also parts of the parietal cortex; the middle network includes the postcentral sulcus, precentral sulcus, anterior insula, and mPFC; finally, the anterior network includes IFS, SFS, mPFC, and parts of the parietal cortex. While these three networks all represent navigation-related information, the posterior network also represents more visual features, the middle network also represents more motor-related features, and the anterior network represents more cognitive features. These results suggest that active, naturalistic navigation is likely mediated by three distinct functional brain networks: one largely for the cognitive aspects of navigation, one for bridging navigation to visual perception, and one for bridging navigation to motor actions.

Disclosures: **T. Zhang:** None. **E.X. Meschke:** None. **J.L. Gallant:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.20/WW36

Topic: H.09. Spatial Navigation

Support: NIH Grant R01HD099165-02S1
NIH Grant R01HD099165

Title: Hippocampal subfield contributions to the co-development of episodic and spatial memory

Authors: *K. V. NGUYEN, J. J. ERARDI, G. A. O. CAMPOS, N. S. NEWCOMBE, I. R. OLSON;
Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: The hippocampus (HC) plays a key role in both spatial navigation and episodic memory. These processes are behaviorally linked by reliance on accurate retrieval of spatial and temporal context. However, the extent and nature of interdependence at behavioral and neural levels is unclear. In development, hippocampal subfields cornu ammonis (CA) 1-3, dentate gyrus (DG), and the subiculum have different maturational patterns, hinting that processes differentially reliant on these subfields may have different maturational profiles (Keresztes et al., 2018). In adults, these subfields are variably linked to memory processes with more free recall of internal details related to greater left CA3/DG and bilateral subiculum volume (Palombo et al., 2017), and faster place learning associated to subiculum and CA1-2 volumes (Daugherty et al., 2016). We know little about the joint development of episodic and spatial memory and how they may differentiate or converge into adulthood. **Using a naturalistic paradigm in which both episodic memory and spatial knowledge are assessed for the same encoding experience, we asked whether these processes are related to one another behaviorally and neurally, and how developmental age affects these processes.** During real-world encoding, 40 8-10-year-olds, 40 11-13-year-olds, and 40 young adults (18-31 years) took a guided walk through a novel environment and encoded distinct events. Episodic memory and spatial knowledge of the environment was assessed. On the second day, participants underwent MRI scanning to obtain T2-weighted images for HC subfields segmentation using a lifespan atlas. Preliminary linear modeling shows spatial performance as a significant predictor of episodic recall (controlling for age, IQ, and biological sex). In ongoing analyses, CA1-2, CA3/DG, and subiculum subfields are being traced within the HC body. We aim to replicate existing profiles of subfield volumetric development. Further, we will model these developmental trajectories with behavioral measures of memory. We predict HC lateralization with left subregions associated with recall and right subregions associated with navigation. Further, we predict an extended trajectory of navigation-related regions that parallels behavioral maturity into adolescence. This neural-behavioral profile will tease apart how episodic and spatial memory relate and inform how development can shape existing memory models.

Disclosures: K.V. Nguyen: None. **J.J. Erardi:** None. **G.A.O. Campos:** None. **N.S. Newcombe:** None. **I.R. Olson:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.21/WW37

Topic: H.09. Spatial Navigation

Support: Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT) CVU 409832

Title: Brain activity in children during navigation in a virtual environment.

Authors: *C. O. ZURITA BAUTISTA[1], G. D. ORTIZ-LAGUNES[2], Y. DEL RÍO-PORTILLA[2];
[1]UNAM, Mexico City, Mexico; [2]UNAM, México City, Mexico

Abstract: Electroencephalographic activity in navigation has been controversial. Humans show slower theta oscillations than rodents (Jacobson, 2014), and there is no consensus regarding oscillations correlate with landmarks encoding or recall. Landmark codification has been associated with both 2-4 Hz oscillations (Werweg & Kahana, 2018) and 4-8 Hz oscillations (Pu, Cornwell, Cheyne, & Johnson, 2017). However, fast theta frequencies (4 to 8 Hz) have also been functionally associated with landmarks recall. Previous research found those oscillation differences comparing landmarks codification and recall versus baseline condition. Participants ran or swim through a virtual environment in baseline conditions. Studies have been carried out mainly with adults whose EEG activity has matured. Whether adult studies report differences, we expect to observe even more variation in children's navigation oscillations due to their maturation brain activity process. We investigated scalp EEG activity in 57 children from 6 to 11 years old, dividing this population into three groups, one of 6-7 years, another of 8-9 years and the third group of 10-11 years old. We registered 19 electrodes according to the international 10-20 system. We recorded EEG activity during navigation in a virtual arena while participants ran through a virtual environment (before landmarks encoding and recall condition), eyes open (EO) and eyes-closed (EC) resting condition. We analyzed age effect according to the three groups described. As preliminary results of this investigation, we found significant differences in absolute power ($p<05$) associated with age groups during EC condition in frontal delta (.2-4 Hz, Fp1, Fp2), frontal theta (4-7 Hz, as well as frontal absolut power (Fp1, Fp2) oscillations. Such differences provide precedent results regarding navigation EEG activity in children, helping to guide EEG variations age-associated. In addition, we highlight the relevance of analyzing age as a factor that impacts oscillation variance reported in adults during navigation.

Disclosures: C.O. Zurita Bautista: None. **G.D. Ortiz-lagunes:** None. **Y. del Río-Portilla:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.22/WW38

Topic: H.09. Spatial Navigation

Support: Marga und Walter Boll-Stiftung grant 210-05. 01-21

Title: "The route to success is paved with hard work": Online experiments about the determinants of difficulty in route following tasks

Authors: *O. BOCK, J.-Y. HUANG;
Inst. of Exercise Training and Sport Informatics, German Sport Univ. Cologne, Koeln, Germany

Abstract: When following a route from A to B, we need to decide at each intersection which way to proceed. The present work addressed several factors that might influence the difficulty of such decision making. In an experiment administered remotely via internet, 96 participants repeatedly followed a route through a virtual maze with twelve or eighteen intersections, and with two or three choices per intersection. One group performed *task S*, where all intersections looked alike to promote decision making by the serial order strategy (e.g.: "first left, then straight, ⋯"). Another group performed *task SA*, where unique visual cues were presented at each intersection to enable decision making both by the serial order strategy and by the associative cue strategy (e.g.: turn left at the train station"). In both tasks, decisions were more accurate on routes with twelve rather than eighteen intersections, and were more accurate by a similar amount on routes with two rather than three choices. Reaction time in task SA followed a pattern that was reciprocal to accuracy; reaction time in task S was generally lower, and was route-independent. Accuracy in task SA was similar in the present study, where participants were transported smoothly across intersections, and an earlier study, where they were transported abruptly across intersections. In contrast, reaction time in task S was lower with smooth rather than abrupt transport across intersections. We conclude that the number of intersections and the number of choices were equivalent determinants of route following difficulty. We further conclude that decision making in task S was anticipatory, occurring even before the next decision was due. We finally conclude that optic flow during turns at intersections interfered with anticipatory decision making, and that natural (smooth) transport across intersections did not enhance route following performance.

Disclosures: O. Bock: None. **J. Huang:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.23/WW39

Topic: H.09. Spatial Navigation

Title: Investigating grid-like spatial navigation in sensorimotor domain

Authors: *J. LEE[1,3], Y. WANG[1,3], A. CASAMENTO-MORAN[1,3], D. MCNAMEE[4], V. S. CHIB[1,3,2];
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Abstract: During sports training, athletes must associate and recall different features of the motor actions they perform with different playing contexts. For example, basketball players associate different shooting techniques with their court location. Works in cognitive psychology have demonstrated that an imaginal technique facilitates learning by associating abstract information (e.g., faces, numbers) with specified locations in a familiar spatial environment (e.g.,

home, office). We explored whether such a spatial structuring of information extends to sensorimotor representations by having participants perform and associate effortful isometric contractions with arbitrary cues. Specifically, each cue was associated with independent force and duration of hand-grip effort exertion. All cues were organized into a 2D grid-like force-duration space. Throughout this association phase, participants were tested on their retention of cue-exertion pairs. Importantly, we did not provide explicit information on the force-duration levels or how the cues were organized. Following this association phase, participants performed a force-duration space navigation task, which assessed their ability to flexibly recall these associations to make novel inferences. Specifically, participants first performed an effortful exertion and then decided which of two presented cues was more different from the exerted contraction in both force and duration levels. Finally, participants were asked to arrange the cues based on the force and duration levels to assess their understanding of the organization of the force-duration space. Throughout the association, all participants learned and retained the force and duration levels for each cue. However, only the participants who correctly formed a spatial grid-like map performed the force-duration space navigation task accurately. These results suggest that sensorimotor information can be spatially organized, which allows individuals to flexibly distinguish and recall different features of their motor actions. Because the hippocampus (HC) and the medial entorhinal cortex (EC) encode a cognitive map of spatial and abstract information, our subsequent fMRI experiments will examine whether grid-like encoding of sensorimotor information is represented by the EC and how the motor and HC-EC networks interact during flexible recall of sensorimotor information.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.24/WW40

Topic: H.09. Spatial Navigation

Title: Full-body motion through a human-scale virtual Morris watermaze modulates EEG dynamics in patients with hippocampal lesions

Authors: *S. JEUNG[1,2,3], D. IGGENA[4,5], P. M. MAIER[4,5], B. HOSGOREN[6], C. J. PLONER[4], C. FINKE[4,5], K. GRAMANN[1];
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Abstract: In previous work (Iggena et al., under review), individuals with lesions in their right medial temporal lobe displayed better spatial memory when they had access to multisensory input from physical movement, displaying distinct strategies to leverage this benefit compared to healthy controls. Here, we used mobile brain-body imaging (Gramann et al., 2011) to investigate cortical activity underlying the compensation of compromised hippocampal input in the patient group. Thirty (18 female, 12 male) subjects were included with 10 patients who had undergone a partial resection of the right medio-temporal lobe including the hippocampus. Patients were matched with two healthy controls in age, sex, and education level. The virtual water maze environment consisted of a circular arena surrounded by environmental cues. During two sessions in i) mobile VR with head-mounted goggles and ii) stationary screen presentation, six blocks of Water Mazes were presented while high-density mobile EEG was continuously recorded. Each block started with three learning trials, during which participants searched for an hidden object in the arena. During the following four probe-trials, they were asked to navigate back to and report the remembered target location. While the target location remained fixed, the starting position varied between probe trials (rotated by 0, 90, 180, and 270 degrees). Power of theta band in frontal-midline electrode group, typically associated with physical locomotion, was compared to baseline activity with participants walking without any memory task. We identified differential event-related spectral perturbation profiles for patients and controls. Significant clusters of spectral perturbation difference showed between mobile and stationary setups, event-locked to the onset of learning trials. In patient group, the comparison between mobile and stationary conditions did not yield a significant difference during the initial 3 seconds of probe trials, whereas in control groups a broad difference over time and a wide frequency range was observed.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.25/WW41

Topic: H.09. Spatial Navigation

Support: DFG

Title: Reliance on spatial cues across multiple virtual navigation tasks

Authors: *V. CHANDRESWARAN[1], A. BIERBRAUER[2], D. STAWARCZYK[3], L. KUNZ[4], D. V. CLEWETT[5], D. PINK[1], J. D. OZUBKO[6], M. SILVA[7], I. BRUNEC[8], G. COUGHLAN[9], M. HORNBERGER[9], H. J. SPIERS[10], L. FUENTEMILLA[7], M. BARENSE[8], L. DAVACHI[11], N. AXMACHER[1], H. ZHANG[1];
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Univ., New York, NY; [5]Psychology, UCLA, Los Angeles, CA; [6]Dept. of Psychology, SUNY Geneseo, Geneseo, NY; [7]Univ. of Barcelona, Barcelona, Spain; [8]Univ. of Toronto, Toronto, ON, Canada; [9]Norwich Med. Sch., Norwich, United Kingdom; [10]Univ. Col. London, London, United Kingdom; [11]Dept. of Psychology, New York Univ., New York, NY

Abstract: Different strategies can be used to navigate towards a goal. Previous research has demonstrated that individuals with an increased genetic risk for Alzheimer's disease exhibit altered navigational patterns in a variety of virtual navigation tasks. Specifically, risk carriers tend to move closer to boundaries and landmarks in virtual environments, but since this has been shown in different tasks, the relationship between these patterns remains unclear. In the present study, we aimed to investigate how the effects of spatial cues are related by conducting a battery of spatial navigation tasks with the same sample of participants. Additionally, we included event segmentation tasks to explore a potential link between event boundaries and spatial boundaries. Hierarchical muti-level analysis showed numerous relationships between navigational patterns. Our results suggest effects of navigation proximity to salient locations on performance in all tasks. Reliance on spatial cues differed substantially between participants, and these differences predicted performance not only within a task but also across tasks. Results in the event segmentation tasks replicated some previously observed effects of episodic boundaries on memory recall, which however were not significantly correlated to the spatial navigation patterns. Overall, our findings show both effects that are specific to an individual task and those that occur across multiple tasks. More generally, this study demonstrate the potential of using a battery of paradigms to reveal general mechanisms of spatial navigation.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

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Program #/Poster #: PSTR240.26/WW42

Topic: H.09. Spatial Navigation

Support: NIH Grant R01MH124761
NIH Grant R01MH120194
NSF Grant 2124252

Title: Cognitive and environmental context shifts modulate human temporal lobe activity during real-world navigation

Authors: *C. INMAN[1], L. GARCIA[2], U. TOPALOVIC[6], M. VALLEJO MARTELO[6], M. STANGL[7], T. DAVIS[3], M. HOLLEARN[4], J. CAMPBELL[5], L. AUGUSTIN[4], D. ELIASHIV[8], V. R. RAO[10], I. FRIED[9], N. HASULAK[11], S. HILLER[7], N. A. SUTHANA[7];
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Abstract: The ultimate goal of neuroscience is to understand and explain real-world behavior in terms of brain activity, and to use these insights to develop therapeutic approaches for neural disorders. Traditional neuroimaging methods, like fMRI, require participants to be stationary, limiting research studies' complexity and realism. By using mobile recording devices synchronized with intracranial EEG recordings in epilepsy patients with an implanted deep brain recording system (NeuroPace Responsive Neurostimulator; RNS), we can study the neural basis of everyday human activities such as navigation and memory encoding in a more natural way that captures the complexity, scale, and functional characteristics of real-world experiences. We asked five RNS participants to learn a 0.75-mile route around campus with only instructions to remember the route well enough to navigate back to the beginning. Subjects walked the route 7-8 times across two days, with the 1st walk guided (encoding) and 6-7 of the walks navigated by the participant themselves (navigation retrieval; 28.5 total miles). Local field potential data between 1-85 Hz was continuously collected throughout each participant's walk synchronized with a suite of 1st person experience sensors at millisecond precision. Findings across all participants suggest that theta band power (5-8 Hz) in the medial and lateral temporal lobe significantly increases when participants are navigating outdoors relative to indoor navigation. This effect persists when controlling for walking speed, although walking speed is also a significant predictor of changes in theta power as well. We also find evidence that temporal lobe theta power increases immediately after participants passed through spatial event boundaries (i.e. doorways, turns, etc). These changes in theta power at event boundaries are immediately followed a few seconds later by co-occurring increases in gamma power (35-75 Hz) and decreases in theta power (3-7 Hz), suggestive of the shift in 1/f spectral tilt often observed during successful memory encoding. Cognitive event boundaries were determined by assessing temporal agreement among 20 separate observers who were asked to segment each of the participant’s first-person videos by indicating when one meaningful unit ended, and another began. These cognitive event boundaries also provoke changes in oscillatory power throughout the temporal lobe. Taken together, these initial findings support our hypotheses that medial and lateral temporal lobe activity reliably changes around real-world spatial and cognitive event boundaries.

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Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

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Program #/Poster #: PSTR240.27/Web Only

Topic: H.09. Spatial Navigation

Support: JSP Grant JPMJCR18A3

Title: Can Brain Structure and Early Vision Predict Calculation Abilities?

Authors: *K. HOSOKAWA[1], T. MATSUHASHI[2], C. HOSODA[2];
[1]Inst. of Development, Aging and Cancer, [2]Tohoku Univ., Sendai, Japan

Abstract: Spatial perception, process through which humans become aware of the relative positions of their own bodies and objects around them. Space perception provides cues, such as depth and distance, that are important for not only orientation to the environment but the mathematical skills(e.g., Uttal et al, 2013). However, the direct relationship between motion-in-depth perception and mathematics and its neural basis was still unclear.. Therefore, we aimed to examine whether the neural basis involved in the sensitivity to depth at earlier stages could predict individual difference of mathematical skills. Ninety-eight adults were participated in the experiment, 50 were female and 48 were male. They undertook the the 3T-MRI (Siemens PRISM) scanning to obtain the T1-weighted images, calculation test at the junior high school-level, and task of the sensitivity for depth from motion. T1w images were normalized, segmented, and smoothed to 8 mm resolution with SPM8 toolbox. The tasks of the sensitivity for depth were set for 2D and 3D conditions. In the 2D conditions depth were hard to be perceived from the stimuli and in 3D condition the stimuli simulated velocity field generated by forward movement. the participants were asked to point the center of the random dot kinematgram. In the analysis, the participants divided into two groups based on median score on the 3D task of sensitivity for depth: 3D depth-high and 3D depth-low. CatBoost model was trained with normalized grey matter volume to classify participants into the high or low 3D depth. Finally, a model predicting 3D depth-high and 3D depth-low from normalized grey matter volume was tested if it could also predict high and low math ability. The trained model was able to classify 3D depth-high and 3D depth-low (70%). In addition, this model was also able to classify the high and low performance of the calculation(60%). The region that contributed to the classification was mainly the angular gyrus (Brodmann area 39). These results suggest that higher-order computational performance may depend on the ability to detect depth cues earlier in spatial perception and it is reflected in the grey matter volume of angular gyrus.

Disclosures: K. Hosokawa: None. **T. Matsuhashi:** None. **C. Hosoda:** None.

Poster

PSTR240. Human Navigation: Cellular Physiology and Sensorimotor Aspects

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR240.28/WW43

Topic: H.09. Spatial Navigation

Support: Joy Ventures 802096

Title: Structural features of spatial configurations modulate anticipation towards movement - a contingent negativity variation ERP study

Authors: *N. R. ZUR[1], L. PELED-AVRON[2], S. G. SHAMAY-TSOORY[3], D. FISHER-GEWIRTZMAN[4];
[1]Georgetown Univ. Med. Ctr., Arlington, VA; [2]Bar-Ilan Univ., Ramat Gan, Israel; [3]Univ. of Haifa, Haifa, Israel; [4]Technion, Haifa, Israel

Abstract: Perceived density has been considered subjective in architecture design and differences in spatial configuration can affect the relationship between perceived and real density. To assess perceived density of spatial configurations in this study we used two architectural design features: location of the wider wall in trapezoid-shaped apartments (at the entrance or edge of the apartment) and window size (wide or narrow). Importantly, while these features were varied, the absolute amount of space depicted within the apartment remained constant. Both subjective ratings as well as electroencephalogram (EEG) measures were used to assess the effect of these design features. Previously, we have shown that these design features modulate event-related desynchronization (ERD or Mu-Rhythm) and Theta power - both measures associated with movement planning. We have also shown that ratings of perceived density were higher in apartments where the wide wall was located at the entrance (in which the apartment appears as narrowing) and in conditions with a narrow window. In the current study, we are looking into the effect of these design features on anticipation towards movement initiation using contingent negativity variation (CNV) an event-related potential (ERP) component. CNV, an anticipatory motor preparation ERP component, which is progressively negative and associated with in thalamo-cortico-striatal networks, was examined by separately looking into the mean amplitude of three 200 ms time windows preceding the initiation of movement within the depicted apartment. Analysis of nine subjects indicate that there is a main effect of the CNV time course, such that there is a decrease of mean amplitude as the time window gets closer to the initiation of movement within the depicted apartment. Additionally, there is an interaction of CNV time course with the location of the wall. Specifically, there is a more pronounced decrease in amplitude (CNV) in conditions where the spatial configuration appears as widening (wider wall is at the edge) as compared to conditions that appear as a narrowing spatial configuration (wider wall is at the entrance of the apartment). Based on these preliminary results we conclude that the depiction of movement within a spatial configuration involves sensory feedback anticipation that is modulated by structural features of the spatial configuration.

Disclosures: **N.R. Zur:** None. **L. Peled-Avron:** None. **S.G. Shamay-Tsoory:** None. **D. Fisher-Gewirtzman:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.01/WW44

Topic: I.04. Physiological Methods

Support: ONR grant N00014-21-1-2343
McDonnell Center Systems Neuroscience Grant

Title: New single lens 2P Lightsheet for real time dual population dynamic olfaction studies in fruit fly.

Authors: *Q. R. R. COQUEREL, C. ZHOU, B. RAMAN;
Biomed. Engin., Washington university, Saint Louis, MO

Abstract: Olfaction is a highly dynamic sensory modality. To understand the basic signal representation and processing features of this sensory modality, we are developing a lightsheet microscopy technique that would allow us to characterize responses of a large population of neurons residing in different neural circuits and with high temporal resolution. Our lightsheet imaging system uses 2P illumination, 2 laser wavelengths to excite sequentially two independent neural populations. Other features such as bessel beam engineering, a single objective geometry, and fast remote focusing makes them ideal for the goal of fast imaging of neural networks. We present our approach and show proof of concept result in the fly olfactory system.
The single lens lightsheet technology is designed to image from thick tissues with limited access. Further, since the setup only requires minimally-invasive surgery to access the fruit fly brain structures, it allows for a longer duration neural recording. To shift our lightsheet through the sample, we use a remote focusing approach with galvo mirrors. In addition to being fast, this approach has the added benefit of suppressing mechanical movements and imaging artefacts associated with such micromovements. Finally, the 2P bessel beam lightsheet generates a needle of light that is more homogenous, penetrates deeper, with reduced scattering, and self -healing properties than regular gaussian beam based lightsheets. This overcomes the limited occlusion issues associated with gaussian 1P lightsheet where shadows are encountered along the light path that limit the spatial resolution in deeper layers of the tissue.
The microscope is designed to record from whole region of interest in the fly brain, with a resolution down to the single neuron, with a volume acquisition frequency of up to 20Hz. We will present proof-of-concept results by monitoring calcium signals from transgenic flies that express GCaMP in the antennal lobes and mushroom bodies. Calcium signals from both antennal lobe and mushroom bodies will be monitored simultaneously to illustrate the strength of this imaging approach.

Disclosures: Q.R.R. Coquerel: None. **C. Zhou:** None. **B. Raman:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.02/WW45

Topic: I.04. Physiological Methods

Support: R21NS122055
R21DA048252

Title: Current applications of a blood-brain barrier permeable substrate for nanoluciferase based reporters

Authors: C. GAO[1], Y. SU[2], *S. WOODS[1], M. LUEDEMAN[1], M. LIN[2], **T. KIRKLAND**[1];
[1]Promega Corp., Madison, WI; [2]Stanford Univ., Stanford, CA

Abstract: This poster will provide and overview of the latest developments in applications for a fluorinated analog of furimazine dubbed cephalofurimazine (CFz). This nanoluciferase substrate has been optimized for bioluminescent imaging of the mouse brain and we demonstrate that it is sufficient to image brain activity in freely moving mice using a head mounted camera. We also demonstrate that it can be utilized in conjunction with nanoluciferase based biosensors to provide real-time physiological data in the murine brain.

Disclosures: C. Gao: A. Employment/Salary (full or part-time):; Promega Corporation. **Y. Su:** None. **S. Woods:** A. Employment/Salary (full or part-time):; Promega Corporation. **M. Luedeman:** A. Employment/Salary (full or part-time):; Promega Corporation. **M. Lin:** None. **T. Kirkland:** A. Employment/Salary (full or part-time):; Promega Corporation.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.03/WW46

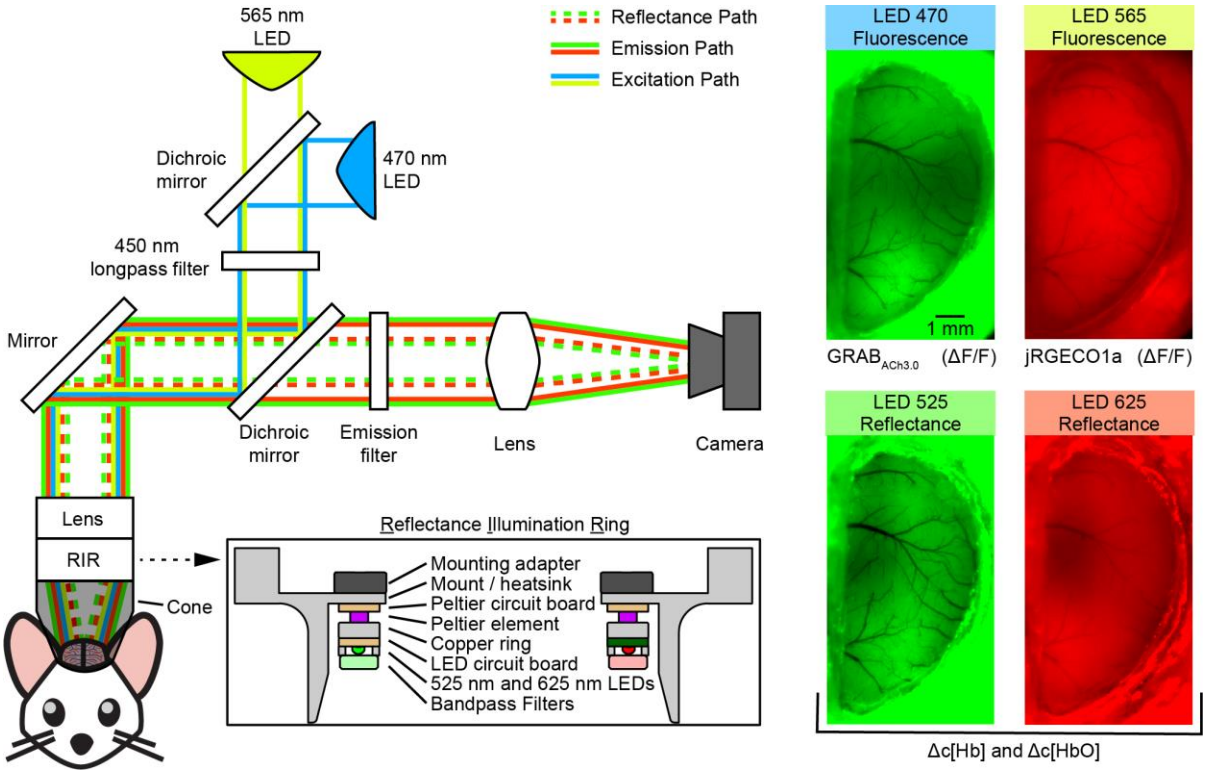
Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative Grant U19-NS123717
NIH Grant R01-DA050159
NIH Fellowship F31-NS118949 (PD)

Title: A widefield in vivo imaging system for two fluorescent probes and quantitative hemodynamics using a single CMOS detector

Authors: *P. DORAN[1], N. FOMIN-THUNEMANN[1], R. TANG[1], B. ZIMMERMANN[1], S. KURA[1], E. A. MARTIN[1], K. KıLıÇ[1], G. CHABBOTT[1], J. X. JIANG[1], P. PEREZ[1], B. FU[2], J. MANDEVILLE[2], S. SAKADZIC[2], D. A. BOAS[1], A. DEVOR[1,2], I. A. CHEN[1], M. THUNEMANN[1];
[1]Boston Univ., Boston, MA; [2]Martinos Ctr. for Biomed. Imaging, Dept. of Radiology, Harvard Med. Sch., Charlestown, MA

Abstract: Widefield microscopy of the mouse cerebral cortex enables mesoscale imaging of spontaneous and evoked neuronal activity. An array of fluorescent reporters is used to visualize distinct aspects of brain function. Reporters with different colors enable spectral multiplexing. A change in neuronal activity induces changes in blood volume and -oxygenation. The resulting dynamic changes in light absorption by oxy- and deoxyhemoglobin (HbO and Hb, respectively) can interfere with fluorescence signal detection. Quantification of Hb/HbO concentrations is a central technique in neurovascular imaging and enables correction of hemodynamic artifacts in fluorescence recordings. We aimed to build an instrument for concurrent imaging of red and green fluorophores as well as changes in Hb and HbO concentrations across mouse cortex. Two-color fluorescence imaging is performed in epi-illumination mode. For reflectance imaging of Hb/HbO absorption, 525- and 625-nm LEDs are placed on a custom-made illumination ring around the objective. An opaque cone between objective and cranial window prevents the animal from perceiving the strobing illumination light. A single sCMOS camera is used to detect fluorescence and reflectance signals. The instrument is controlled through MATLAB-generated trigger signals. We strobe four LED channels to acquire two fluorescence and two reflectance time series at an effective rate of 10 Hz. We illustrate instrument performance by conducting cortex-wide imaging of spontaneous and evoked neuronal and hemodynamic activity in mice expressing the red calcium indicator jRGECO1a and the green acetylcholine indicator $GRAB_{ACh3.0}$. We show signal changes in response to tactile whisker and visual stimulation. In parallel, we built a system for concurrent widefield fluorescence imaging and BOLD fMRI in awake mice to compare results from optical Hb/HbO measurements with BOLD fMRI-based measurements of hemoglobin oxygenation. We developed a versatile system for widefield imaging of two fluorescent reporters and changes in hemoglobin oxygenation with a single camera.



Disclosures: **P. Doran:** None. **N. Fomin-Thunemann:** None. **R. Tang:** None. **B. Zimmermann:** None. **S. Kura:** None. **E.A. Martin:** None. **K. Kılıç:** None. **G. Chabbott:** None. **J.X. Jiang:** None. **P. Perez:** None. **B. Fu:** None. **J. Mandeville:** None. **S. Sakadzic:** None. **D.A. Boas:** None. **A. Devor:** None. **I.A. Chen:** None. **M. Thunemann:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.04/WW47

Topic: I.04. Physiological Methods

Support: Grant-in-Aid for Transformative Research Areas (A) 'Glial Decoding' JP21H05621
JSPS KAKENHI JP19K06883
JSPS KAKENHI 15KK0340
JSPS KAKENHI JP22H00432
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JSPS KAKENHI JP17H06312
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Brain/ MINDS JP19dm0207079
Brain/ MINDS JP19dm0207080
Narishige Neuroscience Research Foundation
Grant for Young Researcher from Yamanashi Prefecture
Takeda Science Foundation

Title: The application of a large cranial window for wide-field and two-photon calcium imaging from the same mouse

Authors: *S. MANITA[1], E. SHIGETOMI[2,3], H. BITO[4], S. KOIZUMI[2,3], K. KITAMURA[1]; [1]Dept. of Neurophysiol., [2]Dept. of Neuropharm., [3]Yamanashi GLIA Ctr., Univ. of Yamanashi, Yamanashi, Japan; [4]The Univ. of Tokyo, Tokyo, Japan

Abstract: Single-neuron activity during animal behavior has been extensively studied using electrophysiological techniques and two-photon calcium imaging. However, understanding complex brain functions like movement requires studying neuronal activity not only at the single-cell level but also at the population level.
In this study, we developed a novel cranial window that enables single-cell and population-level activity imaging in the same mouse. This window, approximately 3×6 mm^2 in size, can be easily and inexpensively fabricated using a food wrap, transparent silicone, and cover glass. It allows the removal of the dura mater and suppression of brain vibrations, facilitating long-term observations of one month or more. By installing this window in transgenic mice expressing calcium sensors specifically in astrocytes, wide-field imaging revealed synchronized responses

over a few millimeters in the awake state. In the same mouse, two-photon imaging captured calcium responses in fine astrocyte processes displaying activity at various timings within a range of approximately 10 micrometers over a few seconds, consistent with previous reports (Stobart et al., 2018). Furthermore, using this window, we could measure macroscopic calcium activity from astrocytes in the prefrontal cortex of mice performing a forelimb reaching and grasping task (Manita et al., 2022).
Additionally, we have succeeded in widely expressing calcium sensors in the cerebral cortex or cerebellar cortex by forming the calcium sensor-expressing adeno-associated viruses (AAVs) membrane onto a food wrap with silk fibroin, a constituent protein of silkworm cocoons (Jackmann et al., 2018).
This technique is expected to be applied to experiments using a wide variety of biosensors, such as voltage and neurotransmitter sensors in various species, from rodents to non-human primates, and thus, holds promise for various neuroscience research applications.

Disclosures: **S. Manita:** None. **E. Shigetomi:** None. **H. Bito:** None. **S. Koizumi:** None. **K. Kitamura:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.05/WW48

Topic: I.04. Physiological Methods

Support: STI2030-Major Projects 2021ZD0202205, 2022ZD0212100
National Natural Science Foundation of China 8200907151, 61975002, 31830036
National Postdoctoral Program for Innovative Talents BX20190011

Title: Multi-color Miniature Two-photon Microscopy for Deep Brain Imaging

Authors: ***R. WU**[1], Z. CHUNZHU[1], Z. YUFEI[1], F. HUAQIANG[3], Z. LIFENG[3], Z. DONG[1], F. QIANG[4], H. YANHUI[4], W. CONGHAO[5], F. LISHUANG[5], W. AIMIN[2], C. HEPING[1];
[1]Col. of Future Technol., [2]State Key Lab. of Advanced Optical Communication Syst. and Networks, Peking Univ., Beijing, China; [3]PKU-Nanjing Inst. of Translational Med., Nanjing, China; [4]Beijing Transcend Vivoscope Biotech Co., Ltd, Beijing, China; [5]Beihang Univ., Beijing, China

Abstract: Simultaneous multicolor imaging of deep brain structure and functional dynamics in freely moving animals is an urgent need for decoding neural networks. Here, we report a multicolor miniature two-photon microscope (FHIRM-TPM 3.0) weighting 2.6 g, which uses a hollow anti-resonant fiber with broad transmission characteristics, an apochromatic headpiece, and interchangeable objectives with different magnification. The FHIRM-TPM 3.0 enables multicolor two-photon excitation at wavelengths of 780nm, 920nm and 1030 nm, with the

imaging resolutions reach to the theoretical optical diffraction limit. Three interchangeable objectives OBJ-HR/U/LF provide 35 times scalable imaging field of view from 0.0225 mm^2 to 0.8 mm^2. Next, the practicability of FHIRM-TPM 3.0 was illustrated by different static and dynamic indicator imaging in awake or freely moving mice. Equipped with OBJ-U, FHIRM-TPM 3.0 enables dual-color calcium imaging with GCaMP6 and jRGECO indicators, deep brain calcium imaging up to a depth of 820 μm through a cranial window, and single spine level calcium dynamics in the hippocampal CA1 subregion through a GRIN lens.

Disclosures: R. Wu: None. **Z. Chunzhu:** None. **Z. Yufei:** None. **F. Huaqiang:** None. **Z. Lifeng:** None. **Z. Dong:** None. **F. Qiang:** None. **H. Yanhui:** None. **W. Conghao:** None. **F. Lishuang:** None. **W. Aimin:** None. **C. Heping:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.06/WW49

Topic: I.04. Physiological Methods

Support: NIH (R01 CA241618)

Title: Fiber-optic nonlinear wavelength converter as an accessible and adaptive femtosecond laser source for neuroscientists

Authors: *H. TU;
Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Fiber-optic nonlinear wavelength converter as an accessible and adaptive femtosecond laser source for neuroscientists

Haohua Tu
Cellular resolution neurophotonics, including the prominent example of optogenetics, has been the driving force for brain research. In contrast to the genetic and biophotonic advancements that have transformed this field, the progress in ultrafast laser technology underlying these advancements has lagged. The lack of collaboration between laser engineers and neuroscientists has produced three technical barriers: (1) neuroscientists and biophotonic scientists have been limited by readily available commercial lasers that may not be the best solutions for their intended applications, due largely to the lack of full tunability in parameters such as wavelength, power, and temporal profile; (2) user-unfriendliness of tunable customer or commercial lasers has hindered the extension of ultrafast laser technology beyond non-laser experts and dedicated optical laboratories, and (3) the lack of adaptation of installed lasers with free-space beam delivery often render them obsolete when new neuroscience needs and applications emerge. Here I develop a versatile and user-friendly femtosecond laser based on the coherent supercontinuum generation in a specific photonic crystal fiber, termed as fiber-optic nonlinear wavelength converter (FOWC), to overcome these barriers. FOWC delivers fully tunable ~20 nJ pulses with

central wavelength across 950-1150 nm, repetition rate across 1-20 MHz, and pulse width across 40-400 fs, with longer-term stability over 2000 hr. Wide accessibility is uniquely enabled by designing FOWC as a mobile facility, and by switching between different applications via standard fiber-optic telecommunication which allows researchers to make quick on-the-fly adjustments as research needs evolve and diversify. The adaptivity of FOWC has been demonstrated in *in vitro* imaging of culture cells or brain slices (and related drug discovery or therapeutics development), *in vivo* neural activity imaging of head-fixed animals, *in vivo* patch camping and volumetric stimulation of targeted neurons, all-optical stimulation and recording of neural activity, functional brain imaging beyond action potential (e.g. metabolism), label-free phenotyping diverse neural populations, and histopathology or intraoperative assessment of *ex vivo* brain tissue. In contrast to alternative lasers, FOWC offers on-demand service (without owning/maintaining the laser) and thus paves the way for adaptive and affordable ultrafast laser technology widely assessable by the neuroscience community.

Disclosures: H. Tu: None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.07/WW50

Topic: I.04. Physiological Methods

Support: NIH Grant 1UF1NS107696
NIH Grant U01NS118300

Title: Ultrafast two-photon fluorescence imaging of activities across large neuronal populations in vivo.

Authors: *J. ZHONG[1,2], R. G. NATAN[3], Q. ZHANG[5], J. ZHU[2], Y. YANG[2], J. S. J. WONG[6], K. BOSE[8], X. LU[9], G. ZHANG[10], S. GUO[12], F. ST-PIERRE[13], M. Z. LIN[11], K. K. TSIA[7], N. JI[4]; [1]Physics, UC Berkeley, Berkeley, CA; [2]Physics, [4]Physics and MCB, [3]Univ. of California, Berkeley, Berkeley, CA; [5]Physics, Univ. of California, Berkeley, United States, Berkeley, CA; [6]Electrical and Electronic Engin., [7]Dept. of Electrical and Electronic Engin., The Univ. of Hong Kong, Hong Kong, China; [8]Bioengineering, Univ. of California, San Francisco, San Francisco, CA; [9]Systems, synthetic and physical biology, Rice Univ., Houston, TX; [10]Neurobio., [11]NEUROBIOLOGY, Stanford Univ., Stanford, CA; [12]Bioengineering, Univ. California - San Francisco, SAN FRANCISCO, CA; [13]Dept. of Neurosci. and Dept. of Electrical and Computer Engin., Baylor Col. of Med. and Rice Univ., Houston, TX

Abstract: Monitoring activity at high spatiotemporal resolution across large populations of neurons is crucial to understanding information processing within the brain. By combining rapid all-optical linescanning with mechanical scanning, we developed a two-photon microscope that allows ultrafast imaging across large areas and volumes with subcellular resolution. Applying the

microscope to voltage, glutamate, and calcium imaging in vivo, we achieved order-of-magnitude improvements in frame rate, volume speed, and cell counts.

Disclosures: **J. Zhong:** None. **R.G. Natan:** None. **Q. Zhang:** None. **Y. Yang:** None. **J.S.J. Wong:** None. **K. Bose:** None. **X. Lu:** None. **G. Zhang:** None. **S. Guo:** None. **F. St-Pierre:** None. **M.Z. Lin:** None. **K.K. Tsia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US patent 14/733,454. **N. Ji:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.08/WW51

Topic: I.04. Physiological Methods

Support: STI2030-Major Projects 2021ZD0204503
National Science Foundation of China 32125020
'Strategic Priority Research Program' of the Chinese Academy of Sciences XDB32030200
Shanghai Municipal Science and Technology Major Project 2018SHZDZX05 and 18JC1410100
National Key R&D Program of China 2018YFA0801000 and 2018YFA0801001

Title: Imaging spinule dynamics at super-resolution in awake mouse brain

Authors: *Y. ZHANG, L. BAI, X. WANG, Y. ZHAO, T. ZHANG, L. YE, X. DU, Z. ZHANG, J. DU, K. WANG;
Inst. of Neuroscience, CAS, Shanghai, China

Abstract: Spines are the major building blocks of neuronal networks, holding the postsynaptic part of synaptic transmission. The morphology of spines, such as their shape, volume, and postsynaptic density area, is highly correlated with the number of AMPA receptors and other parameters of functional synaptic connections, as discovered by electron microscopy. In an ever-changing environment, animals undergo constant learning and neural plasticity to adapt their behaviors, during which spines also display morphological changes. Dendritic spinules are thin spine protrusions that frequently appear on mature spines and potentially play a role in synaptic remodeling and retrograde signaling. Current understanding of spinule dynamics and functions has largely been inferred from ex vivo observations, with their functions under physiological circumstances remaining to be studied. In vivo two-photon microscopy (TPM) has been used to observe morphological spine dynamics during learning, sleep, and disease. However, conventional optical imaging technologies such as TPM and confocal microscopy are limited in resolution and cannot capture the fine details of spinules. Super-resolution microscopies (SRMs)

surpass the resolution limit (~300 nm) but face great challenges when adapted to imaging living animals, mainly due to sample motion caused by heartbeat and breathing. To fill this gap and perform super-resolution imaging in living and behaving animals, we introduce a new imaging paradigm that can tolerate motion artifacts while achieving large field-of-view ($80 \times 80 \times 8$ μm^3) super-resolution imaging. To observe spinule dynamics in vivo, we expressed a membrane-localized fluorescent protein in excitatory neurons in the mouse cortex by stereotactic injection of AAV and performed imaging of dendrites in the superficial layer. We found that spinules in the cortex were small, frequently active, and widely spread on various types of spines. They transiently emerged and disappeared with a lifetime of seconds. The spatial characteristics and temporal characteristics of spinules were correlated in that long-lived spinules explored a wider range of nearby space. Taking advantage of high-speed and long-duration imaging, we found that some active sites on spines showed repeated generation of spinules, indicating potential mechanisms for area-specific plasticity. In conclusion, we have established a new super-resolution imaging technology and characterized the dynamics of spinules in awake mice, leaving potential for further investigation of other subcellular morphological activities and their functions in neural plasticity.

Disclosures: **Y. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent No. 202310207189.0. **L. Bai:** None. **X. Wang:** None. **Y. Zhao:** None. **T. Zhang:** None. **L. Ye:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent No. 202310207189.0. **X. Du:** None. **Z. Zhang:** None. **J. Du:** None. **K. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent No. 202310207189.0.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.09/WW52

Topic: I.04. Physiological Methods

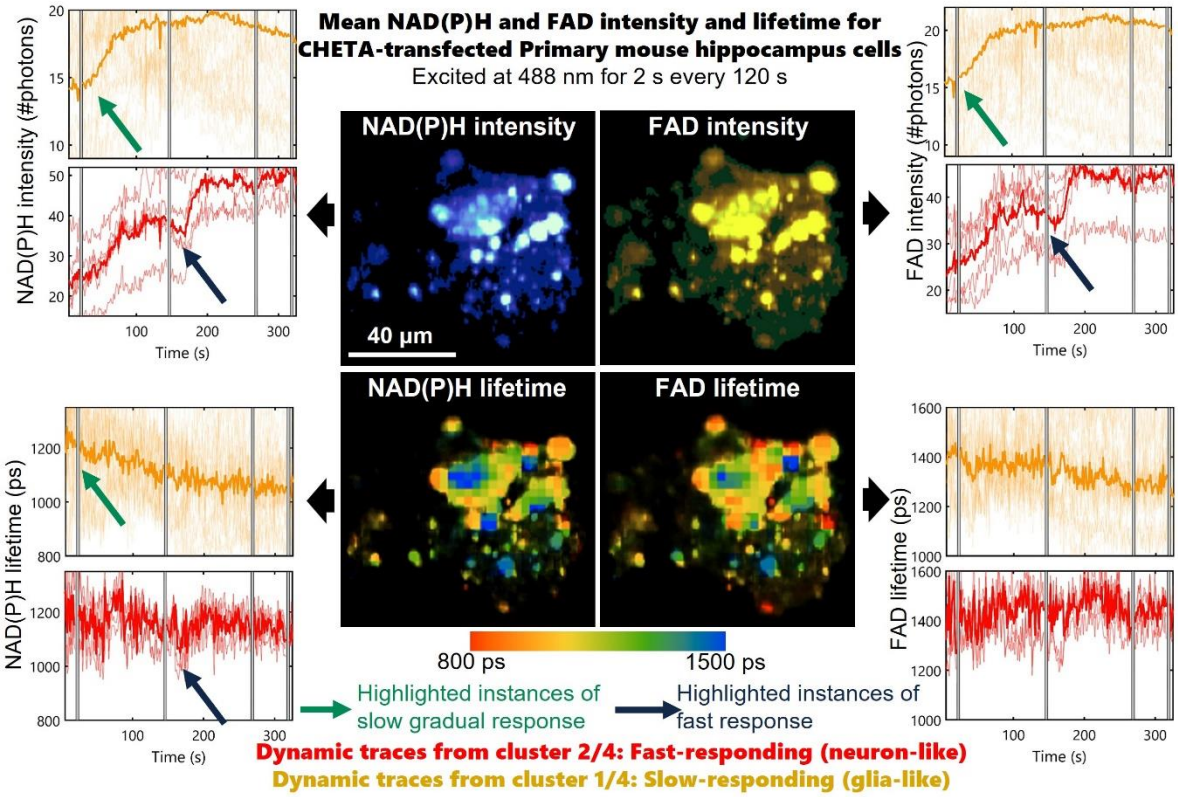
Support: NIH Grant R01CA213149
NIH Grant R01CA241618
NIH Grant T32EB019944
AFOSR Grant FA9550-17-1-0387

Title: Label-free metabolic microscopy of neuronal activity using fluorescence lifetime imaging

Authors: *R. R. IYER[1], J. E. SORRELLS[2], C. A. RENTERIA[3], L. YANG[2], S. A. BOPPART[2]; [1]Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois at Urbana Champaign, Urbana, IL; [2]Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois at Urbana-Champaign,

Urbana, IL; [3]Univ. of Illinois At Urbana-Champaign, Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Neurons that are actively engaged in signaling and communication undergo rapid changes in their metabolic states due to their dynamic energy requirements. The autofluorescence properties of metabolic cofactors such as NAD(P)H and FAD, specifically their intensity and lifetime, respond to this neuronal activity. While conventional fluorescence lifetime imaging microscopy (FLIM) is not capable of capturing these fast dynamics, advanced FLIM techniques that sample photocurrents at gigahertz frequencies offer higher sensing and imaging frame rates. In our previous work, we demonstrated fast FLIM using computational photon counting and real-time processing on a GPU for a single channel, which encountered communication bottlenecks for faster frame rates for two-channel imaging. We demonstrate a new approach for performing fast dual-channel label-free FLIM of NAD(P)H and FAD as a method for studying the dynamic physiology of neurons and neural circuits. This is achieved by implementing computational photon counting on the on-board FPGA of the digitizer. To reduce data throughput, we compress photocurrents to photon counts, resulting in a 4x reduction for each channel. The FPGA's parallel processing capability ensures no acquisition delay or lag in the system. We conducted experiments using this setup on stem-cell-derived neurons (NE-4C), subjecting them to glutamate stimulation. The FLIM data captured the dynamics of NAD(P)H and FAD in response to these manipulations. The temporal dynamics observed in FLIM were used to extract both single-cell responses and network dynamics. Also, the setup was validated on an optogenetic primary mouse hippocampus culture model. The multimodal network responses were clustered using principal component analysis that showed a functional contrast between fast (neuron-like) and slow responding (glia-like) cells (Fig). The use of fast FLIM on the FPGA enables real-time, dual-channel label-free metabolic optophysiology to study neural activity and has promising applications in understanding the intricate dynamics of neuronal networks.



Disclosures: **R.R. Iyer:** None. **J.E. Sorrells:** None. **C.A. Renteria:** None. **L. Yang:** None. **S.A. Boppart:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.10/WW53

Topic: I.04. Physiological Methods

Title: A temporal regression method for removing hemodynamic artifacts in wide-field fluorescent imaging

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Abstract: For *in vivo* wide-field fluorescent imaging experiments, the removal of hemodynamic noise is of critical importance. The classic Beer-lambert-law-based method, however, didn't consider the reflection from non-neuronal substances. This reflection noise affects the spatiotemporal dynamic of fluorescent data in a complicated way. Without reflection noise correction, the classic method cannot achieve complete removal of the hemodynamic noise, resulting in an inaccurate interpretation of the neuronal activity. We have developed a novel temporal regression procedure to calculate the reflection from the non-neuronal substance using the temporal window where the neuronal activity was quiet. The non-neuronal reflection can be subtracted from recorded hemodynamic data to achieve pure hemodynamic change. Using pure hemodynamic change, the hemodynamic artifact can be correctly removed, and the fluorescent data can reflect the real neuronal activity. This technique is demonstrated in different data sets and has been shown to be superior to the classic method. Our research also questioned the previously fluorescent imaging data.

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Poster

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Topic: I.04. Physiological Methods

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Title: Whole-brain, single-cell resolution imaging of neural voltage in larval zebrafish

Authors: *Z. WANG[1], J. ZHANG[2], P. SYMVOULIDIS[2], M. A. WILSON[2], E. S. BOYDEN[2,3]; [1]McGovern Inst. for Brain Res., [2]MIT, Cambridge, MA; [3]HHMI, Cambridge, MA

Abstract: Behavior emerges from the coordinated activity of many neurons distributed throughout entire brains. The ability to monitor the voltage of individual neurons across entire brains would open up new opportunities to examine how neurons work together to generate

behavior. Recent advances in genetically encoded voltage indicators (GEVIs) can allow direct imaging of neural membrane voltage dynamics at high spatiotemporal resolution. However, due to the rigorous imaging speed and signal-to-noise ratio (SNR) demands of existing GEVIs, microscopy hardware to date has only been able to image the voltage of neurons within subregions of the brain, even for small animals like the larval zebrafish. Here we adapted high-speed remote scanning light-sheet microscopy to image the voltage dynamics of neurons expressing GEVIs across entire brains of behaving larval zebrafish. Using these tools, we monitored cellular fast spiking and subthreshold voltage activity across the entire zebrafish brain, while animals were behaving. Our microscope will enable exploration of whole-brain neural computations and mechanisms underlying animal behaviors at the single-cell, single-spike, and subthreshold potential levels.

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Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant R21 NS112948
NIH Grant S10 OD021773
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The Mirowski Family Foundation

Title: A red fluorescent protein-based calcium indicator to study rapid calcium dynamics in the endoplasmic reticulum

Authors: *K. BERGLUND[1], M. A. STERN[1], C.-A. N. GUTEKUNST[1], J. J. YANG[2], R. E. GROSS[1];
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Abstract: Calcium indicator proteins are versatile and indispensable tools for cell type-specific and subcellular imaging in the biomedical field, especially in neuroscience. Here we report a novel calcium indicator, R-CatchER, engineered from a red fluorescent protein that targets the endoplasmic reticulum (ER). Unlike conventional calcium indicator proteins, R-CatchER does not utilize the EF-hand motif as a calcium sensing mechanism. Instead, a single calcium binding site was engineered within the scaffold of mApple near the chromophore through point mutations and molecular dynamics simulation. *In vitro* characterization of the recombinant protein confirmed one-to-one binding stoichiometry and showed that the new indicator had affinity and kinetics suitable to study rapid calcium dynamics in the ER. When transiently expressed in primary cortical neurons in culture, R-CatchER showed proper targeting in neuronal

ER. We quantified ER calcium responses to electrical stimulation in different compartments of neurons. R-CatchER was sensitive to reveal an ER calcium transient to a single electrical stimulus and its red-shifted spectrum allowed for simultaneous monitoring of cytosolic calcium through commonly used calcium-dependent green/yellow fluorescent proteins, such as jGCaMP7s and XCaMP-Y. We further developed a recombinant adeno-associated viral vector to express R-CatchER and XCaMP-Y in the same neurons *in vivo*. Two-photon imaging in the mouse motor cortex revealed calcium dynamics in ER and the cytosol in an awake animal during and after a seizure elicited by a chemical convulsant, pentylenetetrazol. Thus, R-CatchER provides unique opportunities to study ER calcium dynamics in neurons while leaving green/yellow spectra for different imaging purposes.

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Poster

PSTR241. Optical Methodology-Development

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.13/WW56

Topic: I.04. Physiological Methods

Title: Simultaneous and cross-validated one-photon and two-photon imaging of thousands of neurons

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Abstract: One- and two-photon (1P and 2P) Ca2+ imaging are widely used to monitor the activity of large neural ensembles. The two methods are usually thought to have complementary strengths and limitations, but detailed comparisons of activity traces concurrently acquired from the same cells with the two modalities have rarely been performed. To this end, we created the 'Multiscope', a custom microscope allowing simultaneous 1P and 2P Ca2+ imaging in head-fixed mice. Image acquisitions for the two modalities sample overlapping fields-of-view (FOVs) at ~25 Hz and are mutually phase-locked but shifted by a fraction of the image frame duration. The collection pathways are decoupled so as to retain normal detection efficiencies. 1P images are taken between the acquisition of laser-scanning 2P image frames, during flyback of the slow-axis laser-scanning mirror. The 2P illumination pathway has a rapid, remote focusing capability allowing multi-plane imaging at different tissue depths without changing the axial plane or frame rate of 1P imaging.
To evaluate Ca2+ traces from the two modalities, we studied a mouse line expressing GCaMP6f

in cortical layer 2/3 pyramidal cells. With a 0.8 NA objective lens (16x), we extracted cell activity traces from each video dataset using the cell-sorting algorithm, EXTRACT, and identified matched cells across the two modalities using spatial and temporal metrics. Across the overlapping part (~400 μm wide) of the FOVs, 1P Ca2+ traces were nearly identical to their matched 2P traces but often had a comparable signal-to-noise ratio (SNR) across a range of tissue depths. This suggests that 1P imaging benefits from its high fluorescence flux and provides high-fidelity Ca2+ recordings. Further, the numbers of cells found by 1P imaging were higher than those found by 2P imaging within the shared FOV. Percentages of cells found by both modalities varied with tissue depth. At 150 μm and 200 μm depths, 68±3% and 71±7% of cells, respectively, detected by 2P imaging were found by 1P imaging (n=5 mice, mean±s.e.m.). At 300 μm deep, this percentage declined to 30±4%. Using a mouse line targeting layer 4 cells, percentages of matched cells at 350 μm depth rose to 47±14%. Using low-magnification objective lenses, we tracked ~2000-5000 cells at once by 1P imaging and validated the traces of those lying in the FOV sampled by 2P imaging. In studies of CA1 hippocampus using a GRIN lens, levels of matched cells and their trace SNR values were similar as in cortex. Overall, for many studies of neurons as deep as cortical layer 4, 1P imaging can serve as a cost-effective, high-fidelity way to track large numbers of cell bodies at comparable or better SNR values than those from 2P imaging.

Disclosures: **R. Chrapkiewicz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stanford University. **T.H. Kim:** None. **Y. Zhang:** None. **T. Rogerson:** None. **G. Chattree:** None. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stanford University.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

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Topic: I.04. Physiological Methods

Support: Strategic Priority Research Program of the Chinese Academy of Sciences (XDB32030200)
NSFC-GuangdongJointFund-U20A6005, NSFC (Grant No. 32100903)
the Key-Area Research and Development Program of Guangdong Province (2018B030331001)

Title: Multi-region calcium imaging in freely behaving mice with ultra-compact head-mounted fluorescence microscopes

Authors: *F. XUE[1], F. LI[4], K. ZHANG[2], L. DING[4], Y. WANG[5], X. ZHAO[6], F. XU[4], D. ZHANG[4], M. SUN[3], P.-M. LAU[3], Q. ZHU[3], P. ZHOU[7], G.-Q. BI[1];
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Technol. of China, Hefei, China; [4]Shenzhen Inst. of Advanced Technology, Chinese Acad. of Sci., Shenzhen, China; [5]Inst. of Semiconductors, Chinese Acad. of Sci., Beijing, China; [6]Xiongan Inst. of Innovation, Xiongan New Area, China; [7]Shenzhen Inst. of Advanced Technol., Shenzhen, China

Abstract: A fundamental goal of neuroscience is to understand the neural basis of behavior and cognition, which involve the coordinated activity of multiple brain structures distributed across cortical and subcortical areas. Such studies heavily rely on multi-site recordings to reveal the brainwide circuit-level interactions in unrestrained animals. Miniature head-mounted fluorescence microscope have enabled cellular-resolution calcium imaging while the animal is freely behaving. However, the flexible implantation of multiple microscopes on the mouse brain remains challenging due to constraints in available head space and the considerable weight of the equipment. In this work, we developed a novel head-mounted miniature microscope, named TINIscope, designed especially for multiple-site cellular-resolution calcium imaging. Through optimizing electronics and opto-mechanical design, the TINIscope achieves an ultra-compact size and a weight merely **0.43** g, substantially reducing the weight compared to weight of most state-of-the-art miniscopes, which typically weigh around 2 g. Consequently, the TINIscope enables unprecedented simultaneous imaging of up to four brain regions within a freely moving mouse. In proof-of-concept experiments, we achieved simultaneous neural activity recording of over one thousand neurons in four hippocampal subregions of free-moving mice, and detailed analyses revealed spatially-modulated neurons and neuronal assemblies spanning all four subregions. The compact design of TINIscope facilitates seamless integration with optogenetic or electrophysiological tools, allowing for a more versatile experimental setup. In our demonstration experiments, we simultaneously collected individual neuronal activities in four hippocampal subregions in response to optogenetic or electrical stimulations in anterior cingulate cortex (ACC). Additionally, we conducted jointly recordings of calcium signals and local field potentials in each hippocampal subregion, enabling the analysis of population activity patterns concurrent with ripple onsets. These initial efforts suggest that TINIscope can be applied to imaging multiple cortical and sub-cortical regions spanning different depths (i.e., thalamus, hippocampus, and cortex) simultaneously during unrestrained behaviors, as well as integrating with a variety of techniques to become a multifunctional tool for exploring neural mechanisms.

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Poster

PSTR241. Optical Methodology-Development

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Topic: I.04. Physiological Methods

Support: NIH Grant U01NS120820
W.M. Keck Foundation

Title: Imaging dopamine dynamics with a novel red optical sensor with improved brightness and dynamic range

Authors: *K. MAN, A. ANDREONI, L. TIAN;
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Abstract: Dopamine (DA) is an important neuromodulator for brain function as it is essential for salience detection, reward processing, reinforcement learning and motor control. Although it is known that DA mediates these behaviors, the circuit and cellular mechanisms which transform DA release to behavior are not well understood. Bridging this gap calls for direct visualization of DA release in vivo, time-locked to behavior and simultaneous imaging of neuronal or astrocytic activities, ideally at high spatiotemporal resolution to dissect the dynamics of DA release and cellular activities. To this end, we engineered a novel red DA sensor by inserting a red fluorescent protein (FP) into a scaffold consisting of the D1 dopamine receptor (DRD1). This red FP has higher quantum yield and extinction coefficient than mApple, on which the first-generation red DA sensor developed by our lab (RdLight1) is based, resulting in increased brightness and eliminating issues of photoactivation with blue/green light that were of concern with the early generation of red sensors. Using a rational engineering approach, we generated and screened libraries of mutants to obtain top variants with large fluorescence changes upon DA binding, and high basal brightness for easier imaging. We are employing this new sensor for multiplex imaging experiments in combination with the green PKA activity sensor ExRai-AKAR2, to investigate the response of astrocytes in the dorsolateral striatum (DLS) to dopaminergic stimulation. In preliminary experiments, we observed that DA stimulation triggered PKA activity in DLS astrocytes. Interestingly, the peak PKA activity in the astrocytic soma is higher than in astrocytic processes. PKA activity in both soma and processes is partially blocked by either of the D1-like or D2-like receptor antagonists, SCH23390 and sulpiride, demonstrating the involvement of both types of receptors in triggering astrocytic PKA activity. The development of an improved red DA sensor allows multiplex imaging with green genetically-encoded sensors for imaging of Ca^{2+} or voltage, either in the same or different cell-types. Enhanced brightness and dynamic range of the new sensor will improve and facilitate imaging in vivo. As DA is a neuromodulator strongly implicated in different neuropsychiatric diseases, including Parkinson's disease, addiction and schizophrenia, this next-generation sensor can also be employed for drug screening in cell culture or in brain slices to identify small molecules interacting with, and therefore influencing D1 receptors and their response to DA.

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Poster

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Program #/Poster #: PSTR241.16/WW59

Topic: I.04. Physiological Methods

Support: DoD-NDSEG Fellowship

Title: Tunable heterostructures for near-infrared optoelectronic neural stimulation

Authors: *D. RANKE[1], Y. WANG[1], B. CHACON[2], G. GAHRAMANOVA[2], Y. GOGOTSI[2], T. COHEN-KARNI[1];
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Abstract: Light has proven itself as a powerful tool to remotely modulate neuronal activity with minimal invasiveness. Bio-optoelectronic interfaces apply this advantage through optically active material heterostructures capable of generating illumination-induced photovoltages for the capacitive stimulation of local neurons. In comparison to optogenetics and direct stimulation, two alternate light-based techniques for photostimulation which respectively require the introduction of light-sensitive channels and high illumination intensities, bio-optoelectronic interfaces can trigger neuron activation with significantly greater incident light conversion efficiency. Near-infrared light in the range of 650-900 nm (NIR-I window) demonstrates low absorptivity in tissue and in conjunction with optoelectronic stimulation, poses a high potential route for bio-optoelectronic interfaces beyond in-vitro experimentation. Despite this potential, materials systems and heterojunctions optimized towards high photo-response in the NIR-I window have not been thoroughly explored and applied. In this work, we present novel nanomaterial heterostructures composed of Si_xGe_{1-x} possessing enhanced, tunable optoelectronic responses from NIR light for the photo-capacitive stimulation of neurons. Through in-situ tuning of Si/Ge ratios with chemical vapor deposition, absorption band-edges from 500-750 nm are demonstrated with tunability to specific values in the NIR-I optical window and band alignment with carrier-selective contacts. Various electron-selective nanomaterials, including niobium carbide MXenes, are applied to enhance carrier separation and optoelectronic stimulation of neurons, quantified through micropipette and photoelectrochemical cell measurements. Devices developed with these nanostructures are engineered towards maintaining cellular size-scales and high biocompatibility for direct translatability to in-vivo studies. With optimized NIR absorption and tunable band-structures, this work demonstrates a route toward high-performance, remote stimulation of neurons through application-engineered material heterostructures.

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Poster

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KAKENHI JP19K12190

Title: Light scattering along with postsynaptic membrane potential changes as the plausible cause of the fast intrinsic optical signal (FIOS) from label-free mice hippocampal slices

Authors: *Y. TOMINAGA[1], M. KOIKE-TANI[2,3], T. TANI[4], T. TOMINAGA[1,5];
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Abstract: Intrinsic optical signal (IOS) refers to changes in optical properties such as absorption, scattering, and birefringence, which are observed to correlate with neuronal activity. At the level of neural tissue, IOS is commonly used to monitor in vivo brain activity, often due to changes in oxy- and deoxyhemoglobin and blood volume. Single-cell level IOS has been well studied since the late 1960s by using isolated neural tissues such as crab leg nerves and squid giant axons. Recent studies have revealed that the cell swelling induced by intense neuronal excitation is related to cortical spreading depression (CSD) in brain slice preparations.
We recently reported birefringence changes associated with the neuronal excitation in mouse hippocampal slices by using an instantaneous polarized light microscope (PolScope) with a short electrical stimulation train, resulting in a time constant of about 30 seconds (Koike-Tani et al., 2020).
This study reports a novel IOS with a fast time course (FIOS), which is faster than the previously reported birefringence changes. Using a high-speed imaging system (MiCAM Ultima and MiCAM05, BrainVision, Tokyo), we observed that a single electrical stimulation elicited a fast optical signal ($\Delta F/F$ of 10^{-5}-10^{-4}) followed by a slow and massive drift of the optical signal at the frame rate of 5-10 kHz observed through a linear polarizer with a transmission axis that is perpendicular to the illuminated linearly polarized light.
With squid giant axons, Cohen et al. (1968) concluded the cell-level optical signal with a fast time course as a light-scattering signal and birefringence closely tied to membrane potential change. It is not yet confirmed if the fast IOS (FIOS) is caused by the light scattering changes in the brain slice preparation. This FIOS was abolished by applying an AMPA-type glutamate receptor blocker (CNQX, 10 μM), suggesting a close relationship with postsynaptic activity. FIOS was also inhibited by TTX and Ca-free solutions but not by the NMDA receptor blocker (APV, 50 μM). In addition, the magnitude of the signal increased when a $GABA_A$ receptor inhibitor (SR95531, 10 μM) application. FIOS followed physiological modifications such as paired-pulse facilitation (PPF) and long-term potentiation (LTP). FIOS captured activity in CA3 and DG. That property mirrors that of the VSD signal.
Our results suggest that FIOS is a light-scattering change associated with postsynaptic membrane

potential activity in brain tissue. The rapid optical signal related to postsynaptic neuronal activity in unlabeled brain tissue has significant implications.

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Poster

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Program #/Poster #: PSTR241.18/WW61

Topic: I.04. Physiological Methods

Support: NIH Grant NS055251

Title: Strategies for 2-photon calcium imaging and modulation of neuronal calcium dynamics in skin in vivo

Authors: *D. CELINSKIS[1,2], R. MEIR[1,2], A. ANDRADE ANDRADE[1,2], S. DASTE[1,2], A. FLEISCHMANN[1,2], C. I. MOORE[1,2], D. LIPSCOMBE[1,2];
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Abstract: Calcium dynamics are critical signals that alter the excitability of neurons over a range of time scales from milliseconds to years. As the key integrator of chemical and electrical stimuli, intracellular calcium is fundamental to understanding neuronal plasticity which is essential for behavioral adaptation to changes in the external environment. The rapid development of hypersensitivity in heat- and mechano- sensitive nerve endings in skin is a robust, widely studied example of a protective adaptive change in behavioral responses to these stimuli. However, the mechanisms that underlie this phenomenon are not well understood. The inaccessibility of peripheral nerve endings in skin to standard electrophysiological recording methods has, at least in part, limited progress. We recently used two-photon (2P) microscopy in mouse hind paw in vivo to establish the presence, and demonstrated the critical importance, of voltage-gated *$Ca_V2.2$* channels in peripheral nerve endings to control local calcium signals (DuBreuil et al., 2021). Here we expand the use of 2P microscopy using intrathecal viral delivery of jGCaMP7f (AAV2/PHP.S-CAG-FLEX-jGCaMP7f-WPRE) to *Trpv1*-expressing neurons (*Trpv1-Cre*$^{+/+}$ mice; Cavanaugh et al., 2011) and *Cacna1h*-expressing mechanoreceptors (mice generated by our lab) (primarily Aδ-LTMR and C-LTMR) to record calcium dynamics in skin nerve endings of different subpopulations of neurons. To limit motion artifacts associated with mechanical and chemical stimuli, we combine calcium imaging with optogenetics by expressing jRGECO1a (AAV-MaCPNS1-FLEX-CAG-jRGECO1a) and ChannelRhodopsin (ChR2) in *Trpv1* nociceptors (*$Trpv1^{ChR2EYFP+/-}$*) and low threshold mechanoreceptors (*$Cacna1h^{ChR2EYFP+/-}$*). Importantly, optogenetics allows us to use the same stimuli to elicit behavioral and calcium responses in peripheral nerve endings in vivo to synergistically study the relationship between sub-second scale behavioral and calcium data.

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Poster

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Program #/Poster #: PSTR241.19/WW62

Topic: I.04. Physiological Methods

Support: Department of Defense project no. W81XWH2110209

Title: Development of a calcium imaging-based drug screening platform for iPSC-derived heterogeneous neuronal cultures

Authors: *N. A. TEANEY, W. AFSHAR SABER, R. CHEN, K. D. WINDEN, M. SAHIN; Neurobio., The Rosamund Stone Zander Translational Neurosci. Ctr. (RSZ TNC) - The F.M. Kirby Neurobio. Ctr. – Harvard Med. Sch. – Boston Children's Hosp., Boston, MA

Abstract: Calcium imaging serves as a functional assay to measure single-cell neuronal activity and the connectivity of neuronal networks. In neurological disorders where neuronal signaling and network activity are altered, calcium imaging in 2D and 3D *in vitro* models may provide further insight into understanding the underlying cellular mechanisms driving the disorders. Furthermore, calcium imaging has the potential to serve as a drug screening platform. While multi-electrode arrays provide critical insight into the overall electrical activity of a complex heterogeneous cell culture system, there is low spatial resolution in the readout (Gross et al., 1995). To our knowledge, there have been no methods developed to measure the functional activity of a heterogeneous population of cell culture system at a single-cell resolution. In the present study, we demonstrate the feasibility of measuring the functional activity of two distinct populations of neurons derived from induced pluripotent stem cells (iPSCs) in a 2D co-culture system in a method described as *dual-color calcium imaging*. Our imaging system utilizes a fully automated imaging platform with the Nikon ECLIPSE Ti2-E epifluorescence microscope, Micromanager for acquisition, and a Python-based analysis software. The high-throughput imaging system combines unbiased acquisition and analysis using randomly selected regions of interests and automated segmentation. The heterogeneous neuronal culture includes co-culturing NGN2 (Zhang et al., 2013) and iGABA (Yang et al., 2017) control GON0515-03 #5 iPSC-derived neurons after transducing each population with a separate genetically encoded calcium indicator (GECI). Calcium imaging recordings are collected to measure the changes in functional activity of the iGABA and NGN2 neurons before, during, and after the addition of synaptic blockers. As proof of concept, synaptic blockers are added to the co-culture system to elicit changes in neuronal activity in each neuron population within the co-culture. Such synaptic blockers include Vigabatrin, an irreversible inhibitor of GABA transaminase; CNQX, a competitive AMPA receptor antagonist; and DAP-V, a competitive NMDA receptor antagonist (Ben-Menachem, 2011; Honore et al., 1998; Davies et al., 1981). Continuous blue and green

illumination excites the green-shifted and red-shifted GECIs, respectively, within the different sub-types of neurons, which provides a novel platform for single-cell resolution drug screening. Future work will include utilizing the dual-color calcium imaging screening platform in iPSC-derived disease models in which neuronal signaling and connectivity are altered.

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Poster

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Support: NIH Trainee Grant T32MH073526
NIH NRSA Fellowship F31MH123111
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Title: Development of open-source, high performance miniature multiphoton microscopy systems for freely behaving animals

Authors: *B. MADRUGA[1], C. DORIAN[1], D. AHARONI[1], M. SHTRAHMAN[2], P. GOLSHANI[1];
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Abstract: Since the early 2000's several groups have designed and developed transformative multiphoton miniaturized microscopes which surmount many of the optical limitations facing 1P systems and are able to image high resolution fields of view deep into tissue during free behavior. We expand on these advances by developing two versions of an easily adopted miniature 2P microscope. First, we built the largest FOV multiphoton miniature microscope to date, capable of resolving 750um x 750um FOVs in freely behaving mice. Second, we designed a system with a higher excitation NA to resolve dynamics from fine structures like axons and dendrites over a 400um x 400um FOV during free behavior. The systems weigh < 4g and the large FOV version records an imaging area ~4x that of top-of-the-line systems reported in the literature. All hardware is designed to be as straightforward to assemble and as low cost as possible. The miniature microscopes themselves and all supporting equipment are completely open source and all files needed for reproduction by anyone can be accessed through the UCLA Miniscope project's GitHub repository following publication.

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Poster

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Topic: I.04. Physiological Methods

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Burroughs Wellcome Fund Career Award at the Scientific Interface 1015761

Title: High-speed miniaturized two-photon microscopy with elliptical beam excitation

Authors: *B. MATTISON, S.-J. LIU, F. TIAN, W. YANG;
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Abstract: Miniaturized two-photon microscopes are powerful tools in neuroscience to study the neural circuits that underpin the perception and behavior of animals in their natural and freely moving states. However, conventional two-photon microscopy suffers from a limited imaging speed as it builds the image pixel by pixel by raster scanning the laser excitation spot over the field-of-view (FOV). Here, we report a novel miniaturized two-photon microscope that could significantly increase the imaging speed compared to conventional devices while maintaining a cellular resolution. Instead of scanning a diffraction limited spot over the sample, we shape the laser excitation into an ellipse with a long-axis of 5-10 μm. This reduces the number of rows in a single frame, and hence increases the overall imaging frame rate. Such an approach is well-suited for functional imaging of neural population activity in rodents with cellular resolution where the imaging regions of interest are typically the neuronal cell bodies with a diameter of ~10-15 μm. We developed a miniaturized elliptical beam shaper (MEBS) which collimates 920 nm laser light from a hollow-core photonic crystal fiber through two orthogonal cylindrical collimating lenses. The two cylindrical lenses collimate the beam in orthogonal axes and output a collimated elliptical beam, which is then fed to a miniaturized two-photon microscope modified from an open-source two-photon miniscope design, MINI2P (Zong et al. 2022). The elliptical beam is then scanned over the sample through a microelectromechanical scanner and a commercially available objective lens. Fluorescence from the sample is collected through a supple optical fiber bundle. The point-spread-function is experimentally measured to be ~1.9 μm × ~9.0 μm laterally, and ~18 μm axially (full-width-half-maximum). This significantly reduces the number of rows that need to be scanned in a frame and thus increases the overall frame rate. Our miniaturized two-photon microscope holds great promise in high-speed functional imaging of neuronal activity in freely-moving mice.

Disclosures: B. Mattison: None. **S. Liu:** None. **F. Tian:** None. **W. Yang:** None.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.22/WW65

Topic: I.04. Physiological Methods

Support: NIH Grant 1DP2MH129986

Title: Reimagining Archives and Scholarly Communication with Social-First Peer-to-Peer Infrastructures

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Abstract: If our wildest dreams for scholarly infrastructure are to outsource our archives to Amazon and pay Elsevier for prestigious PDFs, it might be time to wake up. One can only admire the boldness of neuroscientists grappling with the staggering complexity of the brain 20 pages and 20 mice at a time. More than our ambition, skill, or resources, it is our infrastructures that constrain our work. If we consider our digital infrastructural problems separately, we find ourselves tracing familiar patterns: a mutually incompatible string of journal-like venues, cloud storage, and SaaS platforms. We present a different strategy, a new generation of peer-to-peer (p2p) protocols for social information infrastructures to bridge data, computation, and communication. Learning from decades of prior art from p2p, decentralized messaging, Semantic Web, federated social media, and wiki communities, we propose a protocol for content-addressed containers of linked data triples in an explicitly social system, blending the best of traditional p2p and federated systems. In this first phase of development, we will present results from a proof of concept protocol designed to integrate data from prevailing neurophysiology formats and the wild vernacularism of hand-structured data. Rather than a backwards-incompatible system-of-everything that requires radically reconfiguring existing practice, we show how p2p can bridge resources from rig computers, lab and institutional servers, and existing cloud archives to make each more useful than in isolation. We plot a course for future work where by taking the social reality of infrastructure seriously we can reimagine publishing data and results as a continual process of collaboration, communication, and cooperative governance, rather than some exogenous burden at the end of an experiment where we throw ourselves at the mercy of multiple adversarial industries. Beyond the practical limitations of archives with always-higher storage and egress bills and a communication system riddled with perverse incentives, sleepwalking into a near-future where we hand ownership of scientific infrastructure to a few for-profit information conglomerates would be an ethical nightmare. We can either become yet another engine of the enclosure of digital infrastructure, or we can use our rare position as publicly funded researchers not beholden to profit to seed information systems for the public good. This work is an invitation to my colleagues to join us in future phases of development, integrating existing projects with new ones, and reclaiming our joy and agency rebuilding the systems that define the daily practice of science

Disclosures: **J. Saunders:** None. **D. Aharoni:** None.

Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant 1DP2MH129986
NIH Grant 1UF1NS122124
JSPS KAKENHI Grant Number 23H03378
JST ACT-X Grant Number JPMJAX190F

Title: Untethering open-source Miniscopes with wireless power and data transfer

Authors: *T. SASATANI[1,5], M. BROSCH[1], F. SANGIULIANO JIMKA[1], H. SEMWAL[1,2], A. GUHA[1,3], H. CHORSI[1,3], K. KEUS[1,4], D. AHARONI[1];
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Abstract: Investigating the relationship between complex behavior and neural activity is an established methodology for systems neuroscience. The development of miniature head-mounted fluorescent microscopes enables in vivo recording of neural activity in freely moving animals and significantly expands the range of behaviors that can be studied. However, supplying power to head-mounted systems and acquiring recording data is a critical bottleneck in many applications, imposing a need for a wired interface that can disrupt behavior or a battery that limits the recording time and increases weight. To this end, prior work demonstrated wirelessly driving neuroscience tools with low power consumption and data rate demand. However, wirelessly interfacing power-hungry and high-bandwidth recording devices such as image sensors with miniaturized form factors for small rodents remains a fundamental challenge. Here we present a fully-wireless miniature microscope enabled by novel, wide-range magnetic resonance coupling wireless power transfer, and free-space optical communication technologies. Unlike prior battery-based wireless miniature microscopes with short recording lengths, our method technically offers unlimited recording time by remotely supplying energy to the untethered head-mounted device. Furthermore, the high-bandwidth communication enabled by the custom-made miniaturized optical communication system will allow online monitoring and processing of recording data. Evaluations reveal that a fully-wireless design can achieve an imaging performance comparable to a typical open-source UCLA Miniscope V4 configuration, and we demonstrate the feasibility of the system in several standardized freely-moving rodent tasks. The presented technology overcomes the issues provoked by wires and batteries and has the promise of unlocking experiment designs that were previously inaccessible, such as the long-term recording of complex social activities of multiple freely-moving animals. In the future, the presented wireless technologies could be designed in a modular form that can seamlessly augment various high-end neural recording devices.

Disclosures: **T. Sasatani:** None. **M. Brosch:** None. **F. Sangiuliano Jimka:** None. **H. Semwal:** None. **A. Guha:** None. **H. Chorsi:** None. **K. Keus:** None. **D. Aharoni:** None.

Poster

PSTR241. Optical Methodology-Development

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Support: NIH R56 MH132959
Friedman Brain Institute Research Scholars Award
Irma T. Hirschl/Monique Weill-Caulier Research Award
NIH DP2 MH122399
NIH R01 MH120162

Title: Simultaneous dual-color calcium imaging in freely-behaving mice

Authors: *Z. DONG[1], Y. FENG[3], K. DIEGO[5], S. I. LAMSIFER[6], A. BAGGETTA[4], B. SWEIS[1], Z. PENNINGTON[3], Y. ZAKI[1], D. MORALES-RODRIGUEZ[1], F. SANGIULIANO JIMKA[7], D. M. KIRCHER[2], P. SLESINGER[9], T. SHUMAN[9], D. AHARONI[8], D. CAI[1]; [2]Neurosci., [1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Neurosci., [3]Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [5]Neurosci., Mount Sinai Sch. of Med., New York, NY; [6]Nash Family Dept. of Neurosci., Mount Sinai Sch. of Med., Queens, NY; [8]Dept. of Neurol., [7]UCLA, Los Angeles, CA; [9]Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Miniaturized fluorescence microscopes (miniscopes) enable imaging of calcium events from a large population of neurons. Traditionally, miniscopes have only been able to record from a single fluorescence wavelength. However, recording from multiple wavelengths simultaneously would provide much more flexibility and function. For example, simultaneous imaging of calcium signals along with a static signal can be beneficial, as the static signal can be used as a landmark for registration of neurons across recording sessions, which remains a major challenge in long-term calcium imaging. Another use case is to image two dynamic signals simultaneously. For example, imaging from two different populations of neurons can provide insight of how different cell-types interact to underlie behavioral function. Imaging FRET (Fluorescence Resonance Energy Transfer)-based signals can uncover the molecular mechanism underlying signal transmission in neural circuits. Here, we present a new open-source dual-color miniscope. To enable simultaneous acquisition of two fluorescent wavelengths, we incorporated two CMOS sensors with two independent sets of emission filters into a single miniscope. This enables us to acquire images at the full frame rate that the CMOS sensor supports. An additional benefit of using two sets of emission filters is that the crosstalk between channels can be eliminated, which enables imaging fluorophores that heavily overlap in the emission spectrums (for example, GFP and tdTomato). To correct for the axial difference of focal plane between the two channels caused by chromatic aberration of the GRIN lens, we adopted a design that enables adjusting the position of one imaging sensor along the light path to match the focal plane of the other sensor, providing additional flexibility for calibrations. We have validated and calibrated

our dual-channel miniscope on benchtop, imaging two wavelengths on a test slide to ensure that we can overlay the two images and record from the same focal plane. We have also validated our dual-channel miniscope in freely behaving animals running on a linear track. We have imaged mouse hippocampus that expressed dynamic GCaMP and constitutively active tdTomato signals during behavior. Our results suggest that our miniscope can image two fluorescence wavelengths in the same focal plane and that there is minimal crosstalk between the two channels.

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Poster

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Program #/Poster #: PSTR241.25/WW68

Topic: I.04. Physiological Methods

Support: JSPS JP23H054JP

Title: Multi-regional brain imaging and sensing based on CMOS imaging device applied to freely moving rodents

Authors: *Y. SUNAGA, Y. OHTA, V. CASTILLO, L. AKBAR, J. OLOROCISIMO, H. TAKEHARA, M. HARUTA, H. TASHIRO, K. SASAGAWA, J. OHTA;
Nara Inst. of Sci. and Technol., Ikoma, Japan

Abstract: The brain comprises distinct regions that perform specialized functions, and these regions are interconnected through neural networks. While significant progress has been made in unrevealed the mechanisms of the brain, there remain numerous challenging diseases for which the etiology and treatments are yet to be established. To gain insights into the causes of such brain disorders, it is crucial to simultaneously access and measure multiple brain regions.However, existing experimental methods to monitor neural activity face limitations in accessing multiple brain regions, particularly the deep brain, without imposing undue constraints on experimental animals or causing substantial invasiveness. Therefore, we have endeavored to develop a system for multiple measurements based on a CMOS imaging device that can be directly implanted into the mouse brain. Our objective is to achieve an ultra-minimally invasive approach to measuring and modulating cranial nerve activity [1],[2]. By focusing on establishing novel systems, we aimed to enable new measurements of rodent brains in a freely moving state.Our research efforts have resulted in the development of combination measurement systems, such as imaging coupled with microdialysis, optogenetics in conjunction with microdialysis, and imaging combined with electrophysiology. These integrated systems facilitate measurements and/or access to multiple brain regions in mice and rats. Furthermore, we have

applied these systems to animals in freely moving conditions, investigating their involvement in reward pathways[1]-[2], pain processing[3], and seizure models.Presently, we have also introduced a novel measurement system that combines optical stimulation with ChR2 and fluorescence imaging with RGECO. This integration aims to mitigate any adverse impact on the quality of fluorescence imaging caused by the optical stimulation light observed when using a combination of GCaMP fluorescence imaging and ChrimsonR optical stimulation. Our ongoing efforts are focused on establishing this system as one of the primary objectives of our research.
[1] A. Ganaway and Y. Sunaga *et al*," Int. J. Mol. Sci., vol. 24, no. 3, 2023.[2] Y. Sunaga *et al*," IEEE Access, vol. 9, pp. 55871-55878, 2021.[3] L. Akbar et al.," Int. J. Mol. Sci., vol. 24, no. 7, 2023.

Disclosures: **Y. Sunaga:** None. **Y. Ohta:** None. **V. Castillo:** None. **L. Akbar:** None. **J. Olorocisimo:** None. **H. Takehara:** None. **M. Haruta:** None. **H. Tashiro:** None. **K. Sasagawa:** None. **J. Ohta:** None.

Poster

PSTR241. Optical Methodology-Development

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Program #/Poster #: PSTR241.26/WW69

Topic: I.04. Physiological Methods

Support: JSPS JP23H054JP

Title: Ultrasmall compact CMOS-based imaging device with laser carbonized electrode for simultaneous deep-brain optical and electrophysiological measurements

Authors: ***V. G. CASTILLO**[1], R. OKADA[1], Y. SUNAGA[2], Y. OHTA[1], H. TAKEHARA[1], H. TASHIRO[1], K. SASAGAWA[1], J. OHTA[1,2];
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Abstract: We developed an ultrasmall CMOS-based image sensor device for recording calcium imaging signals in the deep brain of mice. While detection of calcium activity following nociceptive and addicting stimuli has been done with this device, identification of regions of interest (ROI) has been difficult due to the lensless nature of the device which results in lack of distinguishable features. To improve device function, we developed an algorithm for automatic detection of ROIs based on adaptive binarization. Unlike a global binarization which sets one threshold value for the whole image, adaptive binarization calculates different cut-off values for each pixel in the image depending on the brightness of the surrounding pixels. First, adaptive binarization was applied to each frame and the consequent noise was removed by morphological image processing. The binarized frames were averaged and then binarized again. ROIs were then drawn from the denoised image. The effect of the parameters in the algorithm such as sigma value, kernel size and opening footprint was studied.

Although calcium imaging offers advantages such as high spatial resolution, simultaneous recording of multiple locations and neuron-type specificity, there are disadvantages such as low signal-to-noise ratio (SNR), low sampling rate and the need for a reporter since light does not directly interact with tissue. In electrophysiology, direct contact is established with the brain to probe electrical activity caused by neuronal firing. There is no need for a reporter molecule, SNR is low, and the sampling rate is high. Thus, we took advantage of both methods to better investigate brain dynamics.
To incorporate electrophysiological recording function to our devices, we added carbon electrodes to our CMOS devices using a novel and facile method. Carbon electrodes were created by irradiating thermally evaporated parylene C with laser under ambient atmosphere. The carbonization process was optimized by finding the optimal power and irradiation time on flexible electrode substrates. This method enables the fabrication of a carbon electrode on any surface that can be coated with paralyne C. The integrated electrophysio-imaging device consists of a 450 x 1660 µm CMOS chip and µ-LED mounted on a flexible printed substrate. An absorption filter was fixed on the imaging surface to block excitation light from the LED and allow only emission light to reach the sensor. The device is ultrasmall at only 0.7 mm wide and 0.2 mm thick to reduce implantation invasiveness and tissue damage, and lightweight at only 0.02 g to prevent movement hindrance and facilitate natural behavior when doing freely moving experiments.

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Poster

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.27/WW70

Topic: I.04. Physiological Methods

Title: Mapping structural organization in neurobiology with MINFLUX nanoscopy

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Abstract: According to the fundamental principle of the structure-function relationship, the intricate 3D structure of biomolecules and their assemblies directly governs their specific interactions. Thus, mapping the structural organization of key player proteins in neurobiology with fluorescence microscopy will advance our understanding of neuronal processes and bring us one step closer to unravelling the complexity of brain function. With dimensions smaller than the diffraction limit, specifically synapses and their dense protein population call for advanced super-resolution techniques to be imaged.

MINFLUX nanoscopy has paved the way towards investigating the structural organization of (macro)molecular complexes and their subcellular distribution on a nanoscopic level with unprecedented detail. MINFLUX probes the positions of individual molecules with an excitation intensity minimum, providing the most photon-efficient concept of localizing single emitters [1,2]. While alternative imaging approaches often lack molecular specificity and/or live-cell compatibility, MINFLUX offers single-digit nanometer localization precision on a standard light microscopy setup [3], allowing to easily implement this technique into common workflows in a wide range of neuroscience applications. We demonstrate MINFLUX as a versatile tool to map protein distributions in 2D or 3D in fixed and living neurons at molecular resolution (e.g. [4]).
[1] Balzarotti et al. (2017), Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes, *Science* **355**
[2] Gwosch et al. (2020), MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells, *Nat Methods* **17**
[3] Schmidt et al. (2021), MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope, *Nat Commun* **1**
[4] Grabner et al. (2022), Resolving the molecular architecture of the photoreceptor active zone with 3D-MINFLUX, *Sci Adv* **8**

Disclosures: J. Matthias: A. Employment/Salary (full or part-time):; Abberior Instruments America. **M. Velasco:** A. Employment/Salary (full or part-time):; Abberior Instruments America. **C. Guerth:** A. Employment/Salary (full or part-time):; Abberior Instruments. **J. Waka:** A. Employment/Salary (full or part-time):; Abberior Instruments America. **K. Bahlmann:** A. Employment/Salary (full or part-time):; Abberior Instruments America. **C. Wurm:** A. Employment/Salary (full or part-time):; Abberior Instruments America.

Poster

PSTR241. Optical Methodology-Development

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR241.28/WW71

Topic: I.04. Physiological Methods

Title: Photoacoustic imaging of cortical and subcortical responses to retinal photostimulation in mice

Authors: *G. XU[1], K.-W. CHANG[2], X. WANG[2], K. Y. WONG[3];
[1]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [3]Ophthalmol & Visual Sci. and MCD Biol., [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Physiologic studies of visually-evoked responses in the mouse brain have been hampered by a dearth of noninvasive, high-throughput imaging technologies. Functional magnetic resonance imaging and diffuse optical imaging both suffer from limited spatial resolution. Photoacoustic (PA) computed tomography (PACT) can provide high spatial resolution and deep tissue penetration with real-time frame rates. We have developed a label-free

PACT system to quantify the visually-evoked hemodynamic responses within various vision-related brain regions. All mice were anesthetized by 0.5% isoflurane and acepromazine (Eckley *et al.* 2020, *JAALAS*). Their scalps were removed to reduce optical and acoustic attenuation. The acoustic signals were collected by a 256-element linear ultrasound transducer array with 10 MHz central frequency. Infrared (800 nm) pulsed laser was used as the excitation source to avoid stimulating retinal photoreceptors. Visual stimulation was achieved by presenting onto both eyes 1 Hz flickering white light (~16 mW/cm^2 irradiance) for 10 sec. PACT images were acquired at 10 frames per sec. Temporal traces of the PA variations in the primary visual cortex (V1), superior colliculus (SC), dorsal lateral geniculate nucleus (LGd), olivary pretectal nucleus (OPN) and suprachiasmatic nucleus (SCN) were recorded, as shown in Fig. 1. To assess the utility of PACT for detecting visual deficits in mouse models, we studied wild-type mice, *rd1* mice with rod/cone degeneration, and melanopsin-knockout mice (n=10 per genotype). Compared with the wild type, *rd1* mice showed lower-amplitude and longer-latency hemodynamic responses in most visual regions (p<0.05), while melanopsin-knockout mice had lower amplitudes (p<0.05) but normal latencies. These results agree with the fact that *rd1* mice's visual responses are mediated mainly by melanopsin in ganglion-cell photoreceptors, whereas rods and cones remain functional in melanopsin-knockout mice. This study validates the ability of PACT to rapidly and noninvasively quantify visual responses in the mouse brain.

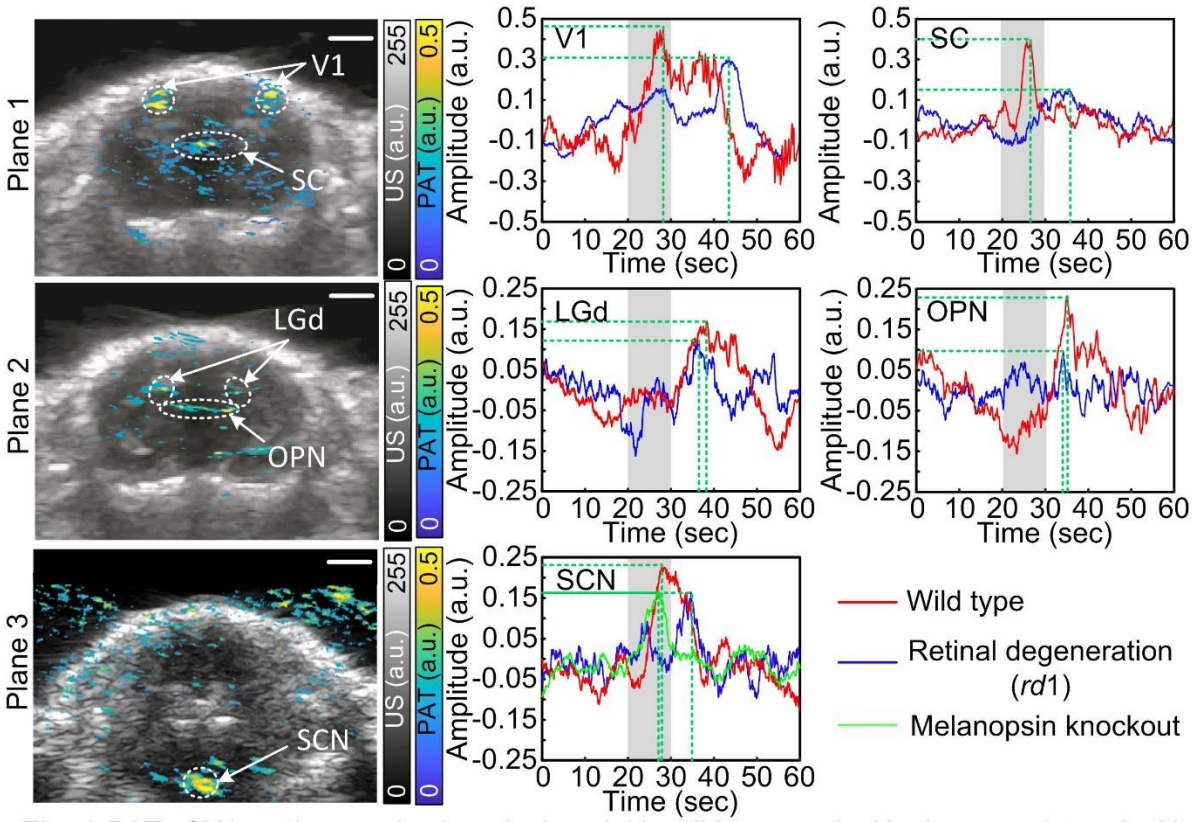

Fig. 1 PAT of V1 cortices and subcortical nuclei in wild-type and *rd1* mice coregistered with US images. In the left column, US images are rendered in gray and PAT responses above the noise threshold are rendered in yellow-blue. The right columns show the temporal traces extracted from PAT images acquired before, during, and after 10 s retinal illumination. Scale bars: 2 mm.

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Poster

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Topic: I.04. Physiological Methods

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NIH BRAIN grant UF1NS107610
NIH BRAIN grant U19NS104590
NSF NeuroNex grant DBI-1707261

Title: Optical sensing of high-frequency voltage dynamics in multiple neuron classes of behaving mammals

Authors: *S. HAZIZA[1], R. CHRAPKIEWICZ[2], Y. ZHANG[3], V. KRUZHILIN[2], M. Z. LIN[4], M. SCHNITZER[5];
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Abstract: Recent methods for optical voltage imaging routinely allow studies of neural spiking at a resolution of single action potentials. However, optical instruments to capture the aggregate voltage activity of genetically identified neuron-types have lacked the sensitivity to track high-frequency (≳10 Hz) oscillations in awake behaving animals. This technology gap has impeded investigations of how specific cell-types shape the spatiotemporal dynamics of the brain's rich set of electrical oscillations. Here, we present two TEMPO (Transmembrane Electrical Measurements Performed Optically) technologies with unprecedented sensitivity for monitoring high-frequency transmembrane voltage activity up to ~100 Hz, for up to ~1 h of continuous recording and from one or two cell-types concurrently in behaving animals. TEMPO has 3 key ingredients: (1) Co-expression of one (or two) GEVIs to track voltage activity, plus a reference fluor to track artifacts; (2) A dual-color fluorescence optical system; and (3) Computational unmixing of optical artifacts (*e.g.,* from hemodynamics) from the fluorescence voltage traces. TEMPO differs from extracellular electric field potential recordings, which capture contributions of multiple unidentified neuron-types, are influenced by electrode shape, orientation, and composition, and include volume-conducted signals originating up to ~1 cm away from the recording electrode. We built a pair of complementary fiber-optic and imaging TEMPO instruments to study voltage dynamics in freely moving and head-fixed behaving animals, respectively. Our fiber-optic apparatus, termed 'uSMAART' (ultra-Sensitive Measurement of Aggregate Activity in Restricted cell-Types), attains ~10-fold greater sensitivity than prior fiber-photometry systems. Our imaging system tracks voltage activity across a ~7-mm-wide field-of-

view with a spatial resolution >10 times finer than that of the densest electrode arrays for electrocorticography. With these instruments, we measured neural population voltage activity at frequencies up to the high-gamma band and captured cross-frequency coupling between distinct rhythms in the transmembrane potentials of single cell-types. With dual cell-type TEMPO, we characterized excitatory and inhibitory cell dynamics during hippocampal ripples and visual cortical processing. Overall, TEMPO technologies are poised to help uncover how specific neuron-types interact to shape the brain's electric field dynamics during active animal behavior, probe the functional roles of propagating neural voltage waves, and advance understanding of high-frequency oscillations in heathy and diseased brains.

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Poster

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Topic: I.04. Physiological Methods

Support: Howard Hughes Medical Institute
Allen Institute for Neural Dynamics

Title: Second-generation scanned line projection microscopy (SLAP2) for in vivo imaging of synaptic input

Authors: *K. PODGORSKI[1], A. CHARLES[2], D. A. FLICKINGER[3], G. JAINDL[4], A. NEGREAN[1], J. ROHDE[1], M. E. XIE[5,6];
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Abstract: Individual neurons act as computational units of the nervous system by transforming synaptic input patterns into firing output, but neuroscientists have lacked experimental methods to observe these input-output relationships in the mammalian brain. We developed SLAP2, a two-photon microscope based on a fast and flexible scan engine that combines a line scanner and two digital micromirror devices (DMDs). Each DMD defines a 2D scanfield over which a line focus is scanned. The DMD dynamically shapes the scanned line into a flexible random access scan. By shaping the line on nanosecond timescales, SLAP2 avoids per-target access time costs and can flexibly trade off resolution against speed even within a single line scan. The two scanfields are scanned interleaved, producing a 100% duty cycle, and steered independently by two fast remote refocusing systems to access a 3D volume (200x300x500 microns per scanfield) at high resolution. SLAP2 records from hundreds of synapses in 2D at >1 kHz per scanfield,

dozens of cell bodies at >10 kHz, and 3D volumes at >100 Hz. We will describe the development of SLAP2, characterize its performance, and demonstrate imaging of glutamate signals at many synapses of individual neurons within the cortex of mice performing a single-neuron brain-machine-interface task. This technology has the potential to identify input-output computations performed by neurons in vivo.

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Poster

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Program #/Poster #: PSTR241.31/WW74

Topic: I.04. Physiological Methods

Title: A three-photon head-mounted microscope for simultaneously imaging cortical input and output layers in freely moving mice

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Abstract: Miniaturized head-mounted two-photon microscopes have enabled imaging of activity from fluorescently labelled neuronal populations in the upper cortical layers with single cell resolution in freely moving rodents. Three-photon excitation (3PE) can considerably extend the imaging depth possible in scattering tissue by utilizing longer wavelengths, that decrease excitation light scattering and eliminating the generation of out-of-focus fluorescence. Miniaturizing this tool to make it adequate for imaging in freely moving mice without impeding behavior has enabled access to all cortical layers and the genetic toolbox. Having gained access to all these tools at the same time allows to dissect the activity of neuronal networks while the freely-moving animal performs self-motivated behavior. However cortical layers are functionally heterogeneous structures featuring different connectivity patterns. Some layers receive most input from subcortical structures and other layers produce most of the output to other cortical and subcortical areas of the brain. We introduce methods to get access to input to cortex and output from it simultaneously in freely moving mice. These technologies opens up the possibility to

monitor neuronal activity of input and output layers of the cortex with single cell resolution and dissect the function of cortical networks in freely moving mice.

Disclosures: A. Klioutchnikov: None. **D.J. Wallace:** None. **J. Sawinski:** None. **K. Voit:** None. **Y. Groemping:** None. **J.N.D. Kerr:** None.

Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.01/WW75

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

Support: National Institute of Mental Health (MH106775)
Vannevar Bush Faculty Fellowship from the US Department of Defense (N00014-20-1-2027)
Air Force Office of Scientific Research (FA9550-22-1-0337)

Title: Enhanced Entrainment and Reduced Excitability as Predictive Neurophysiological Markers of Efficacious EEG-rTMS Treatment for Major Depressive Disorder

Authors: *X. SUN[1], J. DOOSE[7], J. FALLER[1], J. R. MCINTOSH[2,1], G. T. SABER[8,10], S. HUFFMAN[9], S. PANTAZATOS[3], H. YUAN[11], R. I. GOLDMAN[12], T. R. BROWN[8], M. S. GEORGE[9,13], P. SAJDA[1,4,5,6];
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Abstract: Introduction: Repetitive transcranial magnetic stimulation (rTMS) is an effective treatment for major depressive disorder (MDD). However, the optimal combination of stimulation parameters, particularly individualized for each patient, remains unclear. The phase of endogenous EEG alpha rhythms may mediate top-down influences and affect brain dynamics. Under the hypothesis that phase-locked rTMS pulse train delivery may impact brain dynamics and stimulation efficacy, we developed a closed-loop EEG-rTMS system triggering the pulse train at the patient's specific prefrontal EEG alpha rhythm phase.
Methods: 24 MDD patients were enrolled and randomly assigned to the phase-synchronized (SYNC) or unsynchronized (UNSYNC) treatment group (ClinicalTrials.gov listing NCT032421808). The SYNC group received rTMS with phase-locked pulses at the individual preferred phase determined by pre-treatment fMRI scan, while the UNSYNC group used randomized phases. Treatment efficacy was assessed weekly using the Hamilton Depression Rating Scale (HDRS). Each session consisted of 75 rTMS pulse trains, including 40 TMS pulses

delivered at the individual alpha frequency (IAF: 6 to 13Hz) over the left Dorsolateral Prefrontal Cortex. The Global Mean Field Power (GMFP) within the IAF band was calculated to evaluate changes in cortical excitability, and the power-weighted Inter-trial Phase Coherence (wITPC) within the IAF was calculated during the post-stimulation period to assess phase synchronization.
Results: During the six-week treatment, both groups showed GMFP decreases within the IAF, with more significant reductions in the SYNC group. Within the SYNC group, treatment weeks with greater HDRS reductions showed greater pre-vs.-post daily treatment excitability decreases. In terms of entrainment, SYNC patients exhibited increased wITPC (in magnitude) over sessions, while no such increase was observed in the UNSYNC group. Moreover, within the SYNC group, clinical responders who showed better entrainment (in phase) across all treatment sessions—no such association was found within the UNSYNC group. Excitability index and entrainment index were developed to measure weekly changes in excitability and entrainment, respectively. Clinic responders in the SYNC group showed either higher entrainment or lower excitability.
Conclusions: The correlation observed between enhanced entrainment, reduced excitability, and improved antidepressant response in synchronized rTMS highlights the strong potential of our closed-loop EEG-rTMS system in developing personalized treatment plans based on individual neurophysiological markers.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.02/WW76

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

Support: 5R01MH120811-03

Title: Weight map method may not provide a stable estimation of sgACC FC map

Authors: *Y. MA[1], H. LI[1], R. J. DUPRAT[2], D. OATHES[2], Y. FAN[1];
[1]Radiology, [2]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: The subgenual anterior cingulate cortex (sgACC) has been identified to play an important role in patients with major depression disorder (MDD). In clinical practice, transcranial magnetic stimulation (TMS) has been used to aim at modulating sgACC by stimulating cortical-surface regions that are functionally connected to the deep brain region. Due to the fact that the sgACC shows poor signal-to-noise-ratio (SNR) in functional connectivity

MRI (fcMRI), its functional connectivity (FC) map can be challenging to reliably measure. The 'weight map' method was introduced to obtain reliable personalized sgACC FC maps. This method assumes that the sgACC signal can be robustly estimated by a spatially weighted average of individual fcMRI based on a group-averaged sgACC FC map, which can be further used to estimate the personalized sgACC FC map. However, a single iteration of the weight map method may not provide a stable solution. Here, we used an iterative approach to obtain converged results for personalized sgACC FC maps. We tested this approach on fcMRI data of 200 subjects from the HCP dataset. First, results revealed that iterative applications of the weight map method showed decreased spatial similarity to the group-averaged map. The maps became more dissimilar to the 1st iteration results (the conventional weight map result) over iterations. It demonstrated that the weight map method may not provide stable estimations. Second, we found that the converged sgACC FC maps derived from four different fcMRI scans from the same subject show poor spatial similarity to each other. It demonstrates that the stable solutions derived from the iterative weight map method are not reproducible. Interestingly, we found that the major proportion of the converged maps show high spatial similarity to the FC map of a personalized default mode network (DMN) obtained using our nonnegative matrix factorization (NMF)-based personalized functional network modeling method. In addition, more converged maps were assigned to the DMN map by excluding negative values in weights. It indicates that the personalized DMN FC map might be used as an alternative robust approximation, and that the negative weights may bias the estimation of sgACC signal and thus the sgACC FC map.

Disclosures: **Y. Ma:** None. **H. Li:** None. **R.J. Duprat:** None. **D. Oathes:** None. **Y. Fan:** None.

Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.03/WW77

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

Support: Academy of Finland
ERASMUS Mundi
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Magnus Ehrnrooth Foundation
K. Albin Johansson Foundation
Åbo Akademi University
MATTI University of Turku

Title: A multi-parameter zebrafish screen for identifying molecules with anxiolytic properties

Authors: Y. HONG[1], ***C. SOURANDER**[2,1], B. HACKL[1], J. S. PATTON[1], J. JOHN[1], I. PAATERO[1], E. T. COFFEY[1];
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Abstract: Anxiety and depression are highly prevalent disorders with limited treatments that greatly burden both society and individuals. To aid drug screening in this area, we have developed a zebrafish (*D. Rerio*) screening assay and used it to study components of a relevant signalling pathway. We established a screen of 5 cycles of stimuli (a variety of lights and taps) followed by intermission. Classical anti-depressants fluoxetine and imipramine, anxiolytic and mood stabilizing drugs diazepam and LiCl, as well as MK801 and ketamine were tested in 7 dpf larvae. The antidepressants/anxiolytics (AA) elicited strikingly similar effects. A decrease in distance, thigmotaxis, and spurting, together with an increase in turning and pausing, was judged indicative of an AA-like startle profile (SP). The AA-like post-stimuli profiles (PSP) were similar to SP with the exception of thigmotaxis. Notably MK801 (10 µM) and Ketamine (10 & 100 µM) PSPs were different from the SPs (unlike most drugs), and MK801 showed an anxiogenic-like SP but not PSP. As our lab has previously shown c-Jun N-terminal kinase involvement in AA effect we tested JNK inhibitors using previously established stress response methods and our test paradigm. In our tests JNK inhibition (SP600125 10 µM) reversed the stress response. We went on to screen prospective JNK1 pathway signalling hubs identified through a phosphoproteomic screen of *Jnk1-/-* mouse brain which yielded 6 molecules that substantially increased protein:protein interactions in knockout brain. We tested inhibitor and activator drugs (where available) against these downstream targets for potential AA effect using control 7 dpf fish (treated with carrier only) and 3 drug doses (*n*: 20-24 per dose most experiments). SP JNK inhibitor profiles matched AA-L profiles. Of the downstream hubs, 4 of them elicited robust AA-L profiles. Interestingly these hubs are known to be biochemically linked. As the behavioural features measured were complex, we used machine learning algorithims (Random forest, Glmnet and SVM) to identify those targets with the highest AA-L profile. Training was done using classical AA drugs. The highest prediction accuracy (0.996) was obtained in the PSP. We believe that the screen has utility as a first line screen prior to mouse experiments enabling fewer drugs and thus fewer animals to be tested. To conclude, we characterised behavioural profiles of anxiolytic/anti-depressant drugs in zebrafish larvae and utilised them to predict action of JNK pathway relevant drugs.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.04/WW78

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

Title: Human plasma transcriptome analysis in plasma NCAM1 positive extracellular vesicles with major depression

Authors: *Y. KAGEYAMA[1], Y. DEGUCHI[1], S. OKURA[1], K. INOUE[1], C. M. LISTON[2]; [1]Osaka Metropolitan Univ. Grad. Sch. of Med. Dept. of Neuropsychiatry, Osaka, Japan; [2]Dept. of Psychiatry and Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY

Abstract: Background: Extracellular vesicles (EVs) are lipid bilayer-enclosed nanoparticles released by cells into the extracellular space. Intercellular communication through EVs are involved in the pathogenesis of neuropsychiatric disorders. The fact that EVs are released into the bloodstream from the brain and that they express markers that allow their tracking to the cells of origin makes the use of EVs promising for diagnostic purposes and biomarker discovery. Using the brain-derived EVs in the blood samples enables us to obtain the information from the brain indirectly without brain tissue biopsy, allowing for minimally to non-invasive "liquid biopsy" type methods to be used for diagnosis. However, no established liquid biopsy method for the brain has been identified. Here, we developed a novel liquid biopsy method focusing on plasma NCAM1 positive EVs, which are thought to be derived in part from the brain but may also come from other sources. With the method, we evaluated the gene expression levels of major depressive disorder patients (MDD), bipolar disorder patients (BD), and healthy controls. **Methods:** Total circulating EVs were isolated from 500 μL of plasma samples by two sequences of ultracentrifugation. Purified EVs were verified using electron microscopy, a nanoparticle tracking analyzer, and EVs antibody arrays analysis. NCAM1 positive EVs were isolated by immunoprecipitation with anti-NCAM1 antibody beads solution and verified by western blotting. We conducted RNA-seq and Enrichr analysis. Human plasma samples were evaluated in 39 patients with MDD, 13 patients with BD, and 15 healthy controls. **Results:** The existence of EVs from plasma was verified by electron microscopy, nanoparticle tracking analyzer, and copresence of several known EVs markers. The existence of NCAM1 positive EVs was verified by western blotting. Enrichr analysis showed that expression genes were significantly enriched with fetal brain cortex ($p = 6.3e^{-8}$), midbrain ($p = 0.00080$), prefrontal cortex ($p = 0.0041$), and neuronal epithelium ($p = 0.017$). The fetal brain cortex and midbrain survived after correction for multiple testing. As for the comparison of MDD and healthy control, 159 genes were significantly different after multiple testing corrections. As for the comparison of MDD and BD, 279 genes were significantly different after multiple testing corrections. **Conclusions:** Our method indicated that plasma NCAM1 positive EVs contain a part of brain information. The method could apply to neuropsychiatric disorders for biomarker study.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

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

Support: Wellcome Trust, 202397/Z/16/Z to GM
The Royal Society, 202397/Z/16/Z to GMWellcome Trust, 202397/Z/16/Z to GM

Title: Acute Effects of Diazepam on Hippocampal Resting Cerebral Blood Flow in Individuals at Clinical High-Risk for Psychosis

Authors: *N. R. LIVINGSTON[1], A. KIEMES[2], S. KNIGHT[3], P. B. LUKOW[1], L. JELEN[2], T. REILLY[2], A. DIMA[2], A. DIMA[2], M. NETTIS[2], C. CASETTA[2], G. A. DEVENYI[4], T. AGYEKUM[5], F. O. ZELAYA[6], T. SPENCER[2], P. FUSAR-POLI[2], P. FUSAR-POLI[2], S. C. WILLIAMS[1], P. MCGUIRE[7], A. EGERTON[1], M. CHAKRAVARTY[8], G. MODINOS[2];
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Abstract: Background Neuroimaging studies in individuals at clinical high-risk for psychosis (CHR-P) have robustly demonstrated increased resting cerebral blood flow (rCBF) in the hippocampus and subfields, hypothesised to be due to GABAergic dysfunction. Increasing GABAergic signalling in a rodent model of psychosis through administration of diazepam during the premorbid stage prevented the emergence of the psychosis-relevant phenotype. This study aimed to determine whether acute diazepam administration can reduce hippocampal rCBF in CHR-P individuals, and whether this effect is subfield specific. **Methods** We conducted a within-subject, double-blind, placebo-controlled, randomised, cross-over design study in 24 CHR-P individuals (mean [±SD] age: 24.1 [±4.8] years, 15F). Participants underwent two 3T MRI sessions, once under a single dose of 5mg diazepam and once under placebo. rCBF was measured using a pseudocontinuous arterial spin labelling sequence. Following pre-processing of the structural scans, the MAGeT-Brain toolbox was used to segment the hippocampus and its main subfields for each subject. Using the minc-toolkit-v2/1.9.18, these masks were registered and resampled to the rCBF map, and the mean rCBF value was extracted per hemisphere in native space. Individual linear mixed effects models in R/4.2.2 assessed the effect of diazepam vs. placebo on rCBF per region per hemisphere, covarying for age, sex, and days between scans, with subject and drug-scan order as random effects, each individual's contribution to the model weighted according to the degree of CBF variance of that region. Significance was set at pFDR<0.05. **Results** Diazepam significantly reduced rCBF in total brain grey matter (t=-5.66, pFDR<0.001), as well as in right hippocampus (t=-4.30, pFDR<0.001), left hippocampus (t=-2.69, pFDR=0.015), right CA1 (t=-4.09, pFDR=0.001), left CA1 (t=-2.92, pFDR=0.012), right subiculum (t=-3.15, pFDR=0.008), left subiculum (t=-2.74, pFDR=0.015), right CA4/DG (t=-4.53, pFDR<0.001) and left CA4/DG (t=-2.51, pFDR=0.019). After controlling for total brain grey matter rCBF there were no significant effects of diazepam on hippocampal rCBF (all ROIs pFDR>0.05). **Conclusions** Acute diazepam challenge demonstrated a global effect on rCBF in CHR-P individuals including the hippocampus and its subfields. These findings provide proof-of-concept of the efficacy of GABA-enhancing drugs to modulate hippocampal hyperactivity in this clinical group. Our results also encourage the development of more selective

pharmacological agents targeting hippocampal hyperactivity as a promising pharmacological target for psychosis prevention.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.06/WW80

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

Support: R01MH113827
R01MH125816

Title: Reduced thalamocortical connectivity in individuals at clinical high risk for psychosis and schizophrenia relative to healthy control subjects: a 7 Tesla resting state fMRI study

Authors: A. KEIHANI, A. MAYELI, C. A. HUSTON, S. A. JANSSEN, F. DONATI, *F. FERRARELLI;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Previous resting state functional magnetic resonance imaging (rs-fMRI) studies have shown altered thalamo-cortical connectivity in chronic and early course patients with schizophrenia (SCZ). These findings have been recently extended to youth at clinical high risk (CHR), a population uniquely enriched for risk of psychosis and SCZ. However, the magnitude of the thalamic dysconnectivity (i.e., whether it can be detected in whole brain connectivity analysis), the cortical regions mostly involved, as well as the thalamic nuclei most affected in CHR relative to healthy control (HC) individuals remain to be established. In the present study, we used 7 Tesla rs-fMRI to investigate thalamo-cortical dysconnectivity in CHR vs. HC subjects. We recruited 30 HC (16 female, age= 21.27 ± 4.76) and 23 CHR (13 female, age=20.63 ± 3.13) individuals. A Siemens Magnetom scanner was used to acquire rs-fMRI data (matrix size = 98 × 98 × 48, volumes = 220), which were acquired using a multi-band accelerated echo planar imaging (EPI) sequence with a voxel size of 2 mm x 2 mm x 2 mm; rs-fMRI data were then preprocessed with SPM12, and connectivity analysis was performed in CONN toolbox while whole brain, left and right thalamus, and 14 subsections of the thalamus were considered as seed for connectivity analysis. The whole brain analysis revealed that the thalamus was the brain region showing the largest hypoconnectivity in CHR compared to HC (p =0.0005 after FDR

correction). Follow-up analysis using the left and right thalamus as seeds revealed that the brain region showing the largest reduction in connectivity with the thalamus was the dorsolateral prefrontal cortex bilaterally ($p<0.00001$ and $p=0.0212$ respectively). Furthermore, individual thalamic nuclei analysis revealed that the left and right Medio-Dorsal (MD) nuclei [L-MD: Peak cluster coordinates [+00 +44 -08], Voxel no. = 5295, pvalue_fdr_corr<0.001, R-MD: Peak cluster coordinates [-04 +54 +28], Voxel no. = 777, pvalue_fdr_corr<0.001] were the thalamic nuclei showing the largest connectivity reduction in CHR vs. HC individuals. Altogether, these findings indicate that a marked reduction in thalamo-cortical connectivity, involving especially the MD and the DLPFC, is present in individuals at risk for SCZ, thus representing a putative early pathophysiological biomarker for the disorder.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

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Program #/Poster #: PSTR242.07/WW81

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

Support: F32-MH125540
R01-MH110270
R01-MH117323
R01-MH114965

Title: Generalizability and out-of-sample predictive ability of relationships between neuromelanin-sensitive MRI and psychosis in antipsychotic-free individuals

Authors: K. WENGLER[1], S. BAKER[2], A. VELIKOVSKAYA[1], A. FOGELSON[1], R. GIRGIS[1], F. REYES-MADRIGAL[3], S. LEE[4], C. DE LA FUENTE SANDOVAL[3], N. OJEIL[1], ***G. HORGA**[1];
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Abstract: The link between psychosis and dopaminergic dysfunction is established, but no generalizable biomarkers with clear potential for clinical adoption exist. This study aimed to replicate previous findings (Cassidy et al, 2019) relating neuromelanin-sensitive magnetic resonance imaging (NM-MRI), a proxy measure of dopamine function, to psychosis severity in antipsychotic-free individuals in the psychosis spectrum; and to evaluate out-of-sample predictive ability of NM-MRI for psychosis severity. The main samples consisted of 42 antipsychotic-free patients with schizophrenia, 53 antipsychotic-free individuals at clinical high risk for psychosis (CHR), and 52 matched healthy controls. An external validation sample

consisted of 16 antipsychotic-naïve patients with schizophrenia. Using an optimized and reliable protocol, we acquired NM-MRI images of the midbrain and measured contrast within a subregion of the substantia nigra previously linked to psychosis severity (*a priori* $ROI_{psychosis}$; Cassidy et al., 2019). Psychosis severity was measured using the Positive and Negative Syndrome Scale (PANSS) in schizophrenia and the Structured Interview for Psychosis-Risk Syndromes (SIPS) in CHR. In the schizophrenia sample, higher PANSS-PT (positive total) psychosis-severity scores correlated with higher mean NM-MRI contrast in the $ROI_{psychosis}$ (t_{37}=2.24, p=0.031; $r_{partial}$=0.35 [0.05, 0.55]). In the CHR sample, no significant association was found between higher SIPS-PT subsyndromal-psychosis-severity scores and NM-MRI contrast in the $ROI_{psychosis}$ (t_{48}=-0.55, p=0.68; $r_{partial}$=-0.08 [-0.36, 0.23]). Using linear support vector regression, the 10-fold cross-validated prediction accuracy of psychosis severity—across schizophrenia and CHR subjects in the main samples—was above chance in held-out test data (mean r=0.305, p=0.014; mean RMSE=1.001, p=0.005), suggesting the existence of a common psychosis signature across CHR and schizophrenia. Furthermore, external validation prediction accuracy was also above chance (r=0.422, p=0.046; RMSE=0.882, p=0.047). This study provided a direct ROI-based replication of the in-sample association between NM-MRI contrast and psychosis severity in antipsychotic-free patients with schizophrenia. In turn, it failed to replicate such relationship in CHR individuals. Most critically, cross-validated machine-learning analyses provided a proof-of-concept demonstration that NM-MRI patterns can be used to predict psychosis severity in new data, suggesting potential for developing clinically useful tools.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.08/WW82

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

Support: Wellcome Trust (Sir Henry Dale Fellowship 202397/Z/16/Z to GM)
The Royal Society (Sir Henry Dale Fellowship 202397/Z/16/Z to GM)
Mental Health Research UK

Title: Neurochemical Signatures of Regional Cerebral Blood Flow Alterations in Schizophrenia and the Clinical High-Risk state for Psychosis

Authors: *S. KNIGHT[1], O. DIPASQUALE[2], T. T. LIU[3], D. SHIN[4], M. BOSSONG[5], M. AZIS[2], M. ANTONIADES[6], A. EGERTON[2], P. ALLEN[2], O. O'DALY[2], P. MCGUIRE[7], G.

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Abstract: *In vivo* investigations have demonstrated resting regional cerebral blood flow (rCBF) alterations in patients with schizophrenia (SZ) and individuals at clinical high-risk for psychosis (CHR). Recently, availability of neuroreceptor binding atlases derived from positron emission tomography (PET) of healthy volunteers has improved. Combining these atlases with other neuroimaging techniques has been used to validate pharmacologically induced changes in rCBF in healthy volunteers. However, this approach has yet to be applied to investigate the underlying molecular mechanisms of rCBF alterations in psychosis. We compared 129 CHR individuals against 58 healthy controls (HC); and 122 patients with SZ against 117 HC. rCBF data from all participants was obtained using arterial spin labelling (ASL) and pre-processed using the CBFBIRN pipeline (SZvsHC) and the ASL Toolbox. SPM12 was used to derive case-control t-test maps of CHRvsHC and SZvsHC. We next tested the $p<.001$ minimum height thresholded maps for spatial associations with neuroreceptor binding distribution of 19 freely available PET atlases. Case-control rCBF difference maps and receptor atlases were segmented into the same standard space (Desikan-Killiany atlas, 82 regions). A dominance regression analysis was used to determine the unique contribution of mean receptor binding values to the prediction of mean rCBF differences in each region, which included spin permutation testing to correct for spatial autocorrelation between spatial maps. Whole-brain analysis comparing CHRvsHC revealed increased rCBF in the left and right temporal lobe and decreases in the occipital lobe. Comparing SZvsHC, rCBF was reduced in the supramarginal gyrus and insula (all $pFWE<.05$). Receptor distribution significantly predicted SZvsHC and CHRvsHC difference maps ($R^2 = .535$, $p=.029$, $R^2 = .464$, $p=.050$ respectively). Dopamine D1&D2 as well as glutamate, 5-HT4, and acetylcholine maps contributed most to prediction of SZvsHC rCBF differences, while 5-HT1a, mu-opioid, NMDA, and dopamine transporter maps contributed most to the prediction of CHR>HC rCBF differences. Our findings suggest that the profiles of rCBF differences in CHR individuals and in patients with SZ are associated with the distribution of both overlapping and unique neurotransmitter systems. These results may have important implications for understanding the neurochemical pathways underlying key neuroimaging profiles of brain function in SZ and the CHR state. Such hypothesis-generating approaches could be utilised in future to guide the non-invasive stratification of mechanisms of risk which may be amenable to pharmacological intervention.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.09/WW83

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

Title: Selective muscarinic receptor positive allosteric modulator NS-087 for schizophrenia treatment

Authors: *L. WU[1], D. CHEN[2], H. JIA[2], L. ZHAO[2], N. LIU[2], J. SHEN[3];
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Abstract: Muscarinic acetylcholine receptors (mAChRs) are a family of GPCR receptors that are involved in a variety of brain functions, including cognition, memory, emotion, and movement. Muscarinic acetylcholine receptor alterations have been reported in schizophrenia patients. In addition, studies have shown that people with schizophrenia have lower levels of acetylcholine in the brain, and that antipsychotic drugs that are effective in treating schizophrenia can increase acetylcholine levels. Based on these evidences, researchers have hypothesized that modulating mAChRs could be a potential treatment for schizophrenia. In consideration of the great potential of muscarinic acetylcholine receptor modulation for the treatment of schizophrenia, we developed a novel M4 positive allosteric modulator NS-087. This modulator selectively targets the central nervous system distributed M4 subtype function to avoid peripheral cholinergic side effects. NS-087 has shown good in vitro human M4 receptor modulation activity, with an EC_{50} of 370 nM and an E_{max} of 87% at EC_{10} acetylcholine concentration. The bioavailability, clearance, and half-life were measured at 106%, 20.2 ml/min/kg, and 2.4 hr, respectively, in mice. The rat and dog PK exhibited similar trends, all supporting once-daily oral administration. The mice's hyperactivity induced by MK-801 can be dose-dependently suppressed after oral administration of NS-087 at 1, 3, and 5 mg/kg. Oral administration of NS-087 can significantly reduce the immobility time, with 36.7 % and 37.7 % reduction at 5 and 10 mg/kg, respectively. Schizophrenia associated sensorimotor gating can be restored with NS-087 administered at 10 mg/kg in a C57BL/6J mice pre-pulse inhibition model. Next, we evaluated the safety of NS-087. The potential motor function side effects of NS-087 was assessed in the rotarod test using C57BL/6J mice, oral administration of at 10 and 20 mg/kg didn't change the latency of fall. A 28-day repeat-dose toxicity study was performed at dosages of 3, 10, and 30 mg/kg/day with 5 male and 5 female SD rats in each group. No abnormality was observed throughout the study, including daily activity, body weight, food intake, hematology, and biochemistry tests. In summary, NS-087 is a selective muscarinic acetylcholine receptor positive allosteric modulator with good in vitro potency. This candidate has shown potential for the treatment of both positive and negative symptoms of schizophrenia. In addition to its efficacy, the safety profile is also well acceptable. Overall, NS-087 has demonstrated potential as a PCC candidate and is currently undergoing further profiling. It will soon proceed to the development stage.

Disclosures: **L. Wu:** A. Employment/Salary (full or part-time):; NeuShen Therapeutics. **D. Chen:** A. Employment/Salary (full or part-time):; NeuShen Therapeutics. **H. Jia:** A.

Employment/Salary (full or part-time):; NeuShen Therapeutics. **L. Zhao:** A. Employment/Salary (full or part-time):; NeuShen Therapeutics. **N. Liu:** A. Employment/Salary (full or part-time):; NeuShen Therapeutics. **J. Shen:** A. Employment/Salary (full or part-time):; NeuShen Therapeutics.

Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.10/XX1

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

Title: Acutely blocking NMDA receptors in rats reproduces behavioral and electrophysiological features of schizophrenia: a robust and translational tool to assess antipsychotics and investigational drugs indicated for the treatment of schizophrenia

Authors: *F. ADRAOUI[1], K. CARVALHO[1], K. HETTAK[1], M. ALIX[1], G. VIARDOT[2], P. L'HOSTIS[3], C. DRIEU LA ROCHELLE[1];
[1]BIOTRIAL PHARMACOLOGY, RENNES, France; [2]BIOTRIAL NEUROSCIENCE, BRUNSTATT-DIDENHEIM, France; [3]BIOTRIAL NEUROSCIENCE, RENNES, France

Abstract: Schizophrenia (SZ) is one of the most severe psychiatric disorders and affects nearly 1% of the world's population. Yet, there has been no major improvement in the therapeutic management of this disorder since the commercialization of atypical antipsychotics. This is mainly due to the complexity of SZ pathophysiology and the limited translation of output parameters from preclinical to clinical investigations. Recently, the use of behavioral measures combined with electroencephalography has been highlighted as a better approach in SZ drug development. Hence, we report here a simple and translational pre-clinical approach based on behavioral and electroencephalographic assessments to robustly evaluate the efficacy of drugs indicated for the treatment of SZ. SZ pathophysiology being mediated by N-Methyl-D-Aspartate (NMDA) receptor hypofunction, our method relies on acutely blocking NMDA receptors in rats to reproduce key features of SZ including psychosis, social deficit, and abnormal evoked gamma-band oscillations before evaluating the efficacy of various antipsychotics and investigational drugs on these parameters. Separate cohorts of naïve and telemetered Sprague Dawley rats were evaluated in the locomotor activity test (used as an index of psychotic-like behavior), social interaction assay as well as in a 50-Hz auditory steady-state response (ASSR) paradigm following acute NMDA receptor antagonism with phencyclidine (PCP, 1-5 mg/kg, subcutaneously) or MK-801 (0.05-0.2 mg/kg, subcutaneously). The effects of atypical antipsychotics (clozapine: 5-10 mg/kg, intraperitoneally; aripiprazole: 1-30mg/kg, intraperitoneally) and bitopertin (a glycine transporter type 1 blocker whose mechanism of action has recently drawn attention from SZ drug developers and administered *per os* at various doses in this study) were then evaluated on behavioral and electrophysiological changes induced by PCP and MK-801. PCP and MK-801 significantly and dose-dependently increased locomotion, decreased social interactions independently of motor effects, and reduced the phase synchrony of

evoked gamma-band oscillations during the 50-Hz ASSR paradigm. Atypical antipsychotics and bitopertin differentially modulated NMDA-receptor-antagonism-induced behavioral changes and electrophysiological deficits. These results further our understanding on antipsychotics and investigational drugs indicated for the treatment of SZ and indicates that the acute NMDA-receptor-antagonism model could be a reliable and translational pre-clinical tool for drug developers working in the field of SZ.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.11/XX2

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

Title: Taar1-selective compounds as antipsychotics: unanswered questions and lessons learned from ulotaront and ralmitaront

Authors: K. SMART[1], L. E. J. THOMPSON[1], J. BESNARD[1], D. TANAKA[1], A. CHEASTY[1], D. HALLETT[2], ***R. H. J. OLSEN**[2,1];
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Abstract: Trace Amine Receptor 1 (TAAR1) has received substantial attention as a novel drug target for psychiatric disorders, specifically as a novel antipsychotic mechanism distinct from typical (DRD2 targeting) and atypical (5HT2A/DRD2-targeting) antipsychotics. Multiple TAAR1-selective agonists from a range of scaffolds have shown *in vivo* efficacy in classical rodent antipsychotic models without significant adverse sequelae. Despite this, only two TAAR1-targeting molecules have entered the clinic. Ulotaront (TAAR1 agonist, 5HT1A partial agonist) has demonstrated efficacy against both positive and negative symptoms in schizophrenia in a Phase 2 study with results from a Phase 3 trial expected shortly, as well as efficacy in other classes of psychotic disorders. The clinical development of ralmitaront, ostensibly a selective TAAR1 partial agonist, was recently abandoned after failing to demonstrate efficacy. Because of this contrast in clinical efficacy the use of TAAR1-selective agonists as a standalone therapeutic agent has generated controversy, raising questions about their potential effectiveness without additional polypharmacology such as ulotaront's partial agonism at 5HT1A. Here we present detailed pharmacological profiling of ralmitaront and ulotaront in human, rat and mouse receptor signalling assays. We demonstrate that in minimally-amplified systems, ralmitaront is a potent and efficacious agonist of rodent TAAR1, but shows negligible agonist activity at human TAAR1 rather than being a moderate partial agonist as previously reported. We suggest that, in humans, ralmitaront is more likely to function as a TAAR1 antagonist, and that the question of the therapeutic value of selective actual TAAR1 agonists therefore remains unanswered.

Disclosures: **K. Smart:** A. Employment/Salary (full or part-time):; Exscientia. **L.E.J. Thompson:** A. Employment/Salary (full or part-time):; Exscientia. **J. Besnard:** A. Employment/Salary (full or part-time):; Exscientia. **D. Tanaka:** A. Employment/Salary (full or part-time):; Exscientia. **A. Cheasty:** A. Employment/Salary (full or part-time):; Exscientia. **D. Hallett:** A. Employment/Salary (full or part-time):; Exscientia. **R.H.J. Olsen:** A. Employment/Salary (full or part-time):; Exscientia.

Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.12/XX3

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

Support: Stanley Medical Research Institute grants 03-484 and 06T-797
NIH NIMH/FIC/NCCAM grant R21MH095644
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Contract # HHSN-271- 2013-00017-C and HHSN- 271-2018-00023-C (NIMH - PDSP)

Title: Clues for understanding the role of MRGPRX4 signaling in the nervous system and behavior

Authors: *C. GALLO[1], G. POLETTI[1], R. ROJAS[1], J. ALBÁN[2], A. VAISBERG[1];
[1]Univ. Peruana Cayetano Heredia, Lima, Peru; [2]Univ. Nacional Mayor de San Marcos, Lima, Peru

Abstract: MRGPRX4 is still an understudied GPCR. The available evidence supports it is activated by bile acids and other small acid molecules. However, few agonist and antagonist molecules for it have been described so far. Allen Atlas database shows It is highly expressed in the oculomotor system as well as in the hypothalamus, the hippocampus, midbrain, pons, and medulla. Understanding its function will open novel research avenues and treatment opportunities for nervous system disorders. We have a repository of extracts from 87 plants used in Peruvian traditional medicine. Nineteen of these extracts were found active for MRGPRX4 in functional assays performed at the NIMH Psychoactive Drug Screening Program (PDSP) - University of North Carolina, Chapel Hill. The reported traditional use of these extracts ranged diverse behavioral conditions defined either in formal (depression, insomnia, schizophrenia) and informal (“nerves”, “madness”, “sadness”) or traditional (“fright”, “blooming”) terminology. Our results so far show that the plant extracts active for MRGPRX4 have also a combination of activities for oxytocin (OXTR), neurotensin 1 (NTS1), GPR65, delta opioid (OPRD1), mu opioid (OPRM1), and serotonin 5A (HTR5A); all of them previously linked in some way to itch, or to lipid mobilization, absorption, or metabolism.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.13/XX4

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

Title: Investigating blood biomarkers of psychiatric disease in the "Real World" by data mining and Bayesian hierarchical network modelling

Authors: *C. L. EBBESEN[1], K. KOMPUS[2], J. BAHL[2];
[1]Data Sci., [2]Clin. Biomarkers, Lundbeck A/S, Valby, Denmark

Abstract: Electronic Health Records and health insurance claims databases contain a detailed, longitudinal record of individual patients' laboratory results (e.g. blood panels, urine tests), physiological measurements (e.g. weight, blood pressure), and disease and treatment histories (diagnoses, drug prescriptions, procedures performed, etc.). These data can help identify biomarkers to help stratify patients and monitor treatment response and disease outcomes. Biomarkers discovered in Real World Data could have important benefits wrt. robustness, clinical relevance, and health equity, since - compared to participants in clinical studies - Real World Data are in many respects more representative of patients in actual clinical care. The analysis of Real World Data needs to strike a balance between flexible models and clinical interpretability. On the one hand, we want flexible models that can learn dependencies on e.g. diagnostic and prescription history. On the other hand, we also want models that are understandable, so that we can reason with human biology and clinical experts about statistical findings. We propose that this can be solved by viewing patients and e.g. assigned diagnosis as a bipartite network graph, and by using Bayesian hierarchical network models to discover the hierarchical and modular structure of that graph. This approach allows us to discover and understand how characteristic patient types are defined by their medical history, and - in an interpretable and hierarchical way - what aspects of their medical histories make them distinct. Here we present an example of a graph theoretical analysis of Real World Data. We focus on cortisol, a hormone that is routinely measured in clinical care and correlated with stress (a known risk factor for multiple psychiatric diseases). We show that it is possible to discover and quality control cortisol measurements by data mining of Electronic Health Record databases, and that our graph theoretical approach discovers a modular structure of patient types with interpretable disease histories, that we can relate to proxies of psychiatric disease burden mined from health insurance claims databases, such as treatment duration, drug dosages, and classes of psychotherapeutic drugs prescribed by doctors.

Disclosures: **C.L. Ebbesen:** A. Employment/Salary (full or part-time):; Lundbeck A/S. **K. Kompus:** A. Employment/Salary (full or part-time):; Lundbeck A/S. **J. Bahl:** A. Employment/Salary (full or part-time):; Lundbeck A/S.

Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.14/XX5

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

Support: Stanley Medical Research Institute
NIMH R01 MH112704
NIMH 1K23 MH110607

Title: Developing Extracellular Vesicle messenger RNA Biomarkers for the Brain

Authors: L. SMIRNOVA[1], L. M. OSBORNE[2], J. L. PAYNE[3], ***S. SABUNCIYAN**[4]; [1]Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD; [2]Dept. of Obstetrics & Gynecology, Weill Cornell Med., New York, NY; [3]Dept. of Psychiatry and Neurobehavioral Sci., Univ. of Virginia, Charlottesville, VA; [4]Pediatrics, Johns Hopkins Univ., Baltimore, MD

Abstract: The absence of non-invasive tests that can monitor the status of the brain is a major obstacle in the treatment of neurological and psychiatric disorders. As extracellular vesicles (EV) are released by all cell types, including neural cells, brain specific EVs represent a potential reservoir for brain biomarkers. In this work, we assessed the feasibility of using tissue-specific gene expression to determine the origin of EV messenger RNAs in blood. Using the placenta as a model, we discovered that 29 messenger RNAs that were specifically expressed in the placenta were present in EVs circulating in maternal blood. Twenty-seven of these transcripts were present exclusively or in high levels in maternal blood during pregnancy only and not in the postpartum period. As these results demonstrated the feasibility of using tissue-specific gene expression to infer the tissue of origin for EV mRNAs, we applied the same bioinformatic approach to identify 181 mRNAs in blood EVs that are specifically expressed in the brain. These transcripts, which are involved in synaptic functions and myelination, are enriched for genes implicated in mood disorders, schizophrenia and substance use disorder. The levels of thirteen brain specific EV mRNAs in blood were associated with postpartum depression, raising the possibility that they can be used as a proxy for brain health. In order to determine the extent to which EV mRNAs reflect transcription in the brain, we compared mRNAs isolated from cells and EVs in an iPSC-derived brain microphysiological system differentiated for 3 and 9 weeks. Although cellular and extracellular mRNA levels were not identical, they were correlated and EV mRNA levels can be used to extrapolate cellular RNA expression changes in the brain. Our findings establish the potential of EV mRNAs as biomarkers for brain pathology and bring them to the forefront of biomarker development efforts in psychiatric diseases.

Disclosures: **L. Smirnova:** None. **L.M. Osborne:** None. **J.L. Payne:** A. Employment/Salary (full or part-time):; University of Virginia. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Karuna Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Epigenetic Biomarkers of Postpartum Depression. F. Consulting Fees (e.g., advisory boards); SAGE Therapeutics, Brii Biosciences, and Pure Tech Health. **S. Sabunciyan:** None.

Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.15/XX6

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

Support: JSPS KAKENHI 18K15354
JSPS KAKENHI 20H01777
JSPS KAKENHI 19H04887
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JSPS KAKENHI 16H06395
JSPS KAKENHI 20H03951
JSPS KAKENHI 19H04887

Title: Urinrary exosomal microRNAs as predictive biomarkers for persistent psychotic-like experiences

Authors: *Y. TOMITA[1,2], K. SUZUKI[2], S. YAMASAKI[2], M. MIYASHITA[2], K. TORIUMI[2], M. ITOKAWA[2], A. NISIDA[2], M. ARAI[2];
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Abstract: Psychotic-like experiences (PLEs) occasionally occur during adolescence but often disappear as individuals grow older. However, persistent PLEs are considered a robust risk factor for subsequent psychiatric disorders. Few biological markers for predicting persistent PLEs have been explored, highlighting the critical need to identify such markers to prevent the development of psychiatric disorders.Recently, exosomal miRNAs have gained attention as potential biomarkers for psychiatric disorders. However, the relevance of exosomal miRNAs to PLEs remains unexplored. Therefore, our study aimed to investigate the potential of urinary exosomal miRNAs as predictive biomarkers for persistent PLE. Given its non-invasive sampling method, urine is ideal biomarker source for adolescents compared to blood. Our research was conducted as part of a population-based biomarker subsample study within the Tokyo Teen Cohort Study. A total of 345 participants aged 13 (baseline) and 14 (follow-up) years, underwent PLE assessments by experienced psychiatrists using semi-structured interviews. Remitted and persistent PLEs were defined based on longitudinal profiles. Urine samples were collected at

baseline, and the expression levels of urinary exosomal miRNAs were compared between 15 individuals with persistent PLEs and 15 age- and sex-matched individuals with remitted PLEs. A logistic regression model was constructed to examine whether miRNA expression levels could predict persistent PLEs. Our results identified six significantly altered miRNAs (hsa-miR-486-5p, hsa-miR-199a-3p, hsa-miR-144-5p, hsa-miR-451a, hsa-miR-143-3p, and hsa-miR-142-3p) in urinary exosomes of adolescents with persistent PLEs. Moreover, these miRNAs demonstrated high accuracy in predicting the trajectory of PLEs after one year using the logistic regression model. The predictive model showed an area under the curve of 0.860 (95% confidence interval: 0.713-0.993) through five-fold cross-validation. Despite the small sample size, our findings suggest that urinary exosomal miRNAs in adolescents could serve as a novel biomarker for assessing the risk of psychiatric disorders.

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Poster

PSTR242. Biomarkers of Affective Disorders and Schizophrenia

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR242.16/XX7

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: The relationship between the Schumann Resonance and 71Hz with the growth of neurites in PC-12 cells

Authors: *L. M. LEFEBVRE, K. SAROKA, B. DOTTA;
Laurentian Univ., Sudbury, ON, Canada

Abstract: The Schumann resonances are a discreet collection of frequency peaks associated with the background magnetic field of the Earth and are generated by lightning strikes. The frequencies range from 3 Hz through 60 Hz, where the strongest peak is found at 7.8Hz. An interesting research avenue is investigating how this phenomenon interacts with biological systems and other electromagnetic sources. To investigate this, two methods of analysis were conducted on PC-12 cells: (1) physical analysis of both cell viability and neurite outgrowth and (2) the acquisition of the cell's electrical activity by using a novel electrophysiology device. This novel measurement device consisted of two sets of copper wires; one wrapped around the bottom of the cell dish and another in a plate containing only the cell-nourishing medium and a ground. Each of these wires was connected to an amplifier, which was connected to a laptop via USB. The laptop then monitored the electrophysiology activity of the cells and the media allowing for the determination of spectral power density (SPD) of each cell group for frequencies in between 1Hz and 128Hz. A regression analysis revealed that two frequencies (71Hz and 7.8Hz) were able to predict the percentage of cells containing neurites. The prediction equation is as follows: $y = (0.01) \times 71\ Hz - (0.008) \times 7.8Hz + 0.048$, and there was a strong correlation between this equation and actual percentage of cells containing neurites at 71Hz and 7.8Hz frequencies

(R=0.542, p=0.011, n=11). This suggests that both of these frequencies are involved with the outgrowth of neurites in these PC-12 cells. This is interesting as the 7.8Hz frequency coincides with the fundamental mode frequency of the Schumann resonance which may be a potent finding suggesting that neurites could be influenced by the Earth's magnetic field. Studies have found that during cosmic disturbances like solar storms, where we see a perturbation in the Earth's magnetic field, there are certain associated decreases within the right prefrontal cortex, which manifests in marked behaviours like erroneous reconstruction of memory and emotional lability (Mulligan, 2012; Rouleau and Dotta, 2014). As for the 71Hz frequency, Manos *et al.* (2018) demonstrated that a group of 200 Hodgkin-Huxley neurons have an intrinsic abnormally synchronised firing rate of approximately 71Hz before applying Coordinated Reset (CR) stimulation to counteract this abnormality. It can be hypothesized that the arbitrary neurite growth pattern of these PC-12 cells could be considered as an abnormally strong neuronal synchrony, possibly substantiating the significance of this 71Hz frequency.

Disclosures: L.M. Lefebvre: None. **K. Saroka:** None. **B. Dotta:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.01/XX8

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

Support: NIH Grant NS107148
NIH Grant R01NS079507
NIH Grant R01AG075583
NIH Grant R21NS131903

Title: Application of machine learning methods in non-invasive, automated seizure detection in rats

Authors: K. D. DONOHUE[1,2], D. HUFFMAN[1], J. PERDEH[3], B. BAUER[3], *B. F. O'HARA[4,1], *B. O'HARA[5], S. SUNDERAM[6];
[1]Signal Solutions, LLC, Lexington, KY; [2]Electrical and Computer Engin., [3]Dept. of Pharmaceut. Sci., [4]Dept. of Biol., [6]F. Joseph Halcomb III MD Dept. of Biomed. Engin., [5]Univ. of Kentucky, Lexington, KY

Abstract: Preclinical research into epilepsy and other seizure disorders often requires methods to assess the number and severity of seizures. This is primarily accomplished through manual review of electroencephalographic and/or video recordings, which is time- and resource-intensive. Thus, experimental tools for identifying seizure events automatically and without the need for EEG could be of great utility in this field. To this end, we investigate the feasibility of screening for seizures in rats based on changes in activity detected using cage floor pressure sensors. Thirty-seven adult Wistar rats previously treated with lithium chloride/pilocarpine to

induce acute status epilepticus were continuously monitored for several weeks using piezoelectric sensors (located beneath the cage floor) and simultaneous video. Recordings of the piezoelectric signals were processed to identify a preliminary set of candidate seizure events (identified by detecting sudden bursts of activity in the pressure signal). These candidates were then labelled based on manual review of video according to observed behavior (Racine seizure level, grooming, arousals, etc). Labelled events were then compiled into a reference data set (1289 verified seizures, Racine level 3-5; 1481 other behaviors such as arousal or grooming) to validate the predictions of machine learning algorithms. Signals were processed to derive a line length feature time series in 1-second intervals, and peaks in that series were used to identify activity bursts within the signal. A set of 9 additional features were then extracted in the regions surrounding these peaks, and machine learning algorithms were applied to regress a likelihood value for discriminating seizures from non-seizure events using a five-fold cross validation training and testing cycle. The best results were achieved with an ensemble decision tree algorithm, which was successful in detecting 90% of seizures at a 28% false positive detection rate. If only seizures of Racine levels 4-5 were considered, the false positive rate dropped to 10%. Overall, these early results indicate the potential utility of this approach for non-invasive seizure detection in rodents.

Disclosures: **K.D. Donohue:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **D. Huffman:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **J. Perdeh:** None. **B. Bauer:** None. **B.F. O'Hara:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **B. O'Hara:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **S. Sunderam:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.02/XX9

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

Support: NIH U19NS107464

Title: Dynamical state of neural cultures inferred from the configuration vector

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Abstract: Newly available technologies allow for the identification and recording of activity from hundreds to thousands of individual neurons over large areas, both *in vitro* and *in vivo*. However, the information from this many neurons is rarely used for characterizing neuronal network states despite being available. The first experimental results supporting the critical brain hypothesis consisted of the observation that a scale free distribution of neuronal avalanches could be computed from timeseries of neuronal activity (Beggs & Plenz, 2003). Avalanche size distribution was later computed over several neuronal culture conditions, showing that it could be used to infer the dynamical state of the neuronal network (Shew et al. 2009). In this work, we show how we can use the instantaneous configuration vectors (i.e., the identity of firing neurons at a given timeframe), instead of or along with the activity timeseries, to characterize the dynamical state of the network. Here we consider measures based on the scaling of correlations with the observation window (Martin et al. 2021, Camargo et al. 2022). We also introduce a novel measure based on configuration vectors alone, and we compare these results with those obtained from analysis of the whole network, such as avalanche size distribution and time autocorrelations. We run these analyses both with results from a simple numerical model and from primary cultures of rat embryo neurons, where dynamics are captured via calcium imaging.

Disclosures: E.J. Aguilar Trejo: None. **D.A. Martin:** None. **S. Camargo:** None. **A. Emenheiser:** None. **K.M. O'Neill:** None. **D.R. Chialvo:** None. **W. Losert:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.03/XX10

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

Support: BioSUP Project (PR19-CR-P2)

Title: Combining automatic segmentation of nerve histology and computational modeling of neuromodulation

Authors: *A. GIANNOTTI[1], S. ROMENI[2], G. FAORO[1], C. COCCHETTI[3], C. LENZI[3], A. PIRONE[3], E. GIANNESSI[3], V. MIRAGLIOTTA[3], A. MENCIASSI[1], S. MOCCIA[1], S. MICERA[2,1];
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Abstract: Intraneural prostheses selectively stimulate nerve fascicles restoring connections between the nervous system and peripheral organs in cases of injuries or diseases, but their performance is highly sensitive to their placement. Thus, hybrid models (HM) used to optimize stimulation protocols require knowing the exact nerve fascicular structure, currently determined through manual segmentation of nerve histological sections, which is extremely time-consuming.Here, we present a fully automatic procedure cascading a deep encoder-decoder

model to produce a semantic segmentation of histological sections, postprocessing using morphological operations to separate merged fascicles, fascicle simplification and reshaping to reduce the complexity of the produced geometries, and hybrid modeling to estimate the response to stimulation of a given nerve segment.We tested our procedure on a dataset of 87 images of pudendal nerve histological sections collected from pig pudendal nerve samples stained with three different dyes. Images were manually segmented (MS) and given as input to a UNet, trained to minimize Dice loss using Adam optimizer with time-dependent learning rate, early stopping, and data augmentation.We obtained a Dice similarity coefficient of 92.33 ± 2.46 (mean ± standard) using five-fold cross-validation. In most cases, fascicles were correctly separated. Small regions were removed with through thresholding based on fascicle dimensions in the training set. The employed stain did not affect the segmentation results.
We applied repeated erosions and dilations to separate merged fascicles, and simplified fascicle shapes to ellipses, gradually displaced to avoid intersections. Four randomly selected MS and automatically segmented (AS) topographies were used to build HMs, applying point source currents at five given locations. We solved the volume conduction problem using COMSOL with MATLAB, and computed recruitment curves, estimating activation using a threshold on the extracellular electric potential. The recruitment mismatch between MS and AS topographies was computed as the mean absolute deviation (MAD) in the currents required to recruit 20%, 50%, and 80% of a given group. The absolute and relative MAD corresponding the indices did not show evident trends, except for a generally high standard deviation due to the use of four histological sections, while they both were substantially lower in quadrant groups than in fascicular groups. Our results pave the way towards incorporating a large amount of histological data into computational modeling, allowing for the automatic optimization of neural interfaces.

Disclosures: **A. Giannotti:** None. **S. Romeni:** None. **G. Faoro:** None. **C. Cocchetti:** None. **C. Lenzi:** None. **A. Pirone:** None. **E. Giannessi:** None. **V. Miragliotta:** None. **A. Menciassi:** None. **S. Moccia:** None. **S. Micera:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.04/XX11

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

Support: Hacettepe University BAP Grant TOA-2023-20338

Title: A Novel Membrane with Soft Sensors to Directly Measure Mechanical Strain

Authors: *S. KUMBAY YILDIZ[1], N. B. DUZ[1], E. AYDIN[1], S. AKAR[2], H. ARTUNER[1], P. R. DINCER[1], I. UYANIK[1];
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Abstract: Mechanical strain plays a crucial role in understanding cellular responses and tissue development. However, existing cell stretching devices often employ indirect strain measurement methods, basically relying on motor motion, thus introduce uncertainties and inaccuracies. This research aims to address these limitations by developing membranes with soft strain sensors, enabling direct strain measurement and incorporate this information in the closed-loop control of the device's motors. By accurately replicating mechanical forces, this approach contributes to unraveling the complex interaction between mechanical signals and cellular responses. The soft strain sensors used in this study are fabricated using a silicone sandwich structure. The fabrication process involves several steps: First, 3D printed molds are created to match the desired dimensions and serpentine pattern of the sensor. Next, the molds are filled with silicone material, ensuring complete coverage of the serpentine pattern, and then cured to solidify the layers. Once both layers are formed, they are brought together, aligned, and bonded to create a cohesive structure. The serpentine pattern is then filled with an electrically conductive carbon-based liquid. Electrodes are attached to the ends of the pattern to establish electrical connections. Finally, the fabricated soft strain sensor is characterized by subjecting it to known strains, measuring the resistance at each level to create a calibration curve that correlates resistance variations with applied strain. This calibrated sensor provides real-time and direct measurement of the strain applied to the membrane. The integration of soft strain sensors enables the development of a closed-loop control system for the cell stretching device. Real-time strain data obtained from the sensors is fed back into the control system, enabling dynamic and precise adjustment of the device's motors. By incorporating strain measurement directly into the control loop, researchers can maintain a desired strain level on the membranes, ensuring consistent and accurate mechanical loading of cells. This closed-loop control enhances the reproducibility and reliability of cell stretching experiments. The findings from this study can lay the groundwork for the development of more sophisticated cell stretching devices that accurately mimic physiological conditions, provide opportunities for a range of applications, including tissue engineering, regenerative medicine, and drug discovery.

Disclosures: S. Kumbay Yildiz: None. **N.B. Duz:** None. **E. Aydin:** None. **S. Akar:** None. **H. Artuner:** None. **P.R. Dincer:** None. **I. Uyanik:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.05/XX12

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

Support: NIH Grant 1 T32 GM145455-01

Title: Assessing cross-contamination in spike-sorted electrophysiology data

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Univ., Boston, MA; [3]Boston Univ. Ctr. for Systems Neurosci., Boston, MA; [4]Boston Univ. Photonics Ctr., Boston, MA

Abstract: Recent advances in electrophysiological instrumentation now facilitate the recording of spikes from hundreds or thousands of neurons at a time. For many applications, proper interpretation of these data requires accurate spike sorting - identification of the spikes that belong to the same neuron. The growing magnitude of these data necessitates robust *post hoc* quality metrics to effectively quantify spike sorting accuracy. One indication of the false positive rate (FPR) in spike sorting - the frequency with which spikes from one cell are erroneously assigned to another - has been the occurrence of inter-spike-interval (ISI) violations. Yet, implementation of ISI violations as a quality metric have remained *ad hoc*. There is a general understanding that higher ISI violations are associated with higher FPRs, but only a small fraction of false positive spikes produce ISI violations and a quantitative relationship between false positive rates and ISI violations has only been determined under a specific set of assumptions that are unlikely to generally hold (Hill et al., 2011).
We have derived an analytical solution describing the dependence of underlying FPR on observed ISI violation fraction across a range of external conditions, tested this model *in silico* through Monte Carlo simulation, and applied our method to 10 publicly available spike-sorted electrophysiology datasets published between 2016 and 2022. We find that the relationship between ISI violations and unit cross-contamination is highly nonlinear, with additional dependencies on overall unit firing rate, temporal overlap between in-cluster and out-cluster firing, and confounding neuron count. Predicted mean FPRs in public datasets were found to range from 11.7% ± 0.4% to 48.4% ± 0.8% (median FPRs range from 0.1% ± 0.3% to 52.7% ± 0.8%). We also determine the statistical criteria for recording length and spike rate under which ISI violations provide an accurate measure of false positive rates. Our findings provide the community with guidelines for inferring false positive rates in spike sorted data from ISI histograms violations and provide a quantitative assessment of this relationship under typical experimental conditions.
Hill, D. N., Mehta, S. B., & Kleinfeld, D. (2011). Quality Metrics to Accompany Spike Sorting of Extracellular Signals. *Journal of Neuroscience, 31*(24), 8699-8705. https://doi.org/10.1523/JNEUROSCI.0971-11.2011

Disclosures: **J.P. Vincent:** None. **M. Economo:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

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Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.06/XX13

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

Support: Burroughs Wellcome Fund
NIH T-32-NS076401

Title: Real-time prediction of latent neural trajectories and behavior

Authors: J. GOULD[1], *A. DRAELOS[2];
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Abstract: A fundamental goal of systems neuroscience is to obtain a mechanistic understanding of how neural circuit specificity and function regulate behavioral outcomes. Many advances in neural and behavioral measurement techniques have advanced this goal, allowing us to record from thousands of neurons simultaneously and capture relevant behavior at fine resolution. To causally test the relationships between population-level neural circuits and resultant behavior in live organisms would require directly modulating neural activity and measuring the effects of such interventions. And without knowing which groups of neurons are behaviorally relevant ahead of time, or how their dynamics may continuously shape behavior, it is necessary to obtain real-time information to guide the choice of how to intervene throughout an experiment. Thus models are needed that incorporate multiple types of data streams, produce predictions of future neural dynamics and behavior, and generate estimates of neuron-behavior relationships for subsequent testing: all in real time.
We present a method for real-time prediction of both latent neural trajectories and simultaneously measured behavioral variables. Rather than explicitly modeling the mapping between neural trajectories and behavior, we instead focus on prediction of both data types from a shared latent space. Our approach leverages a previously published method that combines streaming dimensionality reduction and a coarse tiling of the resulting space to estimate latent neural dynamics in real time (Draelos, 2021). Here, we incorporate simultaneous streams of behavioral data into these low-dimensional neural spaces and consider a joint space where neural and behavioral components are adaptively weighted to generate future estimates of latent neural trajectories or behavioral metrics. This method supplies predictions of both future neural dynamics and future behavior at high granularity and fine temporal resolution, while maintaining speeds faster than data acquisition. Importantly, it also handles both discrete and continuous behavioral data, without requiring identical sampling rates across data streams. We demonstrate our method on simulated and experimental datasets including neural activity recorded via electrophysiology or calcium imaging and behavior ranging from continuous kinematics to task metrics. We find that the inclusion of behavioral metrics with the neural activity in our latent representations can substantially improve real-time predictions of the ongoing latent neural dynamics.

Disclosures: **J. Gould:** None. **A. Draelos:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.07/XX14

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

Support: Wellcome Trust 209558
Wellcome Trust 216324
National Institutes of Health 1U19NS123716
Simons Foundation

Title: Brain-wide atlas of electrophysiological properties and associated tools

Authors: I. IBL[1], K. BOUGROVA[2], J. BOUSSARD[4], J. BHAGAT[5], G. CHAPUIS[6], J. CATARINO[2], S. HOFER[5], P. LAU[5], L. PANINSKI[4], R. PRÔA[4], Y. SHI[7], N. A. STEINMETZ[8], O. WINTER[3], ***H. YU**[4];
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Abstract: The last decades have seen incredible progress in mapping gene expression, cell types, and connectivity across the entirety of mammalian brains. Yet, to date no attempt has been made to systematically characterize and quantify basic electrophysiological measurements across the brain. Moreover, to make sense of electrophysiological data it is critical to know the location of each recorded neuron. However, precisely localizing electrode channels (and thus recorded neurons) in brain tissue is fraught with uncertainty. Here we use the large dataset acquired at the International Brain Laboratory to build a new brain reference atlas of ephys properties. This dataset comprises the publicly available 354 Neuropixels probe insertions, tiling 194 brain regions of the mouse brain, as well as 551 insertions not yet released.We are developing new methods to compute ephys features (e.g. local field potential (LF) power spectra, spike rate or shape) at such a large scale, and have improved on the pre-processing steps needed to denoise both LF and spike waveforms. We are developing a new website that allows brain-wide exploration of a range of ephys features. Along with this atlas, we are building tools that enable automated localization of recording locations, in any electrophysiology mouse experiment based purely on activity from multi-channel probes (generalizing to many other probe configurations and experiment protocols). Specifically, we are developing decoder algorithms that can predict a brain region label or precise 3D location in the brain based on the ephys signatures (either of a given electrode channel, neuron, or an entire probe; accuracy 64%) - and give a sense for localisation uncertainty. This is the first step towards building on-line tools to be used in real-time experiments.Finally, we build encoding models to construct an electrophysiology atlas of full-brain coverage at high spatial resolution (voxel size 200 um). Specifically, the encoding models use anatomical information (brain region parcellation, spatial coordinates, and gene-expression profiles) as priors, and interpolate the full map from sparse data samples. The encoding models also enable us to explore the link between the brain's anatomical structure and ephys features. In particular, we find that the spatial gene expression profiles capture a large fraction of variance of ephys patterns across the brain.This work summarizes a systematical characterization and quantification of ephys features across the mouse brain. This atlas will provide an open, critical resource for neuroscientists to both guide on-line ephys recordings and post hoc interpretation.

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Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.08/XX15

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

Support: NIH NINDS R01NS115327
NIH T32EB025816

Title: Enhancing the Cleo experiment simulation testbed to support all-optical control, multi-channel optogenetics, and easier integration into data analysis pipelines

Authors: *K. A. JOHNSEN[1], N. A. CRUZADO[2], A. CHARLES[3], C. J. ROZELL[2];
[1]Biomed. Engin., Georgia Inst. of Technol. & Emory Univ., Atlanta, GA; [2]Electrical and Computer Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Johns Hopkins Univ. - Homewood Campus, Lutherville-Timonium, MD.

Abstract: Recent advances in systems neuroscience methods enable exciting new kinds of experiments. One of these is closed-loop optogenetic control, which combines simultaneous photostimulation and recording to precisely control mesoscale neural activity on the order of milliseconds. However, the difficulty of implementing such experiments can slow development and impede adoption. To address this challenge, we have developed the Cleo (Closed Loop, Electrophysiology, and Optogenetics) experiment simulation testbed, which allows researchers to prototype complex experiments *in silico*. It does this by wrapping a Brian spiking neural network model, enabling closed-loop control as well as the injection of recording and stimulation devices.

While we have previously demonstrated Cleo's capabilities to simulate electrode recording and optogenetic stimulation, we now present a number of improvements to enhance Cleo's utility and usability in modern experimental paradigms. The first is support for two-photon imaging with flexible specification of genetically encoded indicator (e.g., for calcium or voltage) models. Second is holographic photostimuation, which, combined with the first, allows for the simulation of all-optical control. Third, we have greatly facilitated the simultaneous use of multiple light sources and opsins, allowing for advanced paradigms such as bidirectional or multi-channel stimulation. Finally, Cleo can now export recording and stimulation data via Neo, which can in turn convert to a number of proprietary formats and thus be easily incorporated into pre-existing data analysis pipelines.

As before, the package is accompanied by detailed online documentation including user-friendly tutorials. This documentation, as well as open-source code, can be found at the links below:
Documentation: https://cleosim.readthedocs.io
Code repository: https://github.com/Sensory-Information-Processing-Lab/Cleo

Disclosures: K.A. Johnsen: None. **N.A. Cruzado:** None. **C.J. Rozell:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.09/XX16

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

Title: Multimodal stereotaxic mouse brain atlas for robot-assisted, high-precision intracranial injection procedures in mice

Authors: *J. PERENS[1], F. LYNGE SØRENSEN[1], A. PARKA[1], T. TOPILKO[1], A. THOMSEN[1], U. ROOSTALU[1], A. DAHL[2], T. DYRBY[2,3], C. SALINAS[1], J. HECKSHER-SØRENSEN[1];
[1]Gubra, Hørsholm, Denmark; [2]Applied Mathematics and Computer Sci., Tech. Univ. Denmark, Kongens Lyngby, Denmark; [3]Ctr. for Functional and Diagnos. Imaging and Res., Danish Res. Ctr. for Magnetic Resonance, Copenhagen Univ. Hosp. Amager and Hvidovre, Copenhagen, Denmark

Abstract: The rise of three-dimensional imaging modalities, such as light-sheet fluorescence microscopy (LSFM), in combination with tissue-clearing techniques, opens an unprecedented opportunity to study complex biological phenomena in whole intact organs. The combination of these techniques is now being used as a high-throughput discovery platform to study the impact of drug treatments, gene therapies, and the evaluation of various disease states. While its application spans multiple organs, the brain is of particular interest as standardized analysis tools enable unbiased region-wise quantifications and statistical comparisons across multiple experimental groups.
Such studies often yield a list of candidate subregions in the brain worth investigating using a more targeted approach. Stereotaxic surgeries enable the precise delivery of drugs or viral vectors to a region of interest in the brain, allowing, for instance, for the targeted manipulation of neural activity or gene expression at the site of injection. Despite its significant benefits, this approach is fraught with challenges. Procedural complexities, labor-intense manual work, and a requirement for highly skilled personnel often restrict its effectiveness and increase the potential for error. Recent developments in robotics have sought to address these limitations by automating and refining the procedure.
Directly translating the complex 3D information procured via LSFM for precision-targeting distinct brain regions holds great potential in preclinical target validation and drug discovery for CNS disorders. Here, we present a novel platform that couples automated, robot-assisted stereotaxic injections with a multimodal brain atlas. In addition to offering significantly improved targeting during stereotaxic surgeries, this atlas also provides a bidirectional transformation capability for results procured using LSFM, STPT, and MRI, promoting the integration of findings across imaging modalities. Combining this approach with the stereotaxic robot's coordinate system can significantly improve stereotaxic injection precision in vivo, bridging the gap between imaging and intervention, for example using intracerebral AAV-based therapeutic modalities. The results of our study could prove significant in neuroscience research,

enabling more accurate interventions and paving the way for new discoveries in understanding and treating neurological conditions. This presentation will cover our experimental methodology, the challenges encountered, proposed solutions, and the outcomes of integrating LSFM with stereotaxic procedures.

Disclosures: **J. Perens:** A. Employment/Salary (full or part-time):; Gubra ApS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra ApS. **F. Lynge Sørensen:** A. Employment/Salary (full or part-time):; Gubra ApS. **A. Parka:** A. Employment/Salary (full or part-time):; Gubra ApS. **T. Topilko:** A. Employment/Salary (full or part-time):; Gubra ApS. **A. Thomsen:** A. Employment/Salary (full or part-time):; Gubra ApS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra ApS. **U. Roostalu:** A. Employment/Salary (full or part-time):; Gubra ApS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra ApS. **A. Dahl:** None. **T. Dyrby:** None. **C. Salinas:** A. Employment/Salary (full or part-time):; Gubra ApS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra ApS. **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time):; Gubra ApS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra ApS.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.10/XX17

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

Title: A leap on large-scale brain simulation performance: NEURON 9.0 and Neurodamus

Authors: *F. LEITE PEREIRA[1], J. G. KING[1], P. S. KUMBHAR[1], O. AWILE[1], A. L. SAVULESCU[1], I. MAGKANARIS[1], O. LUPTON[1], W. JI[1], J. BLANCO[1], N. CORNU[1], W. LYTTON[2], R. A. MCDOUGAL[3], S. DURA-BERNAL[2], M. L. HINES[3];
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Abstract: Over the past years, the Blue Brain Project (BBP) has been developing technology to construct and simulate increasingly complex brain circuits. In collaboration with Yale University, we have been working on enhancing the performance and scalability of the NEURON simulator (Carnevale and Hines 2006), reaching unprecedented levels. Additionally, several tools in our simulation toolchain have adopted the open standard SONATA and are now open-source. NEURON started to be developed over 35 years ago, at a time computing systems were very different and didn't feature hardware accelerators like GPUs. To overcome this limitation the NEURON simulation engine has been reimplemented as an external library, called

CoreNEURON, offering significant performance improvements and support for modern acceleration features. In the past two years significant efforts were made to seamlessly re-integrate CoreNEURON into NEURON. Additionally, the upcoming NEURON major release 9.0 brings various improvements including a fundamental change in the memory layout of data structures. This has enabled us to reduce the simulation time of compute-intensive models by a factor of two.To run morphologically detailed simulations at scale, BBP has developed Neurodamus, a tool to set up and orchestrate NEURON across large clusters via declarative configuration files. Being actively used by BBP researchers, it has enabled the simulation of rodent somatosensory cortical circuits such as the recently published somatosensory cortex (Isbister et al. 2023) and the hippocampus circuit (Romani et al. 2023). After significant development, Neurodamus is now fully integrated with the SONATA format and has been released as open-source software on GitHub in May 2023. This will enable researchers and enthusiasts worldwide to experiment with large neuronal models, benefiting from years of development and scientific work.

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A. Romani et al. (2023) Community-based Reconstruction and Simulation of a Full-scale Model of Region CA1 of Rat Hippocampus, bioRxiv 2023.05.17.541167

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Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.11/XX18

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

Title: Predicting physiological responses of cervical vagus nerve stimulation using realistic computational nerve model

Authors: *N. KATIC SECEROVIC[1], F. CIOTTI[2], R. JOHN[2], N. GOZZI[3], V. TOTH[5], N. JAYAPRAKASH[7], S. ZANOS[6], S. RASPOPOVIC[4];
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Abstract: The vagus nerve is one of the main components of the parasympathetic nervous system, which oversees a number of crucial body functions, including control of mood, immune response, digestion, breathing, and heart rate. It establishes one of the connections between the brain and the gastrointestinal tract. The cervical vagus nerve (cVN) contains more than 100 thousand afferent and efferent nerve fibers from almost all thoracic and abdominal organs, making it a promising target for various neuromodulatory applications. Distinct nerve fiber populations mediate both the intended effects of cervical vagus nerve stimulation (cVNS), like heart or breathing rate changes, as well as undesired events such as voice alterations, coughing, dyspnea, nausea etc. Understanding their involvement can assist in fine-tuning and monitoring VNS therapies. To make a first step in overcoming this limitation, we constructed a novel anatomically detailed and biophysically plausible computational model of the pig cervical vagus nerve. We reconstructed the nerve considering specific fascicular geometry, including three-dimensional curvatures and branching. We employed distinct models for myelinated type A and type B fibers, as well as unmyelinated type C fibers, together with their precise position, obtained by applying computer vision techniques to the experimentally collected data. Finally, we also developed a complete computational tool for cVN that enables the testing of complex stimulation paradigms and arbitrary electrode geometries. During the experimental procedure on pigs, cVN was stimulated with the multi-contact cuff electrode with different stimulation paradigms. In parallel, we recorded compound action potentials (CAP) and physiological responses - heart rate, breathing rate, and laryngeal muscle activation that arise from the cVNS. We developed our predictive algorithm by combining model outcomes that estimate the fiber activation when applying stimulation, with the CAP and observed physiological responses. As a result, a researcher can simulate the cVNS with an arbitrary paradigm and receive as output the expected physiological response. After proper calibration for a specific subject during the experimental procedure, it can be used as a noninvasive estimating tool of cVNS bodily reactions. The insights of this computational procedure can be used for optimizing the stimulation paradigms and therefore advancing future therapeutic applications of VNS.

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Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.12/XX19

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

Support: NINDS RF1NS116450

Title: Capillary transit-time heterogeneity estimation with mesoscopic imaging

Authors: *Y. SHEN[1], A. L. VAZQUEZ[2], B. IORDANOVA[3];
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Abstract: Cerebral blood flow (CBF) varies between regions and CBF change can be an early symptom and even a direct cause of brain disease. Transit-time based biomarkers can reveal regional cerebrovascular health and are accessible with clinical MRI but their interpretation on cellular and capillary level remains unclear. The use of 2-photon imaging for the measurements of mean transit time (MTT) and capillary transit-time heterogeneity (CTH) permits a microscopic assessment of capillary health, but the information is restricted to a small field of view (FOV). To address this gap, we propose to adapt existing methods and use mesoscopic, wide-field scale data to compute both MTT and CTH. We used a 1 mm cranial window for *in vivo* imaging. After immobilizing the animal, fluorescence bolus was injected via tail vein and its transit dynamics was captured by fluorescence imaging. The acquired image time-stack was used to extract a vessel map. Arteries and veins in the FOV can be separated by their peak arrival time (**Figure 1**), and their temporal intensity changes represent the arterial input function (AIF) and the venous output function (VOF). The duration between peaks of AIF and VOF is the computed MTT. Empirically, the transport function in VOF = conv (transport function, AIF) relationship can be modeled as a gamma distribution. Iteratively convolving AIF with transport functions with different parameters, we can identify the optimal transport function and CTH can be estimated from its dispersion. MTT and CTH quantify vascular dysregulation, e.g., lack of vascular reactivity and capillary stall/bleed, which can increase MTT and CTH. Applying our pipeline to 3 mice at different ages, we observed a trend where older animals tend to have longer MTT and CTH, which aligns with prior knowledge that older animals exhibit decrease in CBF. Thus, we validated the effectiveness of mesoscopic scale wide-field fluorescence imaging-based transit time measurements. Our proposed method can reveal vascular health in a larger brain region and constitute a powerful component in a multi-modal optical imaging platform.

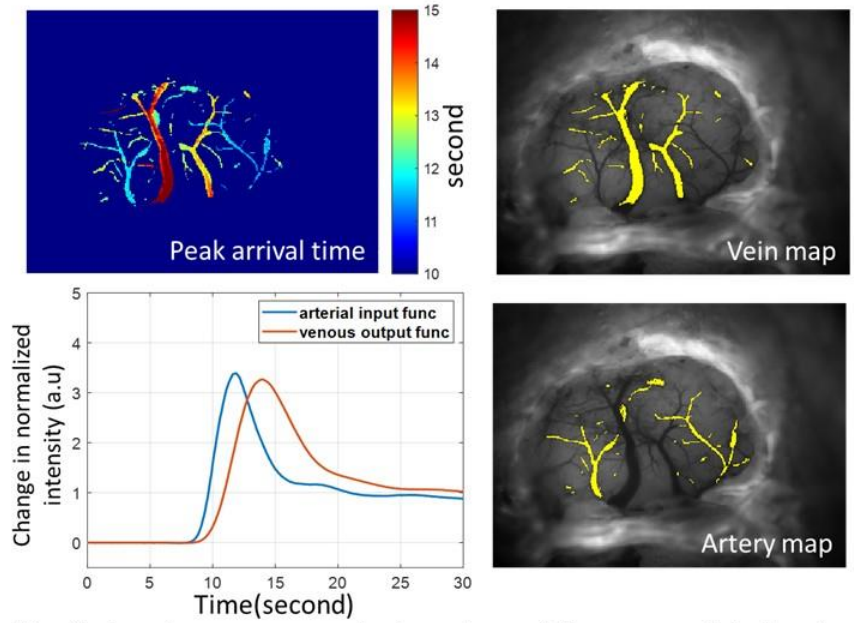

Fig 1. In vivo mesoscopic imaging of fluorescent bolus is used to map vessels and compute transit-time.

Disclosures: **Y. Shen:** None. **A.L. Vazquez:** None. **B. Iordanova:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.13/Web Only

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

Title: Molecular Dynamics Simulation and Deep Learning Analysis of Potential Ligands of G-protein Coupled Receptor 37-like 1 for the Treatment of Neuropathic Pain

Authors: *S. CHANDRA[1,2], S. BANG[2], J. XU[2], R.-R. JI[2];
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Abstract: G-protein coupled receptor 37-like 1 (GPR37L1) is highly expressed in astrocytes in the CNS and satellite glial cells in the PNS such as dorsal root ganglia. However, it remains an orphan receptor. We explore the potential of (GPR37L1 ligands as a promising avenue for mitigating neuropathic pain. Leveraging molecular dynamics (MD) simulations and Deep learning derived kdeep scoring data, we conducted a massive virtual screening including validation of docking approaches as well as shape and chemical features for hit identification and in-depth investigation into the binding dynamics and efficacy of GPR37L1 ligands. Our MD simulations, performed on a computationally generated model of GPR37L1, provided insights into the ligand-receptor interactions at an atomic level. The simulations revealed the stability and flexibility of the ligand-receptor complex, allowing for the characterization of orthosteric binding pockets and amino acid residue ASN190, ARG196, and GLU375 involved in ligand recognition and activation. Our analysis using kdeep scoring further elucidated the ligand-receptor interactions and provided quantitative estimates of binding free energies for a diverse set of GPR37L1 ligands from ten million compounds of Mcule chemical library. These computational predictions were found to be consistent with experimental data, validating the reliability and accuracy of the kdeep scoring method in evaluating ligand binding. We propose that identified Mcule compounds as GPR37L1 ligands hold great promise as potential therapeutics for neuropathic pain in animal models of neuropathic pain. As proof of concept, we found a significant reduction of neuropathic pain following intrathecal injection of our lead compound. The computational insights gained from this study offer a foundation for the rational design and optimization of novel GPR37L1 ligands with improved pharmacological properties and enhanced efficacy. This work highlights the potential of GPR37L1 ligands in addressing neuropathic pain and paves the way for future experimental studies and the development of innovative treatments targeting GPR37L1.

Disclosures: **S. Chandra:** None. **S. Bang:** None. **J. Xu:** None. **R. Ji:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.14/XX20

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

Support: Bertarelli Foundation

Title: A UNet surrogate model of peripheral nerve FEM enables automatic optimization of electrical stimulation implant geometry

Authors: *S. ROMENI[1], L. LIEBI[1], C. SABATINI[1], S. MOCCIA[2], S. MICERA[1,2];
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Abstract: Computational models can be used to optimize any aspect of neuroprosthetic devices estimating the neural response to electric stimulation by providing a digital twin of the target system. Nonetheless, though in the state of the art there are many examples of the use of hybrid models (HMs) of neuromodulation to perform qualitative comparisons of a few hypothetical stimulation setups, there is currently no way to determine the optimal geometry of a neuromodulation implant among the continuous range of values for its several defining variables. Here, we propose a surrogate model of finite element modelling (FEM) for the solution of the volume conduction problem. With the term surrogate model, we mean a system that loyally reproduces its response to perturbation without including all its details and thus that requires only a small fraction of the computational time of the original model. We employed a 3D UNet which took as input two volumes containing the topography of the nerve as binary pixels indicating the presence of fascicles in the nerve volume, and the stimulation protocol, consisting of real-valued isolated pixels representing the injected cathodic or anodic stimulation currents. The output of the network was the distribution of the electric potential in the nerve volume which in the training set is computed using FEM. We tested the effectiveness of applying logarithmic transformations to reduce the imbalance between different value ranges in our dataset, and of computing the loss between the target and predicted fields only inside fascicular structures, and found it to produce worse results than training on the original data. We show that it is possible to predict the fields caused by multipolar stimulation protocols exploiting the superposition principle, training a single UNet with monopolar stimulations and building multipolar stimulations superimposing several predicted fields with performances superior to training directly on multipolar data. We evaluate performance using a variety of metrics, like the mean absolute error, root mean squared error, Huber loss.We cascaded our surrogate model of FEM with biophysical models of nerve fibers, and surrogate models of fiber activation and show that recruitment characteristics in a complex nerve are loyally predicted. Finally, we performed a set of optimizations to maximize the selectivity of stimulation of several fiber groups assigned to different nerve fascicles by evolving using particle swarm optimization both the location and the stimulation amplitudes applied by a set of stimulating sites, showing how our work paves the way for the automatic optimization of the design of peripheral neural prostheses.

Disclosures: S. Romeni: None. **L. Liebi:** None. **C. Sabatini:** None. **S. Moccia:** None. **S. Micera:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.15/XX21

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

Support: NIH Brain Initiative
HHMI
Stanford MBCT Program

Title: Fast, convex training of recurrent neural network models constrained by large-scale neural recordings

Authors: *F. DINC[1], A. S. SHAI[1], L. STORAN[1], I. LANDAU[1], S. HAZIZA[1], S. GANGULI[1], H. TANAKA[2], M. SCHNITZER[1];
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Abstract: Recent optical technologies enable imaging and optogenetic studies of thousands of individual neurons. However, traditional methods for modeling neural population activity are poorly suited for capturing the detailed dynamics of large numbers of cells. To address this gap, recent studies focused on dynamical attractors with far fewer activity dimensions than the dimensionality of the entire observed cell population. Notably, fixed point, line and ring attractors have been identified in various brain areas and linked to specific animal behaviors. One approach to uncovering such structures involves data-constrained recurrent neural networks (dRNNs) and aims first to replicate the observed patterns of activity and then to reverse engineer the learned network to identify the underlying attractors. However, existing training algorithms for dRNNs are inefficient and have limited scalability, making it challenging to analyze large neural recordings. To address this, we created a training method termed Convex training of Recurrent Neural Networks (CoRNN). In studies of simulated recordings of hundreds of cells, CoRNN attained training speeds ~100-fold faster than prior RNN modeling approaches while maintaining or enhancing modeling accuracy. We further validated CoRNN on simulations with thousands of cells, in which the target dRNNs performed simple computations such as those of a 3-bit flip-flop or the execution of a timed response. In ~1 min or less of processing time, we used a desktop computer with one GPU to successfully train a dRNN to replicate the activities of 3,000 simulated cells performing 6,000 trials of 3-bit flip-flop task (roughly equivalent to the data volume from 30 imaging sessions, each ~1 hr in duration). Moreover, CoRNN replicated neural activity traces and captured dynamical attractors even in cases in which recordings from only 10% of the full neural population were used to train the RNN model. Further, CoRNN accurately inferred activity traces and underlying attractors for networks, even when the detailed dynamical equations governing these networks differed moderately from those used by CoRNN in the construction of its RNN models. Finally, we tested CoRNN's applicability to real neural recordings acquired by high-speed voltage or large-scale Ca^{2+} imaging. Overall, by enabling accurate training of dRNNs in ~1 min of processing time, CoRNN constitutes a powerful tool for

experimental and computational neuroscience that will facilitate targeted manipulations of single or select subsets of cells, thereby advancing the understanding of large-scale neural dynamics.

Disclosures: F. Dinc: None. **A.S. Shai:** None. **L. Storan:** None. **I. Landau:** None. **S. Haziza:** None. **S. Ganguli:** None. **H. Tanaka:** None. **M. Schnitzer:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.16/XX22

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

Support: NIH, R01 EB028350

Title: Neuroweaver: a translational platform for embedding artificial intelligent in closed-loop neuromodulation systems

Authors: *P. SARIKHANI[1], H. XU[2], S.-T. WANG[2], S. KINZER[2], H.-L. HSU[3], Y. ZHU[3], J. KRASNEY[1], J. R. MANNS[1], H. ESMAEILZADEH[2], B. MAHMOUDI[1,3];
[1]Emory Univ., Atlanta, GA; [2]Univ. of California San Diego, San Diego, CA; [3]Georgia Inst. of Technol., Atlanta, GA

Abstract: Introduction: Implantable neuromodulation devices provide a powerful paradigm in the treatment of neurological and psychiatric disorders. While relying on battery power, these implantable devices must adapt flexibly to the intricate dynamics of the nervous system. Advances in Artificial Intelligence (AI) and Reinforcement Learning (RL) have paved the way to develop intelligent closed-loop neuromodulation (iCLON) systems for adaptive and precision therapies. However, the computational complexities posed by these types of algorithms surpass the capabilities of typical embedded systems. In this research, we present Neuroweaver, a translational AI platform which aims to facilitate research and development of iCLON systems for a broader community of clinicians, neuroscientists, and engineers. Neuroweaver is an effort towards enabling end-to-end design, simulation, and implementation of embedded AI for closed-loop neuromodulation. **Methods:** To enable algorithm-hardware design space explorations, we offer a framework to explore research ideas by providing a modular experimental design including simulation environments of the brain under stimulation, libraries of RL algorithms for testing and prototyping iCLON systems, a template architecture with a highly parametric design before fabrication to match the requirements. In addition, Neuroweaver enables multi-target cross-domain exploration to adapt its workflow to different compilation targets with a familiar programming interface through a Python-embedded, cross-domain interface (CDI) eliminating the need for programmers to specify low-level execution details of their programs. **Results:** We employed mechanistic models of the brain under electrical stimulation in closed-loop with RL algorithms for testing and prototyping the design of iCLON systems. The simulations of the developed iCLON system with multiple RL algorithms was implemented using Neuroweaver

through the Python-embedded CDI with multiple execution targets, where the control policy networks were implemented on Field Programmable Gate Arrays (FPGAs) to enable prototyping hardware-implementable closed-loop control pipelines. Neuroweaver was additionally tested in an in vivo real-time experimental setting to detect the fluctuations of the hippocampal theta rhythm in behaving rats. **Conclusion:** Our results demonstrated the capabilities of Neuroweaver as an end-to-end platform for designing iCLON systems in silico, testing in vivo and embedding in hardware for clinical deployment of precision neuromodulation therapies.

Disclosures: P. Sarikhani: None. **H. Xu:** None. **S. Wang:** None. **S. Kinzer:** None. **H. Hsu:** None. **Y. Zhu:** None. **J. Krasney:** None. **J.R. Manns:** None. **H. Esmaeilzadeh:** None. **B. Mahmoudi:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.17/XX23

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

Support: Wings for Life Spinal Cord Research Foundation Project #210

Title: Mechanistic characterization of surface electromyography signal alterations after spinal cord injury: insights from computational modeling

Authors: *G. LI[1,4], G. BALBINOT[1,2], J. C. FURLAN[1,5,6,3,7], S. KALSI-RYAN[1,6,8], J. ZARIFFA[1,4,5,9];
[1]KITE Res. Inst., [2]Krembil Res. Inst., [3]Div. of Physical Med. and Rehabilitation, Toronto Rehabil. Inst., Univ. Hlth. Network, Toronto, ON, Canada; [4]Inst. of Biomed. Engin., [5]Rehabil. Sci. Inst., [6]Dept. of Medicine, Div. of Physical Med. and Rehabil., [7]Inst. of Med. Sci., [8]Dept. of Physical Therapy, [9]Edward S. Rogers Sr. Dept. of Electrical and Computer Engin., Univ. of Toronto, Toronto, ON, Canada

Abstract: Background: Spinal cord injury (SCI) can lead to substantial disability and negatively impact the quality of life and independence of individuals. Surface electromyography (SEMG) is a non-invasive yet sensitive technique to measure muscle activity and shows promise in assessing the impact of SCI. However, it is challenging to study in vivo the complex mechanisms behind SEMG signal changes due to SCI.
Objective: We aim to characterize the alterations in SEMG signals following SCI utilizing validated computational models. Additionally, we seek to identify SEMG features that are both sensitive and specific to different aspects of SCI.
Method: Starting from existing computational models for motor neuron pool organization and for motor unit action potential generation for healthy neuromuscular systems, we set up scenarios to model alterations after SCI in upper motor neurons (UMN), lower motor neurons (LMN), the number of muscle fibers within each motor unit, and the subcutaneous fat layer thickness. After

simulating SEMG signals from each scenario, we extracted a range of time and frequency domain features, including root mean square (RMS), mean absolute values (MAV), 4th order autoregressive and cepstrum coefficients, zero crossing (ZC), and slope sign changes (SSC). Subsequently, we investigated the impact of SCI disruptions on SEMG features using the Kendall rank correlation between a feature and the extent of a given disruption. The Kendall rank correlation coefficient τ ranges from -1 to 1 (strong negative to strong positive correlation).
Findings: Commonly used amplitude-based SEMG features cannot differentiate between injury scenarios. Both RMS and MAV strongly correlated with any given disruption ($\tau \geq 0.88$). However, a broader set of features offers greater specificity to different types of damage. For example, ZC and SSC responded to UMN and LMN damages ($\tau \geq 0.63$) rather than muscle fiber loss. Autoregressive and cepstrum coefficients mainly responded to the changes in LMN ($\tau \geq 0.51$ or $\tau \leq 0.60$), whose status often facilitates clinical decision-making for neuromodulation interventions. Most features, besides amplitude-based ones, were not correlated with subcutaneous fat layer thickness, indicating potential benefit in differentiating the impact of body composition on the interpretation of SEMG signal after SCI.
Conclusion: Through our novel approach, we provide mechanistic insights into the changes observed in SEMG signals after SCI. Our findings highlight the potential of SEMG in clinical applications and contribute to a deeper understanding of its utility, ultimately leading to improved patient outcomes after SCI.

Disclosures: G. Li: None. **G. Balbinot:** None. **J.C. Furlan:** None. **S. Kalsi-Ryan:** None. **J. Zariffa:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.18/XX24

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

Support: NIH Grant R21HD107511

Title: Identifying an orthogonalized list of written words for an fMRI multi-voxel pattern analysis study

Authors: *M. BROOKS[1], M. KIELY[2], T. GUNASEELAN[2], A. LYU[2], L. RODGERS[2], D. J. BOLGER[2], W. W. GRAVES[3], J. J. PURCELL[2];
[1]Human Develop. and Quantitative Methods, [2]Univ. of Maryland, College Park, MD; [3]Rutgers Univ., Newark, NJ

Abstract: Reading words is a complex cognitive feat that involves the integration of orthographic (visual word form), phonological (spoken word form), and semantic (word meaning) feature representations. Attempts to parse the neural basis of these representations have used advanced multivariate analyses such as representational similarity analysis. A limitation of

these pattern based approaches is the difficulty in determining how specific the neural representations are to the features being tested. This can be addressed by identifying stimuli that are optimally distant from each other prior to acquiring or analying the data. The objective of this study was to identify a sizable list of words that were orthogonalized for different word features (including letter, phoneme, grapheme, and semantic units) prior to fMRI data acquisition to more accurately determine the brain regions associated with these specific word features. To do this, a stochastic algorithm was generated in MATLAB to derive a random subset of 160 words from a total set of approximately 7,000 words. The letter, phoneme, grapheme, and semantic pairwise edit distances for each possible word pairing were calculated from the random subset. Then, the pairwise distances were put into a matrix, and the Spearman correlations across the dissimilarity matrices were calculated for every combination of features. This process looped through a total of 165,532 iterations, and a word list that was minimally correlated (based on a threshold of $\rho = 0.70$) on our word feature distance matrices was identified. Across the random iterations, the maximum distance matrix correlation values for similar features were highest for graphemes-phonemes ($\rho = 0.80$), graphemes-letters ($\rho = 0.71$), and phonemes-letters ($\rho = 0.628$). The maximum distances for unrelated features such as graphemes and semantic units ($\rho = 0.08$) were lowest. For our selected final list of words, the related and unrelated feature pairs were less correlated (graphemes-phonemes $\rho = 0.64$, graphemes-letters $\rho = 0.57$, phonemes-letters $\rho = 0.48$, and graphemes-semantic $\rho = -0.02$). This list of words was below our orthogonalization threshold, and will thus be sufficient to test for unique variance in neural patterns for these different features. This is the first known optimization algorithm to generate a relatively orthogonalized list of words with sufficiently reduced multi-collinearity based on pairwise distance matrices to be used in a multivariate neuroimaging study. This algorithm will contribute to the understanding of written language representations in the brain that has relevance to adult, developmental, and disordered literacy.

Disclosures: **M. Brooks:** None. **M. Kiely:** None. **T. Gunaseelan:** None. **A. Lyu:** None. **L. Rodgers:** None. **D.J. Bolger:** None. **W.W. Graves:** None. **J.J. Purcell:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.19/XX25

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

Title: Numbers representation in the brain using meg

Authors: *A. SLEMAN;
Bar-Ilan Univ., Ramat Gan, Israel

Abstract: Abstract Most people use natural numbers daily for counting, estimating quantities telling time etc. Numbers are most commonly represented switch order: using words (e.g., three), symbols, (e.g., ♥ ♥ ♥) or Roman numerical (e.g., III) or Arabic digits (e.g.,3). Many functional

neuroimaging studies have investigated the brain regions which support numerical processes, e.g., comparing quantities or performing arithmetic operations such as additions or multiplication. Yet, despite their central position in human cognitive importance, our understanding of the brain correlations of the processes involving numberers is still evolving. This dissertation utilizes the MEG (⋯..) to understand aspects of numerical representation in the brain. To this end, I have focused on the higher perceptual processes of the 'number concept in the human brain' and determined what information can be inferred from it. We tried to find the neural correlation of number shape processing. Our results shed light on higher perceptual processes in humans, and on their spatial-temporal basis. In our study we focused on brain activity while seeing a number in two different forms. In the first stage the number appears as a digit. And in the second stage, the number appears as an amount of white circles on a gray background. The results we obtained shed light on the concept of the number in the brain in particular, and on characterization of the number in particular. and on the study of the brain in general. This research is paving the way for us to study the number conceptual fallout.

Disclosures: A. Sleman: None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.20/XX26

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

Support: Azrieli Foundation
Israel Science Foundation 688/22
Binational Science Foundation 2021746
National Science Foundation 2207891

Title: Open-source architecture for high-throughput imaging with realtime processing of neural activity and behavior

Authors: *R. BARBARA, T. KAWASHIMA;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: With the advancement of optical technologies, the utilization of high-throughput cameras is becoming a standard practice in neuroscience for recording neural activity and animal behavior. Achieving high data throughput for acquiring, processing, and writing poses challenges for computer resources. Here, we constructed an open-source Python software architecture for achieving efficient camera data acquisition. We used an efficient data buffering flow and multi-threading modules to get a stable throughput of >1 GB/s. This developed framework was first applied successfully for light-sheet microscopy (PyZebraScope) to perform whole-brain neural activity imaging and voltage imaging in behaving zebrafish. This framework also enabled wide-field highspeed tracking of unconfined zebrafish behavior under diverse paradigms. Hardware-

accelerated computing further expanded the bandwidth to 10Gb/s, with realtime processing, opening paths for large-scale voltage imaging and behavioral tracking. This platform put forward an open-source, flexible, and modular architecture to lower the overhead of the usage of high-throughput and closed-loop experimental designs.

Disclosures: R. Barbara: None. **T. Kawashima:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.21/XX27

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

Support: NIH 1R01NS118442-01

Title: Structural localization is embedded in the spike trains of neurons

Authors: *G. TOLOSSA[1], A. SCHNEIDER[1], K. B. HENGEN[2];
[1]Dept. of Biol., Washington Univ. in St. Louis, St. Louis, MO; [2]Biol., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Neurons within a brain region have long been observed to exhibit correlated dynamics. Neurons from distinct regions receive diverse inputs and display different connectivity patterns. As a result, the electrophysiological features of neurons may vary across functional brain regions, potentially possessing information about their spatial location. However, the extent to which structural location can reliably be extracted from the neural code remains uncertain. Further, the degree to which the neural activity signatures emerge to be apparent at the circuit level or manifest within individual neurons is unknown. In this study, we investigated the differences in spike trains among neurons across various functional brain regions using machine learning. Using the visual coding neuropixels data from the Allen Brain Observatory, we extracted diverse spike train features such as firing rate, ISI distribution, coefficient of variation and peri-stimulus histogram. We then trained machine learning algorithms of varying complexity to classify neuronal units into their functional brain regions. Our findings demonstrate that discernible patterns exist within individual neurons, which support their localization at arbitrary levels of structural hierarchy such as functional visual cortex subareas, hippocampal subregions, and thalamic nuclei. Additionally, we explored the extent to which higher-order interactions between neurons in a circuit contribute to localization. These results suggest the presence of spatial fingerprints within neuronal spike trains, which may provide an additional level of computational advantage in the brain. Beyond an improved understanding of neuronal computation, these findings have practical implications for neuron-level brain-region localization directly from electrophysiological recording.

Disclosures: G. Tolossa: None. **A. Schneider:** None. **K.B. Hengen:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

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Program #/Poster #: PSTR243.22/Web Only

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

Support: Research Council of Norway via FRIPRO grant #324239
national infrastructure for computational science in Norway, Sigma2, via grant #NN8049K

Title: A fast and scalable solution strategy to simulate ionic electrodiffusion in cellular geometries

Authors: *P. BENEDUSI, M. E. ROGNES, A. ELLINGSRUD, H. HERLYNG;
Simula Res. Lab., Oslo, Norway

Abstract: To fully understand excitable cells, it is crucial to grasp the dynamics of ion concentrations, which are regulated by electrodiffusion and membrane ion channels. However, conventional mathematical models used to simulate excitable tissues often rely on homogenization techniques, with no distinction between intra- and extra-cellular media, and assume constant ionic concentrations. While these models offer valuable insights, they fail to account for the effects of altered ion concentrations and complex cell morphology on electrical potentials. Recently, a more detailed approach has been developed to model electrodiffusion in neural tissue, known as the KNP-EMI model [1]. This model incorporates a geometrically explicit representation of ion concentrations in both intra- and extracellular domains by combining elliptic and parabolic partial differential equations. By doing so, it provides a more accurate portrayal of ion concentration dynamics and their impact on electrical potentials. Computing KNP-EMI solutions, using the finite element method, poses significant computational challenges. As a result, its application has been restricted to fairly simple scenarios. To address this limitation, we introduce a fast and parallel specialized solution approach that significantly reduces the time to solution, surpassing the previous method by more than a factor of ten. The capabilities of this new method, based on state-of-art preconditioning techniques, are demonstrated through electrodiffusion simulations conducted in realistic 3D glial geometries, with several millions of degrees of freedom. We perform numerical investigations to evaluate the scalability, robustness, and efficiency of the proposed strategy on massively parallel machines.
The KNP-EMI model is a valuable tool to investigate and comprehend ion dynamics with remarkable geometric detail. It enables the study of ion concentrations in neuronal and glial cells by utilizing 3D murine data. The model can be used to explore phenomena like ephaptic coupling [2] and the role of potassium and calcium buffering. Furthermore, on a large spatial scale, it allows studying of dramatic ion concentration changes that are trademarks of various pathological conditions, including spreading depression and epilepsy. By employing the KNP-EMI model, researchers can gain insights into these complex processes and their implications for

cellular function and disease states.
[1]: Ellingsrud, Ada J., et al., Frontiers in Neuroinformatics 14 (2020).[2]: Rasmussen, Rune, et al., Progress in neurobiology 193 (2020).

Disclosures: P. Benedusi: None. **M.E. Rognes:** None. **A. Ellingsrud:** None. **H. Herlyng:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.23/XX28

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

Title: Development of technology for carrying out behavioral and physiological tests

Authors: *R. BELTRAN-RAMIREZ[1], J. MARTINEZ-MENDOZA[4], X. BECERRA GONZÁLEZ[1], J. DOMINGUEZ[2], C. ROMAN[3];
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Abstract: The integration of technology with neurophysiology and neurobehavioral testing has revolutionized our ability to study the brain. Advancements in brain imaging techniques, such as high-density EEG and real-time fMRI, offer unprecedented spatial and temporal resolution, enabling researchers to capture detailed neural activity in real-time. Additionally, brain-computer interfaces (BCIs) have emerged as powerful tools for decoding neural signals and enabling direct communication between the brain and external devices. For example, EEG and fMRI can aid in the diagnosis of epilepsy and help identify regions of abnormal brain activity. BCIs hold promise for restoring lost motor or sensory function in individuals with spinal cord injuries or neurodegenerative disorders.In summary, the combination of neurophysiology, neurobehavioral testing, and technology is of paramount importance in advancing our understanding of the brain. This integrated approach offers unprecedented insights into the complexities of neural processes and provides opportunities for groundbreaking discoveries in both research and clinical domains. By continuing to refine these techniques and develop new technologies, we can unlock the full potential of neuroscience and pave the way for novel therapeutic interventions and improved patient care. The present invention describes a device for performing behavioral and physiological tests that have integrated electrodes to monitor the physiological state of the animal, in addition to having a tracker that allows the displacement of said electrodes to be able to monitor the animal during its journey.

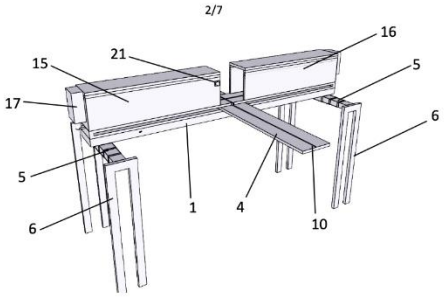

FIGURA 3

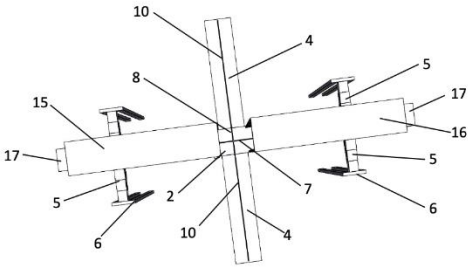

FIGURA 4

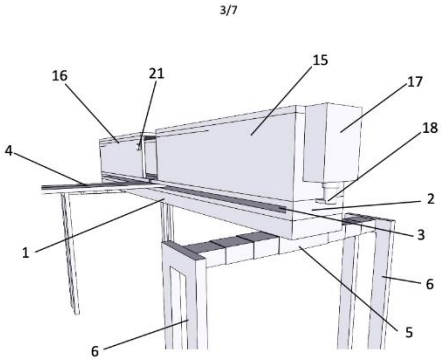

FIGURA 5

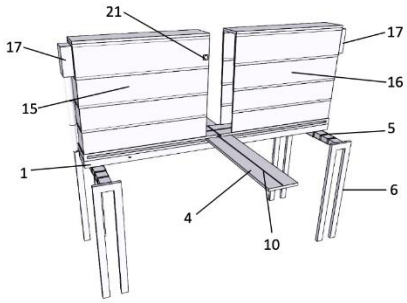

FIGURA 6

Disclosures: R. Beltran-Ramirez: None. **J. Martinez-Mendoza:** None. **X. Becerra González:** None. **J. Dominguez:** None. **C. Roman:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.24/XX29

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

Support: NIH BRAIN Initiative Grant 1U19NS107464

Title: Real time analysis and holographic spatial light modulation for informed photostimulation of neuronal cells

Authors: *A. DE ZOYSA, A. SMIRNOV, W. LOSERT;
Univ. of Maryland, College Park, MD

Abstract: Two-photon (2P) calcium imaging allows for the activity readout of large populations of neurons at single cell resolution, and holographic optogenetics makes the population

accessible for targeted stimulation. However, significant pre- and post-processing is required to interpret signals from large 2P imaging datasets, that leads to the selection of stimulation targets based only on rudimentary qualitative criteria. We have developed **NeuroART** (**Neuro**nal **A**nalysis in **R**eal **T**ime), a software platform that accesses microscope data streams to provide real-time readout of neuronal activity, downstream analysis, which enables informed real-time modulation of neuronal activity through photostimulation during image acquisition. The software package includes automatic neuronal cell identification routines (e.g., CaImAn) to facilitate real time neuronal activity analysis. Utilizing NeuroART, experimenters gain knowledge of the data quality in real time and can identify neurons of interest for targeted photostimulation based on quantitative analysis of the ΔF/F traces of each neuron. Furthermore, NeuroART employs a pairwise correlation-based analysis to construct functional networks and identify the most population correlated neurons in the field of view.The software is interfaced with a fast switching Spatial Light Modulator (SLM) that provides real-time holographic photostimulation at millisecond timescales (1-2ms to switch between photostimulation patterns). After real-time identification of neurons for photostimulation, the corresponding phase masks (generated using the Gerchberg-Saxton algorithm) are pre-loaded to the SLM (Boulder Nonlinear Systems) to enable rapid switching between intensity patterns at the objective focus through real-time modulation of the phase front of the laser beam. Furthermore, we utilize twisted light (optical vortices) as beamlets for photostimulation to demonstrate the utility of optical vortices for photostimulation of neuronal cells. We tested our real-time photostimulation protocol on rat embryonic primary neurons in-vitro which resulted in reliable responses to infrared optical vortex beams during imaging. The excitation source for 2P photostimulation is fixed at 1064 nm (Fianium amplified fiber laser, average output up to 5.25 W; pulse width, 160 fs). The downstream analysis within the real-time loop is rapid enough (at 30Hz for 250 neurons) to operate at widely used image acquisition rates and the graphical user interface (GUI) updates every second during the real-time analysis loop (works even for a laptop with Intel(R) Core i7-8565U CPU @ 1.80 GHz 1.99 GHz, 12 GB RAM, 64-bit Windows 10).

Disclosures: **A. De Zoysa:** None. **A. Smirnov:** None. **W. Losert:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.25/XX30

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

Support: NIMH grant MH108053-01

Title: Registering experimental brain image volumes to standardized rat and mouse atlases

Authors: ***N. O'CONNOR**[1], B. EASTWOOD[1], P. ANGSTMAN[1], A. D. LEDUC[1], N. D. LIESE[1], C. S. GERFEN[1], C. R. GERFEN[2], J. R. GLASER[1];
[1]MBF Biosci., Williston, VT; [2]LNP NIMH, Bethesda, MD

Abstract: Molecular neuroanatomical methods have greatly enhanced the ability to map connections of neuron subtypes in the context of behaviorally or pathologically driven patterns of neuronal activity. Analyses of neuronal connectivity, density, and activity across mouse and rat brains registered to a reference atlas reveal details about the functional organization of brain circuits related to behavior and pathologies that are comparable across animals, experiments, and laboratories.

Here we present advances in our work for automatically registering rodent brain image volumes reconstructed from serial sections, or imaged from cleared brain tissues, to the Allen Mouse Brain Common Coordinate Framework (CCF) or the Waxholm Space Rat Brain Atlas, and anatomically classifying brain-wide cellular populations and reconstructed neuronal projections and circuits.

Specifically, we have extended section registration accuracy using deep learning classifiers for greater positional accuracy within reference atlas coordinates. Additionally, registration of intact rodent brain volumes imaged by light sheet microscopy to the Allen CCF or Waxholm Space has been improved by enhancing our using iterative linear and nonlinear registration methods with fiducials.

We also continue to update our published open specifications for using digital atlases and data structures in our technologies. The atlas specification is documented and available online. Our general data format embraces FAIR data standards and is endorsed by the International Neuroinformatics Coordinating Facility.

We present whole brain analyses of region connectivity and neuronal density. For analysis, labeled neurons were automatically marked or reconstructed using a neural network, and brain volumes and neurons were registered to the Allen or Waxholm atlases. The number of labeled neurons in all brain structures were calculated, allowing for comparative quantitative analysis between animals.

Disclosures: **N. O'Connor:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A.D. LeDuc:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N.D. Liese:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.S. Gerfen:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.R. Gerfen:** None. **J.R. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.26/XX31

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

Support: NIH Project REVEAL (U54AT012307)
Bertarelli Foundation

Title: Modeling the origin of microneurographic recordings in the human vagus nerve

Authors: *C. VERARDO[1], S. ROMENI[2], V. G. MACEFIELD[3], S. MICERA[2,1];
[1]The BioRobotics Inst. and Dept. of Excellence in Robotics and AI, Scuola Superiore Sant'Anna, Pontedera, Italy; [2]Bertarelli Fndn. Chair in Translational Neural Engineering, Neuro-X Inst., EPFL, Genève, Switzerland; [3]Dept. of Neurosci., Monash Univ., Melbourne, Australia

Abstract: The vagus nerve plays a crucial role in the control of many visceral organs by conveying sensory and motor signals to and from the brain, and vagus nerve stimulation (VNS) through implanted electrodes holds the potential to treat many drug-resistant pathologies. However, the clinical efficacy of VNS protocols is limited by their limited functional selectivity and the side effects of stimulating motor axons to the larynx. The effectiveness of VNS may be improved using intraneural recordings as a direct measure of nerve activity and thus feedback signals to control the delivered stimulation. Microneurographic recordings have been recently acquired from the cervical vagus nerve of awake humans for the first time. A clear understanding of the mechanisms of generation of such signals is pivotal if we want to use them as feedback for controlling VNS.
To this aim, we propose a hybrid model of microneurographic recording. It first solves the activity of fibers in NEURON and, in turn, predicts the electrode read-out through finite-element modeling. The model is informed by recent data of vagus nerve structural and functional topography. We employ it to investigate in a controlled fashion how microneurographic recordings of vagal activity are affected by the placement and shape of the electrode, the fascicular organization of the nerve, as well as the electrogenic activity of fibers depending on their type, position, and diameter. This allows us to gain insight into the spatial reach of microneurographic recordings, namely the spatial extent of nerve activity that can be distinguished from the experimental noise floor. Our simulations suggest that the needle electrodes employed in microneurography act as highly selective spatial filters, which mainly collect the activity of single nerve fascicles. Furthermore, the contribution of unmyelinated fibers decays substantially faster in space than that of myelinated fibers, confirming the putative origin of the multi-unit activity observed in experiments.
The proposed framework can be readily adapted to model electroneurographic (ENG) recordings with implantable electrodes (e.g., transverse intrafascicular multichannel electrodes). This sets the basis for a comparison between the spatial reach of ENG and microneurography. Along with the functional topography of the vagus nerve, this paves the way to test the feasibility of using intrafascicular recordings from chronic electrodes as feedback for VNS, with the ultimate goal of human translation. Furthermore, our framework is integrated in our pipeline for peripheral nerve stimulation, enabling the development of *in-silico* models of closed-loop VNS.

Disclosures: **C. Verardo:** None. **S. Romeni:** None. **V.G. Macefield:** None. **S. Micera:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.27/XX32

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

Support: McKnight Brain Research Foundation (MBRF)
NIH Grant AG055544
NIH Grant MH109548
NIH Grant MH126236

Title: Effects of running speed and medial entorhinal cortex inactivation on theta and gamma oscillations in rat hippocampal regions

Authors: *B. ZHAO[1], Y. QIN[2], I. ZUTSHI[4], A. P. MAURER[3];
[1]Univ. of Florida Dept. of Neurosci., Gainesville, FL; [3]Evelyn F. McKnight Brain Inst., [2]Univ. of Florida, Gainesville, FL; [4]New York Univ., New York, NY

Abstract: Using data generously provided by Zutshi and colleagues (2022; PMID: 34890566), this study delves into the complex dynamics of theta and gamma oscillations within the hippocampal regions of rats, oscillations that are hypothesized to play a crucial role in cognitive processes. Previous research has established a correlation between the amplitude and frequency of these oscillations and the running speed of rats, with both parameters increasing as the rats run faster. Moreover, it has been demonstrated that suppression of the medial entorhinal cortex (mEC), a primary generator of hippocampal theta oscillations, leads to a decrease in theta and gamma rhythms, while only moderately impacting cell firing rates. Our research aimed to dissect these phenomena further, focusing on the theta and gamma oscillations within the various strata of the CA1 region across a spectrum of running speeds. We investigated their interaction with spikes from narrow interneurons and pyramidal cells, with a particular emphasis on coherence, under both control and ipsilateral mEC inactivation conditions. In accordance with prior research, our analyses revealed an increase in the frequency of gamma oscillations with higher running speeds in the control animals. Furthermore, the spikes exhibited a relatively stable cross-correlation pattern with the wideband signals. Inactivation of the ipsilateral mEC dramatically decreased the amplitude of theta and gamma oscillations and cross-frequency coupling. Moreover, while the power and frequency of theta increased with running speed during ipsilateral mEC inactivation, the rate of this increase was attenuated compared to the control condition. Contrary to some previous research suggesting that removal of the mEC results in the appearance of a “slow gamma” rhythm (e.g., Bragin et al., 1995), this result was not replicated in the present database. Overall, these findings lend more support to the energy cascade hypothesis, which posits that slower oscillations (like theta waves) modulate faster ones (like gamma waves), creating a kind of "energy cascade" crucial for various cognitive processes. This contrasts with the "routing by gamma" concept, which suggests that gamma oscillations play a primary role in information routing in the brain. This study, therefore, provides a more nuanced understanding of the interplay between running speed, brain oscillations, and the role of the mEC, furthering the body of knowledge established by previous research. It also highlights the remarkable self-organization within the CA1 network, which maintains place field activity and expresses assemblies even when the mEC and other inputs are suppressed.

Disclosures: B. Zhao: None. **Y. Qin:** None. **I. Zutshi:** None. **A.P. Maurer:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.28/XX33

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

Title: Behavioral adaptation to changing energy constraints via altered frequency of movement selection

Authors: T. DARVENIZA[1], S. ZHU[2], Z. PUJIC[5], B. SUN[6], M. LEVENDOSKY[7], R. WONG[3], R. AGARWAL[8], M. MCCULLOUGH[9], ***G. GOODHILL**[4];
[1]Electrical and Systems Engin., Washington Univ. in St. Louis, St Louis, MO; [2]Washington Univ. in St. Louis, Washington Univ. in St. Louis, Stuart, FL; [3]4949 W Pine Blvd, Apt 3M, Washington Univ. in St. Louis, Saint Louis, MO; [4]Washington Univ. in St. Louis, St. Louis, MO; [5]Queensland Brain Inst., Queensland Brain Inst., Brisbane, Australia; [6]Univ. of Queensland, Brisbane, Australia; [7]Washington Univ. Sch. of Med., Washington Univ. Sch. of Med., Saint Louis, MO; [8]Washington Univ. in St Louis, St Louis, MO; [9]Australian Natl. Univ., Canberra, Australia

Abstract: Animal behavior is strongly constrained by energy consumption. A natural manipulation which provides insight into this constraint is development, where an animal must adapt its movement to a changing energy landscape as its body grows. Unlike many other animals, for fish it is relatively easy to estimate the energy consumed by their movements via fluid mechanics. Here we simulated the fluid mechanics of > 100,000 experimentally-recorded movement bouts from larval zebrafish at 3 different ages and 2 different fluid conditions as they hunted paramecia. We find that these fish adapt to their changing relationship with the fluid environment as they grow by adjusting the frequency with which they select different types of movements, so that more expensive movements are chosen less often. This strategy was preserved when fish were raised in an unnaturally viscous environment. This work suggests a general principle by which animals could minimize energy consumption in the face of changing energy costs over development.

Disclosures: T. Darveniza: None. **S. Zhu:** None. **Z. Pujic:** None. **B. Sun:** None. **M. Levendosky:** None. **R. Wong:** None. **R. Agarwal:** None. **M. McCullough:** None. **G. Goodhill:** None.

Poster

PSTR243. Computational Neuroscience: Experiment

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR243.29/XX34

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

Support: NSF Graduate Research Fellowship

Title: Comparison of cross-species functional networks targeted via Transcranial Magnetic Stimulation (TMS)

Authors: *T. BERGER[1], T. XU[2], A. OPITZ[1];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Ctr. for the Developing Brain, Child Mind Inst., New York, NY

Abstract: Transcranial Magnetic Stimulation (TMS) is a non-invasive neuromodulation technique to alter neural activity. Due to its well-established safety profile and ability to target specific brain networks, TMS is used in a wide range of clinical applications. For example, TMS is used in the treatment of depression by targeting prefrontal brain networks and their connection with deeper brain areas. However, despite its widespread use, the underlying mechanisms are not fully understood. Non-human primates (NHPs) offer an ideal model to study TMS mechanisms through invasive electrophysiological recordings. As such, bridging the gap between NHP experiments and human applications is imperative. In this study, we aim to establish a cross-species comparison framework that matches TMS stimulation parameters and target regions between humans and NHPs. This work would enable functional networks targeted by TMS to be translated across species, regardless of the anatomical divergence. To accomplish this goal, we have developed an integrated pipeline that couples cross-species anatomical alignment with resting-state functional magnetic resonance imaging (fMRI) data to compare the functional networks targeted via TMS at a given coil location and orientation. Using data from the Human Connectome Project and PRIMatE-Data Exchange, we developed realistic finite element head models and conducted a comparative investigation of targeting the dorsolateral prefrontal cortex with TMS in macaques and humans. First, we used a 5x5 TMS stimulation grid on the macaque and human head models and simulated TMS-induced electric fields. We then combined the electric field simulations with resting-state fMRI data to categorize network activation across species using the Yeo7 brain network map. We found distinct stimulation zones in both macaque and human models that differed as to which the networks were affected (macaque: frontoparietal, somatomotor, dorsal attention network; human: frontoparietal, dorsal attention) and indicated a sensitivity to coil orientation changes. Our method provides important insight into NHP and human TMS applications by allowing researchers to establish consistency in network activation across species. This will facilitate translational efforts across species crucial for developing improved TMS protocols for clinical use.

Disclosures: T. Berger: None. **T. Xu:** None. **A. Opitz:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.01/XX35

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

Title: Dissecting heterogeneity into classes and continua in scRNA-seq data

Authors: *Y. MARGHI, U. SÜMBÜL;
Allen Inst., Seattle, WA

Abstract: The brain comprises an extensive and diverse group of cell types, each with distinct molecular andfunctional characteristics. Cellular diversity can be represented as a combination of discreteand continuous factors of variability. While grouping cells into types has been accomplishedusing a variety of features, including transcriptomic profiles, arbor morphology, firing patterns,and synaptic connectivity, a complicated continuum of variation still exists within individualgroups in all cases. Understanding the transcriptional basis of cellular heterogeneity is key tounraveling complex biological processes and diseases. For instance, in neurodegenerativedisorders such as Alzheimer's disease, the brain cells that are affected by the disease aretranscriptomically heterogeneous, which makes it difficult to develop an effective treatment.Single-cell RNA-seq (scRNA-seq) technologies provide a powerful tool to study and dissectcellular diversity through their transcriptome. Nonetheless, high-dimensionality of scRNA-seqdata, the large number of expressed genes per cell, and the variability of gene expression levelsacross cells of the same type pose challenges, making it difficult to dissect cellular heterogeneityinto discrete and continuous factors of variability. To address these challenges, we propose a novelunsupervised mixture variational inference method tailored to representing cellular diversity intoclasses and continua in single-cell data, for both mouse and human brains, with a focus onAlzheimer's disease progression. Our method captures the complexity of cellular heterogeneity byinferring discrete (cell type) and continuous (cell state) factors, enabling the identification of keygenes and cellular changes associated with disease progression, and providing insights into brain’scellular landscape.

Disclosures: Y. Marghi: None. **U. Sümbül:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.02/XX36

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

Support: NIMH grant R01-MH087755

Title: Hybrid learning architectures to predict the local field potential

Authors: *D. DOPP;
Univ. of Missouri, Columbia, MO

Abstract: Hybrid learning architectures to predict the local field potential

Daniel Dopp, Vladimir Omelyusik, Ziao Chen , Satish S Nair[1]University Missouri-Columbia, Columbia, MO

Temporal synchronization of the spiking of pyramidal cells is reflected in the local field potential (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. To support such closed-loop approaches, the present study explores the inherent constraints on the estimation problem and then considers the suitability of statistical learning architectures to predict future values of the LFP. 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.As a first step, we used LFP from a physiologically-based computational model of a rodent amygdala that matched the in vivo LFP characteristics, including transient gamma bursts which occur over brief durations of 3-4 cycles. We then compared various types of architectures to predict future model LFP values, using both filtered and raw versions. In comparison to the filtered LFP, accuracy of raw LFP prediction using simple architectures was limited to the 1 to 5 ms horizon. Adding the average firing rates of interneurons increased the prediction horizon. The present effort is to determine constraints placed by noise statistics and signal-to-noise ratio for this class of problems. In parallel, we are exploring hybrid machine learning architectures using both in silico and in vivo LFP data.

Disclosures: D. Dopp: None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.03/XX37

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

Support: NSERC RGPIN - 2020-06757

Title: Intersession reliability of fast motor mapping using transcranial magnetic stimulation

Authors: *S. FOGLIA[1], F. ADAMS[1], C. DRAPEAU[1], C. TURCO[2], A. NELSON[1];
[1]McMaster Univ., Hamilton, ON, Canada; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Transcranial magnetic stimulation (TMS) is a safe and non-invasive method to investigate somatotopic organization of human motor cortex (M1). Pairing the location of multiple TMS pulses delivered to areas including and surrounding M1 with the resulting motor evoked potential can be used to create a 2-dimensional contour plot or “motor map” of the targeted muscle. Motor maps have both clinical and scientific utility as a preoperative planning

tool for tumor resection, functional assessment tool following neurological injury, and a measure for examining mechanisms underlying interventional plasticity. However, traditional motor mapping takes upwards of 30 minutes to complete which reduces its utility in time sensitive protocols measuring corticospinal excitability and motor learning where changes are rapidly evolving. Frameless stereoscopy guided TMS combined with the pseudorandom walk approach can address this issue by reducing map acquisition time to ~3 minutes. This approach, referred to as 'fast motor mapping' is comparable to traditional motor mapping and is reliable within a session when performed at 120% resting motor threshold (RMT). However, the ability for this measure to reliability track changes during and following multi-session interventions has yet to be explored. The goal of the research was to investigate the between session reliability of fast motor maps using both relative and absolute reliability statistics. Intersession reliability was assessed in 32 healthy right-handed male participants at two time points separated by 18 ±11 days. Maps were acquired by delivering pulses at 120% RMT to a 6x6cm grid centred over the motor hotspot for the right first dorsal interossei muscle. Relative reliability was assessed using the intraclass correlation coefficient (ICC). Absolute reliability was measured using standard error of measurement (SEMeas) which was expressed as a percentage of the mean to provide a unitless assessment of the measurement error. Map parameters consisting of the center of gravity (CoG), area, and volume were assessed. CoG in the X (ICC= 0.73; 95% CI [0.45 - 0.87]) and Y (ICC= 0.93; 95% CI [0.85 - 0.97]) coordinate planes demonstrated moderate and excellent reliability with a %SEMeas of 1.75 and 2.62, respectively. Map area (ICC= 0.17; 95% CI [-0.71 - 0.6]) and volume (ICC= 0.46; 95% CI [0.11 - 0.74]) demonstrated poor reliability with %SEMeas of 27.03 and 11.66, respectively. These results demonstrate that the measure of CoG is a reliable intersession metric of fast motor mapping.

Disclosures: S. Foglia: None. **F. Adams:** None. **C. Drapeau:** None. **C. Turco:** None. **A. Nelson:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.04/XX38

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

Support: BMBF Grant 01ZZ2016

Title: Multi-scale digital twin simulations for therapy design and clinical decision support of cervical spinal neurostimulation

Authors: *A. ALASHQAR[1], J. GARCIA ORDONEZ[2], T. NEWTON[3], S.-R. KOH[4], S. DIAZ-PIER[5], K. AMUNTS[6,7], E. NEUFELD[3], N. KUSTER[3,8], A. ROWALD[1];
[1]Friedrich Alexander Univ. Erlangen, Erlangen Nuremberg, Germany; [2]ZMT Zurich MedTech, Zürich, Switzerland; [3]Fndn. for Res. on Information Technologies in Society (IT'IS), Zurich, Switzerland; [4]Simulation and Data Lab. Highly Scalable Fluids and Solids Engineering, Jülich

Supercomputing Ctr., [5]Simulation and Data Lab. Neuroscience, Jülich Supercomputing Ctr. (JSC), Inst. for Advanced Sim, Forschungszentrum Jülich GmbH (Jülich Res. Centre), Jülich, Germany; [6]Inst. of Neurosci. and Medicine, INM-1, Forschungszentrum Jülich GmbH (Jülich Res. Center), Jülich, Germany; [7]C. & O. Vogt-Institute of Brain Res., Heinrich Heine Univ. Düsseldorf, Düsseldorf, Germany; [8]Dept. of Information Technol. and Electrical Engin., Swiss Federal Inst. of Technol. (ETH), Zurich, Switzerland

Abstract: Electrical stimulation of the cervical spinal cord has shown the potential to support upper-limb motor function in paralyzed individuals. Robust clinical translation of cervical spinal neurostimulation technologies is hindered by a lack of tools for the efficient and consistent identification of effective and safe stimulation parameters in large and diverse patient cohorts. Here, we present a computational framework for the comprehensive exploration, optimization, and personalization of invasive and non-invasive cervical spinal neurostimulation technologies. Our modular framework combines medical image processing technologies, adjustable expert-based models, 3D modeling, multi-physics and physiological simulations of electromagnetic exposure, and neural responses to rapidly generate multi-scale digital twins of electrical stimulation in the entire cervicothoracic body. Our multi-scale digital twins accurately approximate the pathophysiology and biophysics of cervical spinal neurostimulation from the (sub-)cellular to the macroanatomical scale. We further introduce novel and robust algorithmic optimization techniques to efficiently identify effective, safe, and clinically relevant neurostimulation parameters that are translatable to other neurostimulation technologies. We have deployed our framework on a high-performance computing cluster and a cloud-based scalable infrastructure to perform large-scale parameter explorations and optimization of spinal neurostimulation strategies. We outline guidelines for the design of cervical spinal neurostimulation technologies that selectively target propriospinal pathways while minimizing safety concerns, including unwanted co-activation of neural structures controlling diverse physiological functions. Our framework can be easily deployed locally within clinics to support clinical decision making, given the substantial inter-subject variabilities in anatomy and pathophysiology and associated patient-specific outcomes.

Disclosures: A. Alashqar: None. **J. Garcia Ordonez:** None. **T. Newton:** None. **S. Koh:** None. **S. Diaz-Pier:** None. **K. Amunts:** None. **E. Neufeld:** None. **N. Kuster:** None. **A. Rowald:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.05/XX39

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

Title: Generalized Contrastive PCA (gcPCA): a generalized framework for finding low-dimensional subspaces that differ between experimental conditions

Authors: *E. F. OLIVEIRA[1], P. GARG[3], L. L. SJULSON[2];
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Abstract: Dimensionality reduction methods are used widely across neuroscience. Their applications range from data visualization to interpretation of high-dimensional data. However, these methods typically explain the low-dimensional structure within a single dataset. In many cases, the experimenter is interested in finding the differences between two conditions, such as stimulus vs. control or drug vs. saline. Contrastive PCA (cPCA) was developed as a tool for this purpose, but it depends critically on computationally-expensive optimization of an opaque free parameter, of which it is not always possible to determine the "correct" value.
Here we intuitively explain the purpose of this parameter and propose Generalized Contrastive PCA (gcPCA), a framework that achieves optimal performance without it. gcPCA comprises three distinct algorithms that are appropriate for different use cases, and we demonstrate their utility in different neuroscientific datasets.

Disclosures: E.F. Oliveira: None. **P. Garg:** None. **L.L. Sjulson:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.06/XX40

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

Support: McNair Foundation

Title: Emulator: rapid estimation of electric fields induced during electromagnetic stimulation using data-driven models

Authors: *F. AHSAN[1], L. LUZI[2], R. BARAINUK[2], S. SHETH[3], W. GOODMAN[4], B. AAZHANG[1];
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Abstract: A common factor across electromagnetic methodologies of brain stimulation (such as deep brain, transcranial electrical and magnetic, and EMvelop stimulation) is the optimization of essential dosimetry parameters, like amplitude, phase, and the location of one or more transducers, which controls the strength and targeting precision of stimulation. Since obtaining in-vivo measurements for the electric field distribution inside the biological tissue is challenging, physics-based solvers are used. However, these physics-based solvers are computationally expensive and time-consuming, making computing the electric field repeatedly for optimization purposes computationally prohibitive. Here we investigate if we can calculate the electric field at any location of the transducers from data-driven models rather than repeatedly running the

computationally expensive physics-based solvers. We propose our methodology from the perspective of EMvelop stimulation. However, it is generalizable across other stimulation modalities. We trained two deep neural networks (DNNs), namely convolutional autoencoder (convAE) and U-net, by providing the electric field generated by transducers placed at thirty-eight specified locations on the brain tissue. We then compared the electric field prediction accuracy of the two DNNs at twenty randomly generated, non-trained locations inside the brain tissue. Finally, we compared the mean square error (MSE) value and the computation time against the ground truth electric field calculated by the state-of-the-art physics-based software COMSOL. As a result of our training, we found that U-net significantly outperforms convAE in terms of MSE with the ground truth. After 3000 training epochs, the convAE has a MSE of 898 V/m, whereas we get a MSE of 1V/m from the ground truth with the help of U-net DNN. With U-net, we could calculate the electric field at any place inside the brain tissue with a mean time of 56ms for a batch of one potential transducer location and a mean time of 9.7ms for a batch of 128 potential transducer locations. This is up to 100 times smaller than the time required by COMSOL. Our results show that DNNs can be a powerful tool to compute the electric field induced by the transducers at new locations. The significance of this work is that it shows the possibility of real-time calculation of the electric field from a set of coordinates for the transducer, making it possible to optimize the amplitude, phase, and location of several different transducers with a complete end-to-end continuous function like stochastic gradient descent.

Disclosures: **F. Ahsan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **L. Luzi:** None. **R. Barainuk:** None. **S. Sheth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent. **W. Goodman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent. **B. Aazhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.07/XX41

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

Support: U19NS104649
RF1 MH114276-01
R01 NS063226-08
22CVD0
542991
R35GM131765

Title: Model-based and empirical analysis pipelines to quantify state-dependent dynamic neural representations of spontaneous behaviors in awake mice

Authors: *K. UGORJI[1], W. XU[1], S. SHAHSAVARANI[2], A. J. YAGIELSKI[2], K. CHEN[3], E. A. MALAN[1], T. PEREZ[1], E. M. HILLMAN[2];
[2]Mortimer B. Zuckerman Mind Brain Behavior Inst., [3]Dept. of Anesthesiol., [1]Columbia Univ., New York, NY

Abstract: Emerging techniques capable of large-scale, real-time recording of brain activity in awake mice have opened up the ability to investigate dynamic neuronal representations of spontaneous behavior. Traditional stimulus or task-based studies typically average together repeated trials to establish the statistical significance of signals in specific neurons or brain regions. However, alternative approaches are needed to enable quantitative analysis of real-time data, and to quantify the way in which neuronal representations of spontaneous behavior change under different conditions.
Leveraging wide-field optical mapping (WFOM) data, which enables the simultaneous recording of behavior and pan-cortical neuronal signals in head-fixed, spontaneously behaving mice, we compare two different approaches to real-time neuronal and behavioral analysis, exploring their ability to detect differences in neuronal representations of behavior in different brain states induced by drugs and disease states, specifically during acute alcohol administration, and in a progressive Alzheimer's disease model.
The first approach uses convolutional neural networks to establish a predictive model of behaviors such as locomotion, grooming, whisking and pupil diameter from brain-wide neuronal recordings. By generating models that decode such behavior parameters using neural data collected under different conditions, we can compare how the accuracy of prediction of each model is altered by state. The second, more empirical approach uses event-triggered averaging to establish motifs of activity patterns across the cortex in response to behaviors such as locomotion onset. Although the former approach can be more generalized to diverse behaviors, we find that the latter approach permits more specific analysis of different aspects of spontaneous behavior, and the way in which perturbations disrupt the relationship between neural representations and behavior. Both methods improve our ability to explore brain region-specific relationships between cortical neural activity and behavior and the way in which both activity and behavior are perturbed in different brain states.

Disclosures: K. Ugorji: None. **W. Xu:** None. **S. Shahsavarani:** None. **A.J. Yagielski:** None. **K. Chen:** None. **E.A. Malan:** None. **T. Perez:** None. **E.M. Hillman:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.08/XX42

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

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

Title: Biophysics-based Frequency Domain Pattern Separation of Local Field Potential Signals

Authors: *W. CHEN, A. SIROTA;
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Abstract: Local field potentials (LFP) are generated mainly by synchronous synaptic currents of local and upstream neuronal populations onto local neurons and provide an insight into mean-field network dynamics of these populations. Unmixing distinct sources of the LFP requires multichannel extracellular recording along the somato-dendritic axis of the local layer-arranged populations. But, it is challenging due to various factors, such as multiple inputs sharing correlated dynamics, nonlinear and non-instantaneous mixing of inputs via dendrites, volume conduction, and bias towards slower frequencies due to the colored spectrum of the LFP. To overcome these limitations, we incorporate the biophysical prior knowledge into the blind source separation approach to disentangle the synaptic sources. First, we derive analytical Green's function for realistic hippocampal neurons based on their detailed 3D reconstructions and use it to efficiently generate synthetic LFP signals and infer parameters of neuronal location and geometry from synthetic LFP data. Second, we use a mixture model of Green's functions to guide a frequency-domain independent component analysis for unmixing the sources from synthetically generated or extracellular multichannel LFP recordings. Validation on synthetic data proved that the developed method accurately recovers the true synaptic inputs, even under high input coherence regimes, in contrast to conventional ICA. We tested the performance of the method on the laminar hippocampal and entorhinal recordings, revealing multiple synaptic sources with distinct oscillatory modes and presumed cellular substrates consistent with the entorhino-hippocampal anatomy. Our biophysically-grounded model of the LFP captures the main aspects of dendritic processing and electrical field volume conduction, reducing the cross-contamination and improving the interpretability of the ICA results.

Disclosures: W. Chen: A. Employment/Salary (full or part-time):; Ludwig-Maximilians-Universität München. 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.; European Union's Horizon 2020 research and innovation program under Grant Agreement No. 732032 (BrainCom). **A. Sirota:** A. Employment/Salary (full or part-time):; Ludwig-Maximilians-Universität München. 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.; European Union's Horizon 2020 research and innovation program under Grant Agreement No. 732032 (BrainCom).

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.09/XX43

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

Title: Random variations in spike clustering account for up to 25% of disagreement when spike sorting human intracranial microwire recordings

Authors: *P. N. STEINMETZ;
NeurTex Brain Res. Inst., Dallas, TX

Abstract: Prior work has shown that the four spike sorting techniques commonly used to identify single neuron activity in human intracranial recordings have relatively low agreement with one another (0.3 on a 0-1 scale). Furthermore, this low level of agreement results in differing estimates of the fractions of neurons in a population which respond to experimental stimuli. This low level of agreement may be caused by a number of factors: low levels of signal to noise in the clinical recording environment, differences between techniques such as the method of event detection, thresholds used, or methods of clustering waveforms based on similar waveform shapes. This latter step normally uses a pseudo-random number generator (PRNG) to assign event waveforms to initial clusters and to divide up potential clusters.
To understand the impact of the PRNG on spike sorting, 64 channels of actual recordings from two sites, Barrow Neurological Institute (BNI) and UCLA, and 200 simulated recordings were repeatedly sorted. Two commonly used spike sorting techniques, BML and WaveClus (WC), were used. Each iteration used different seeds for the PRNG. Agreement between the sorting outputs was measured using the AMI_{match} measure which examines differences between the clustering output only on a 0-1 scale.
For data recorded at the BNI, mean AMI_{match} was 0.83 for BML and 0.87 for WC. The standard deviations (SD) were 0.33 and 0.25. For data recorded at UCLA, means were 0.81 and 0.83; SDs were 0.31 and 0.21. For simulated data with the same power spectrum for noise and background firing activity as typical recordings, mean AMI_{match} was 1.0 for BML and .81 for WC. SDs were 1.1×10^{-13} and 0.36.
These results show that for actual recordings, the variations in output produced by random number generation during spike clustering account for up to 25% = (1-0.82)/(1-0.3) of the decrease from perfect agreement in many cases. The differences between BML and WaveClus methods on simulated data show that the optimal spike sorting method likely depends on the specific power spectrum of the recordings and thus the site of recording. These results also provide a limit to the precision which should be assigned to the output of spike sorting techniques in these types of recordings.

Disclosures: P.N. Steinmetz: None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.10/XX44

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

Support: R01AG064066
R01AG064066-S1

Title: Tracking Neurons Across Intermittent Multielectrode Recordings

Authors: *O. SHETLER[1], A. AOUN[2], S. A. HUSSAINI[3];
[2]Columbia Univ., [1]Columbia Univ., New York, NY; [3]Taub Institute; Dept. of Pathology and Cell Biol., Columbia Univ. Med. Ctr., NEW YORK, NY

Abstract: Longitudinal studies of neural activity are crucial for understanding changes in neural representations and dynamics over time. Numerous experimental frameworks necessitate that each subject take part in multiple recording sessions, which can be spaced out by intervals ranging from several hours to several days. Tracking neurons across multiple electrophysiology recording sessions is a critical component of these studies, enabling researchers to investigate neuronal stability, functionality, and plasticity. While neuronal tracking is well studied in calcium imaging, methods for tracking neurons with single-unit extracellular recording electrodes have not been systematized. Moreover, even less work has been done on neuron tracking across intermittent electrophysiology recordings. A typical paper that tracks neurons across intermittent recording sessions will dedicate as little as a single sentence to to the technique. For example, "To follow cells across recording sessions, clusters of successive sessions were compared and identified to be the same unit if the locations of the spike clusters in the multidimensional parameter space were stable" (Nagelhus et al., 2023). Despite this lack of care, neuron tracking has been necessary for a wide variety of study designs dating back over thirty years. Moreover, mis-matched spike trains can result in false inferences which could call into question many studies that use simplistic and subjective neuron tracking techniques. This risk is especially pronounced when smaller ensembles, such as those collected from tetrodes recordings, are used. Our paper aims to fill the knowledge gap on best neuron tracking practices for electrode recordings. We discuss the major neuron tracking steps that should be taken, but seldom are, such as cluster trimming, feature projection and comparison graph trimming. We show how robust cluster template extraction, outlier trimming, bootstrapping and non-linear feature projections can drastically improve cluster separation without the need to drop inconveniently similar units (as is common practice). We develop a taxonomy of automated unit matching algorithms and explore best practices for their use guided by performance on simulated and in vivo ground truth data sets. Moreover, we demonstrate how to perform these steps using a Python package we developed for this purpose.

Disclosures: O. Shetler: None. **A. Aoun:** None. **S.A. Hussaini:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.11/XX45

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

Title: Linear discriminant analysis in machine learning with application to macro face feature discrimination

Authors: *H. YUAN[1], M. CHAO[2];
[1]Independent Lab., San Marino, CA; [2]Independent Lab., Palos Verdes Estates, CA

Abstract: This poster investigates the use of linear discriminant analysis in Machine Learning, and using the Olivetti face database as an example of how this can be applied to macro face feature discrimination for glasses, facial hair (beard and/or moustache), and gender. Macro face feature (as glasses, facial hair) discrimination can be used in preliminary screening for reasons of safety or gender specificity for facility entrance. Though not seemingly as structured as a macro face feature as are glasses, estimating the age of a person through visual facial observation is cited in the journal Cognitive Research - Principles and Implications (February 2022). The study found that the ability to accurately estimate the age of subjects wearing sunglasses or surgical-style face masks is no less accurate than when the subject is wearing sunglasses. However, it was found that the age is overestimated when the subject is wearing a face mask. In contrast to estimating the age of a person, a machine learning approach to discriminating if a person is wearing glasses might be prudent in situations prior to entry into secure areas or laboratories that need eye protection from exposure to chemicals or laser light.
Classic techniques for face recognition in machine learning can be an approach to discriminate faces belonging to a group or class of faces with a particular macro face feature such as glasses or facial hair and not necessarily to identify a face to a specific individual person. The classic technique would assume Gaussian statistics and estimate the class probability densities to assign the face with a specific macro face feature to the macro face feature class with the highest posterior probability. Linear discriminant analysis can be used to maximize the separation between multiple macro face feature classes and minimize the variance within a macro face feature class. Linear discriminant analysis assumes all macro face feature classes are linearly separable, computes hyperplanes in the macro face feature space to discriminate among the macro face feature classes. The classifier performance for macro face feature discrimination is counted as good if a test face with a certain macro face feature is correctly classified. Macro face feature discrimination is investigated in this poster using the Olivetti face dataset and linear discriminant analysis as a classifier trained on groups of faces with certain macro face features such as glasses or facial hair to discriminate as face into a macro face feature group or class. Macro face feature discrimination performance is computed for single-look presentation of a test face to the classifier.

Disclosures: H. Yuan: None. **M. Chao:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.12/XX46

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

Support: scientific research on innovative areas Hyper-adaptability for Overcoming Body-brain Dysfunction 19H05728
scientific research on innovative areas Hyper-adaptability for Overcoming Body-brain Dysfunction 19H05724
scientific research on innovative areas Hyper-adaptability for Overcoming Body-brain Dysfunction 22H04769

Title: Upper Limb Musculoskeletal Model of Macaque Monkey for Approaching Adaptation Mechanism to Tendon Transfer

Authors: *N. NAKAJIMA[1], S. WANG[1], N. OGIHARA[2], T. OYA[3], K. SEKI[4], T. FUNATO[1]; [1]Dept. of Mechanical and Intelligent Systems Engin., Univ. of Electro-Communications, Tokyo, Japan; [2]The Univ. of Tokyo, Tokyo, Japan; [3]Dept. of Neurophysiol., Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan; [4]Natl.inst.Neurosci., Tokyo, Japan

Abstract: After tendon transfer surgery, patients gradually recover to use the transferred muscles, but the adaptation mechanism is still unclear. To approach this mechanism, it is useful to conduct an experimental approach using animal model with tendon transfer surgery, and a constructivist approach using the animal's musculoskeletal model with adaptive control to new musculoskeletal profiles in parallel. In this study, for the aim of constructing a platform to investigate adaptive control before and after tendon transfer, we constructed a monkey musculoskeletal model on MuJoCo software. The constructed musculoskeletal model is the right upper limb model of a macaque monkey, which has six degrees of freedoms (DOFs) in total: three DOFs for the shoulder joint between the scapula and humerus, one DOF each for elbow flexion, forearm internal rotation, and wrist flexion. The musculoskeletal model also has 29 muscles based on the arrangement of the muscles of the macaque monkey. To evaluate the correctness of the musculoskeletal model, we estimated torques and muscle activities from a monkey's motion to pick up foods, and compared the muscle activities between the estimated muscle activities and measured muscle activities of the monkey. Torques are estimated using Inverse dynamics function of the MuJoCo software. Muscle activities are estimated using custom-made static optimization program, in which the sum of the cubic activities of each muscle is minimized under the constraint that the sum of joint torques and torques due to muscle activities is balanced. The measured muscle activity consisted of the activities around one second of extending the arm to the food and the activities after three seconds of bringing the hand to the mouth, totaling approximately 5.5 seconds of activities. These activities were well-reproduced in the estimated muscle activities using the constructed musculoskeletal model.

Disclosures: **N. Nakajima:** None. **S. Wang:** None. **N. Ogihara:** None. **T. Oya:** None. **K. Seki:** None. **T. Funato:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

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Program #/Poster #: PSTR244.13/XX47

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

Support: NIH Grant 5P50DA044121
F31NS126012
ERC - PAINSTRAT

Title: Novel task-fMRI analyses reveal whole-brain multivoxel neural assemblies

Authors: *R. JABAKHANJI[1], A. D. VIGOTSKY[1], L. HUANG[1], G. IANNETTI[2,3], J. I. GLASER[1], A. APKARIAN[1];
[1]Northwestern Univ., Chicago, IL; [2]Italian Inst. of Technol., Rome, Italy; [3]UCL, London, United Kingdom

Abstract: fMRI research has uncovered brain-wide task-specific information that may be tied to perception. There is still much to learn about the characteristics and functions of this information, especially in brain regions previously thought to contain only noise. We propose multivoxel dimensionality reduction methods to study brain-wide neural activity and glean the task-encoding properties and dynamics underlying perception.

To illustrate, we analyzed data from Gonzales-Castillo et al., where subjects were scanned 100 times performing the same visuomotor task. It shows that the number of statistically significant task-related voxels increases with the number of averaged scans, ultimately including the entire brain at 100 averaged scans. Instead of a standard general linear model, we performed whole-brain principal components analysis on the BOLD activity averaged across scans. The explained variance of the 100 scan average using PCA can be captured with a moderate number of scans and relatively few components (>75%; 20 scans, 10 components). Task-specific information is captured by the principal components, e.g., the first component captures the temporal pattern of the task block design and includes both the primary visual cortex and sensorimotor cortex, consistent with the task design.

Standard PCA generates orthogonal components that maximize the variance explained. There is no a priori reason that brain activity spatiotemporally would obey this rule. So, we performed rotations on the first 10 components to make them sparser in time or space. Spatial sparsity distinguishes between visual and somatosensory activity, including regions involved at the start/end of tasks. Temporal sparsity separates the start/end components into two, pointing to distinct brain regions engaged at the start and end.

We quantified the extent of information integration across the brain. We obtained each component's contribution to the original BOLD timeseries at each voxel. We then calculated the geometric mean of these coefficients and used it as a metric "integration" - if more components had a high coefficient at a particular voxel, we have high integration. Visual and somatosensory cortices exhibited the lowest integration (specialized areas), while more anterior brain regions showed localized clusters of high integration.

We leveraged an information-rich dataset to develop and apply multivariate methods to study task-fMRI from a whole-brain perspective. By applying dimensionality reduction to task-fMRI data, we can estimate how regions across the brain work together, from specialized local processing to rich integration, to form multivoxel neural assemblies.

Disclosures: **R. Jabakhanji:** None. **A.D. Vigotsky:** None. **L. Huang:** None. **G. Iannetti:** None. **J.I. Glaser:** None. **A. Apkarian:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

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

Support: NSF DBI 2015317 as part of the NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program

Title: A comparison of absolute and relative neural encoding schemes in arithmetic functional subnetworks

Authors: *C. SCHARZENBERGER-BRAET, A. J. HUNT;
Portland State Univ., Portland, OR

Abstract: As neural networks have become increasingly prolific solutions to modern problems in science and engineering, there has been a congruent rise in the popularity of the numerical machine learning techniques used to design them. While numerical methods are highly generalizable, they also tend to produce unintuitive networks with inscrutable behavior. One solution to the problem of network interpretability is to use analytical design techniques, but these methods are relatively underdeveloped compared to their numerical alternatives. To increase the utilization of analytical techniques and eventually facilitate the integration of both design strategies, it is necessary to improve the efficacy of analytical methods on fundamental function approximation tasks. Toward this end, this work extends previous efforts to quantify the impact that different non-spiking neural encoding schemes have on the approximation quality of the arithmetic subnetworks of the functional subnetwork approach (FSA). In particular, novel design constraints are derived for inversion, division, and multiplication functional subnetworks using each of two different neural encoding schemes. The first of these encoding strategies is an "absolute" scheme in which numerical values are represented directly by the membrane voltages of the subnetwork's constituent neurons, while the second is a "relative" scheme wherein values are represented by the percent activation of the subnetwork's constituent neurons. Numerical simulation results for each of the aforementioned subnetworks indicate that there are both qualitative and quantitative advantages to selecting a relative encoding scheme over an absolute one, including an increased approximation accuracy of 4%-6% for normal operational ranges, greater numerical conditioning, and the freedom to choose more biologically realistic subnetwork parameters. The extent to which a relative encoding scheme improves subnetwork approximation accuracy is found to depend on the operation of interest, but in all cases increases monotonically with subnetwork gain. Future work will extend the study of non-spiking neural encoding schemes beyond arithmetic operations to the more sophisticated calculus subnetworks of the FSA.

Disclosures: C. Scharzenberger-Braet: None. **A.J. Hunt:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

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Program #/Poster #: PSTR244.15/XX49

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

Title: Comparing amygdala, hippocampus, piriform cortex in classification accuracy on spatial bins and airpuff using Linear Discriminate Analysis and Naive Base

Authors: *L. YANG, C. MIKKELSEN;
Neurosci. Dept., Smith Col., Northampton, MA

Abstract: Previous studies have demonstrated the role of the medial temporal lobe in combining different dimensions of memory, such as valence and space (Eichenbaum, 2000). Specifically, the amygdala has been particularly related to memory for highly valent stimuli (Richardson et al., 2004), the hippocampus has strong ties to spatial memory (Fritch et al., 2020), and the piriform cortex has strong odor-coding properties (Bolding & Frank, 2020). However, it is unknown how these regions can relationally encode mixed spatial and valence stimuli within a single experiment. To investigate the difference in their spatial and valence encoding ability, we use the data from Girardeau et al. (2017). In this experiment, rats ran on a linear track with a negative stimulus (airpuff) applied in a random location and direction that changed daily. Uniquely, this single experiment contains single cells recorded from the central amygdala (CeA), Basolateral amygdala (BLA), hippocampus (HPC), and the piriform cortex (PIR). Based on the previous research, we hypothesize that the hippocampus will perform the best in spatial encoding, while the amygdala regions will perform best in valence coding. We then used Linear Discriminant Analysis (LDA) and Naive Bayes (NB) to classify the spatial location, and airpuff vs non-airpuff locations, using spike data collected from the CeA, BLA, HPC, and PIR. We were able to decode both spatial location and valence (air puff vs. non-air puff) successfully as compared to shuffled data ($p<0.05$) for all regions. The preliminary results also show that the hippocampus has the highest classification accuracy on both spatial and valence coding whereas the piriform cortex has the lowest. This research advances the field by demonstrating the unique contributions of the regions that comprise the medial temporal lobe.

Disclosures: L. Yang: None. **C. Mikkelsen:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

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Program #/Poster #: PSTR244.16/XX50

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

Support: NIH NINDS RF1NS116450
NSF IIS 2045848

Title: Analyzing Effects of Cerebral Microbleeds on Brain Connectivity in Alzheimer's Disease

Authors: *E. L. HUNTER[1], L. ZHAN[1,2], Y. ZHAO[1], M. WU[1,3], H. AIZENSTEIN[1,3], B. IORDANOVA[1];
[1]Bioengineering, [2]Electrical and Computer Engin., [3]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cerebral microbleeds (MBs) are common pathological findings in the brain of subjects that later develop Alzheimer's Disease (AD). The impact of MBs on the structural integrity of white matter remains to be understood. Graphical deep learning models provide frameworks that represent the brain as a complex 3D network consisting of nodes and edges to help characterize and detect abnormal brain connectivity. In this study, we aim to analyze the effects of MBs on brain connectivity through the use of graph learning methods, to obtain insights into the overall tissue structural integrity that may drive changes in connections between different regions of the brain. We used 11.7 Tesla Bruker *ex vivo* MRI images of mice expressing amyloid precursor protein and presenilin (APP-PS1) and age matched controls. The APP-PS1 mice are well-characterized models of AD. The mice (N=40, 17 male and 23 female, age 4 -24 months) were deeply anesthetized, transcardially perfused; brains were excised and placed in a 3D printed holder. We used diffusion tensor imaging (DTI, TE/TR=22.38/1200ms, 30 directions) with 104 μm isotropic voxel resolution and whole-brain 3D multi-gradient echo (MGE, TE/TR=3/800ms, 29 echoes, 3 ms apart). R2* maps were computed from a voxel-based exponential fit applied to each MGE, to obtain quantifiable values for MBs. The maps were then aligned and registered in the DTI space to examine the relationship between diffusion tensor indices and R2* relaxation rates in MB locations. The aligned maps were then co-registered to the Waxholm Space Atlas (Johnson et al., 2010) and transformation matrices were saved. A probabilistic graph learning model using graph neural networks was then developed. A connectivity matrix was output to examine relaxation rates and specific brain regions that have abnormal connectivity in local MB locations. We posit that high relaxation values of specific nodes in the brain network containing MBs have abnormally sparse connections and fiber tracts. Using graph learning, our findings showed that brain regions affected by MBs had abnormal degradation of structural integrity of the white matter. These findings help to develop translational MRI-based quantitative biomarkers to detect subtle cellular changes reflective of early AD pathology.

Disclosures: E.L. Hunter: None. **L. Zhan:** 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.; NSF IIS 2045848. **Y. Zhao:** None. **M. Wu:** None. **H. Aizenstein:** None. **B. Iordanova:** 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 NINDS RF1NS116450.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.17/XX51

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

Title: Performance of fully automated spike sorting methods applied to simulated (known ground-truth) human intracranial recordings

Authors: *J. WIXTED[1], P. N. STEINMETZ[2];
[1]UC San Diego, La Jolla, CA; [2]NeurTex Brain Res. Inst., NeurTex Brain Res. Inst., Dallas, TX

Abstract: The primary objective of human intracranial microwire recordings is to identify single or small groups of neurons which change firing rate in response to changes in the mental state of the subject. Here we use simulated firing rate changes to determine how well such changes can be isolated from recorded extracellular potentials when using standard spike sorting techniques. The advantage of this approach is that the performance of these techniques can be assessed relative to known ground truth, rather than to expert opinion (which could be wrong). Simulations were performed of background electrical activity having a typical power spectrum with 5 µV std in the 300-3000 Hz passband and increasing power in lower LFP frequencies. Single neuron activity was simulated using a 25 µV high spike waveform and random background firing of 2.5 sp/s. For 1/3 of one-second trials representing a stimulus with a response, the firing rate was increased by 25%. These conditions were chosen to simulate a typical experiment with visually responsive neurons. For each simulation, spike sorting was performed using two commonly used techniques, BML and WaveClus, and their default settings. Each identified cluster was automatically classified using the Valdez criteria. Cluster spike times were counted during the trials, and the ability to distinguish between trials with and without a response (based on hits and false alarms) was computed using d'. Both BML and Waveclus yielded results on the order of d'=1 for detecting a 25% change in the firing rate. Without spike sorting, discriminating responsive trials using detected events was larger for WaveClus (1.23 vs 1.14 with spike sorting) and lower for BML (0.56 vs 1.14). The best criteria for identifying responsive clusters differed between BML and WaveClus. These results suggest that if electrical potentials in intracranial microwire recordings result from nearby neurons with larger waveforms than surrounding neurons or other electrical sources, commonly used spike sorting techniques work reasonably well to identify changes due to experimental manipulations. Further work is needed to optimize the methods of processing and classifying spike sorting outputs to identify the neural activity underlying cognitive changes.

Disclosures: J. Wixted: None. **P.N. Steinmetz:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.18/XX52

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

Title: Automating neuroscience model identification and characterization

Authors: Z. JI[1], S. GUO[1], ***R. MCDOUGAL**[2];
[2]Biostatistics, [1]Yale Univ., New Haven, CT

Abstract: Our ability to gather neuroscience data at scale is constantly improving, but experimental observations are necessarily incomplete as there are always more details that could be collected. Theoretical and computational models can be used to fill in the gaps and to make predictions beyond the explicit observations, but the development of such models is itself a work-in-progress building on new observations and prior models. Identifying prior models of a given topic (e.g. CA1 Pyramidal Cells in Alzheimer's) is non-trivial as these models are rarely published as stand-alone projects but are instead used and only-briefly described within larger research studies. Some model codes are shared on resources like GitHub, which facilitates code reuse, but requires one to first find the relevant repository. To address this need, ModelDB (https://modeldb.science) serves as a discovery platform for computational neuroscience, offering a single platform with over 1800 published model codes with standardized metadata. These model codes were gathered through mostly organic growth from model author submissions, but this approach is inherently limited, and is estimated to have captured only around one-third of NEURON models and lower fractions for other simulators. To more completely characterize the state of computational neuroscience modeling work, we have applied modern NLP techniques, including SPECTER2 and GPT-4, to identify papers likely to contain results derived from computational neuroscience approaches. In particular, we used ModelDB to identify a set of known computational neuroscience work and identified generic neuroscience work through querying PubMed for specific MeSH terms (A08 Nervous Systems and C10 Nervous System Diseases). SPECTER2 embeddings showed high separability under LDA, with 96.5% classification accuracy as measured by 10-fold cross-validation. GPT-4 was used on papers identified as likely to contain computational neuroscience work to identify relevant metadata annotations (e.g. cell types, research topics); performance on these tests was compared on a per metadata category basis. Results of this analysis are in the process of being added to ModelDB to facilitate further model discovery.

Disclosures: Z. Ji: None. **S. Guo:** None. **R. McDougal:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.19/XX53

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

Title: Temporally-precise inference of neural population dynamics from slow-sampling rate calcium imaging using deconvolution-free dynamics modeling

Authors: *A. AGARWAL[1], F. ZHU[1], Y. YU[2], H. A. GRIER[3], S. L. SMITH[2], M. T. KAUFMAN[4], C. PANDARINATH[1,5];
[1]Wallace H. Coulter Dept. of Biomed. Engin., Emory Univ. and Georgia Inst. of Technol., Atlanta, GA; [2]Dept. of Electrical & Computer Engin., Univ. of California Santa Barbara, Santa Barbara, CA; [3]Committee on Computat. Neurosci., [4]Dept. of Organismal Biol. and Anatomy, Neurosci. Inst., The Univ. of Chicago, Chicago, IL; [5]Dept. of Neurosurg., Emory Univ., Atlanta, GA

Abstract: Two-photon (2p) calcium imaging is a powerful tool to monitor the activity of vast populations of neurons in the brain, but increasing the number of monitored neurons often imposes a critical limitation on the temporal resolution of neural population activity. A recent deep learning method, RADICaL (Zhu et al., Nat Neuro 2022), demonstrated high-resolution inference of neural population dynamics at fast imaging rates (~30 Hz), but relies on deconvolution. This fails at slow rates because sparse sampling of the fluorescence can result in missing peak amplitudes, or missing events entirely. Here we present Free-RADICaL, a novel solution that overcomes this limitation, enabling inference of neural population dynamics at high temporal resolution at low imaging rates. Free-RADICaL incorporates a generative model of fluorescence activity and a novel optimization objective that separates neural dynamics from calcium indicator dynamics, enabling direct application to fluorescence signals. We evaluated Free-RADICaL on simulated data and 2p recordings from the visual cortex and motor cortex comparing its performance to the standard approaches, such as gaussian-smoothing the deconvolved events (s-deconv) or fluorescence traces (s-fluor). In synthetic data, Free-RADICaL achieved significantly better performance in inferring known rates - particularly for high-frequency (7-10 Hz) features sampled at extremely low frame rates (2-4 Hz) - compared to s-deconv and s-fluor (e.g., 7x increase in correlation (r) for 7 Hz signals imaged at a frame rate of 2 Hz). When applied to 2p recordings from the primary visual cortex in a mouse watching a sequence of drifting gratings (imaged at 10.45 Hz), Free-RADICaL successfully denoised individual neurons' responses on single trials (2x increase in PSTH r compared to s-deconv and s-fluor). Free-RADICaL also successfully denoised 2p recordings from the motor and somatosensory cortical areas in mice performing a water grab task (imaged at 31.08 Hz) and produced estimates of neural population states that closely corresponded to single-trial variations in reach kinematics. The denoised rates led to higher performance in decoding the hand position and velocity on a moment-by-moment basis (R^2 0.85 and 0.41, respectively) compared to s-deconv (R^2 0.75 and 0.36, respectively). Free-RADICaL is therefore a promising tool to enable temporally-precise inference of the activity of large-scale neural populations monitored at very slow sampling rates by 2p imaging.

Disclosures: **A. Agarwal:** None. **F. Zhu:** None. **Y. Yu:** None. **H.A. Grier:** None. **S.L. Smith:** None. **M.T. Kaufman:** None. **C. Pandarinath:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

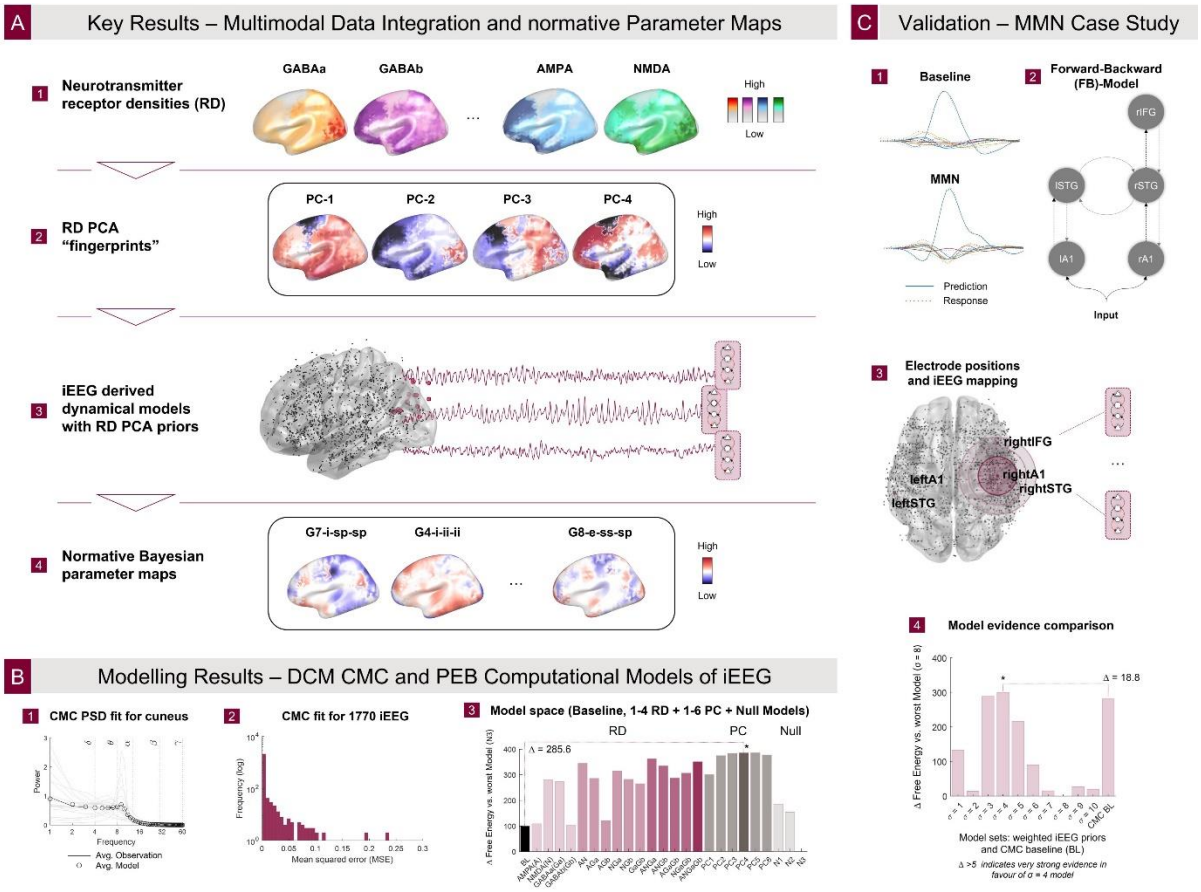
Program #/Poster #: PSTR244.20/XX54

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

Title: Topographic variation in neurotransmitter receptor densities explain differences in intracranial EEG spectra

Authors: *U. STOOF[1], K. FRISTON[1], M. TISDALL[1], G. COORAY[1], R. ROSCH[2];
[1]Univ. Col. London, London, United Kingdom; [2]King's Col. London, London, United Kingdom

Abstract: Introduction: Both molecular characteristics of neurons and electrophysiological recordings of their dynamics show regional variability across the human cortex. However, currently there is an explanatory gap regarding how cortical microarchitecture and electrophysiological measures are linked. Here we fit biophysically informed neural mass models (NMMs) to intracranial EEG (iEEG) data using a dynamic causal modelling (DCM) framework and test whether regional variation in neurotransmitter receptor densities (RD) partially explains between-region differences in iEEG signals.
Method: We first fit generative canonical microcircuit (CMC) NMMs to iEEG spectra of individual brain regions (baseline). Subsequently, using a hierarchical parametric empirical Bayesian (PEB) method, we evaluate whether regional RD data improve our NMMs. Finally, to confirm generalisability, we use RD-informed parameter posteriors as priors for a well-characterised use case for DCM: the mismatch negativity (MNN).
Results: DCM CMCs reproduce iEEG signals with high accuracy, capturing characteristic spectral components of individual brain regions, and including RD data in a hierarchical model (PEB) improves model evidence. Using the resultant parameter posteriors as priors for a NMM of the independent scalp EEG MMN dataset results in increased model accuracy and evidence, demonstrating cross-modal robustness of our findings.
Discussion: In summary, we show that molecular cortical characteristics (i.e., receptor densities) can be incorporated to improve generative, biophysically plausible models of coupled neuronal populations. This work can help to explain regional variations in human electrophysiology and may provide a normative resource for future DCM studies.



Disclosures: **U. Stoof:** None. **K. Friston:** None. **M. Tisdall:** None. **G. Cooray:** None. **R. Rosch:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.21/XX55

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

Support: NIH Grant U01AG076791-02

Title: Curvilinear coordinate systems for visualizing adult and developmental neuroanatomical topography

Authors: *A. A. BHANDIWAD[1,2], F. KRONMAN[3], S.-L. DING[4], Q. WANG[2], L. PUELLES[5], Y. KIM[6], L. NG[2];
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Hershey, PA; [4]Allen Inst. For Brain Sci., Seattle, WA; [5]Univ. Murcia Fac of Med., Murcia, Spain; [6]Penn State Univ., Hershey, PA

Abstract: The complex three-dimensional geometry of the brain poses challenges to our understanding of its organization and function. How the brain's shape and connectivity changes throughout an animal's lifespan allows extraction of invariant topographic features related to its morphogenetic process. To compare these features in the same space, a nonlinear coordinate system is essential. This system would facilitate transformation from Cartesian coordinates (x,y,z) into a flatmap, a visualization that preserves nearest-neighbor relationships while unfolding brain regions into a coherent topological system. Previously, Laplace's equation has been used to generate curvilinear coordinates of the mouse cortex by finding the shortest distance along the columnar axes. Here, we apply Laplace's equation to develop a generalized workflow for generating curvilinear coordinates for multiple brain structures in the mouse. Our flatmaps convert Cartesian axes to antero-posterior, roof plate-floor plate, and radial axes. We provide specific examples from the adult mouse hippocampus. Using anterograde connectivity data and single neuron reconstructions, we show that the resulting flatmaps preserve local topography and nearest-neighbor geodesic distances while revealing regional compartmentalization in the hippocampal dentate gyrus and Ammon's horn. Other examples relate to developmental prosomeric model parcellations. Finally, we show how flatmaps from hindbrain rhombomeres in early development can be aligned with the goal of connecting regional flatmaps into a comprehensive geodesic map of the adult and developing mouse brain common coordinate frameworks.

Disclosures: A.A. Bhandiwad: None. **F. Kronman:** None. **S. Ding:** None. **Q. Wang:** None. **L. Puelles:** None. **Y. Kim:** None. **L. Ng:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.22/XX56

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

Title: On the optimal spatio-temporal resolution for computational modeling of mesoscale neural dynamics

Authors: *T. SAMIEI[1], Z. ZOU[2], M. IMANI[2], E. NOZARI[1];
[1]Univ. of California, Riverside, Riverside, CA; [2]Univ. of California Irvine, Irvine, CA

Abstract: Understanding the neural code poses a significant challenge owing to the complex nature ofneuron participation in multiple circuits and computations. In the context of vision, decoding the neuralcode has been extensively studied, including the critical role of neural dynamics in encoding visual stimuli.However, several questions remain open, including the optimal spatial and temporal resolution that maxi-mizes the neural signal-to-noise ratio. Coarse

resolutions mean more spatiotemporal averaging, which wouldin turn dampen both signal and noise. This creates a delicate correlation-dependent balance between pre-serving information and reducing noise, which is critical for optimal information processing but is currentlypoorly understood.In this work, we use hyper-dimensional computing (HDC) to model the encoding of visual informationin mesoscale neural activity using the Allen Institute Visual Coding - Neuropixels dataset. HDC classifiersare not only symbolic and thus more interpretable than black-box models, but they also do not involveany implicit averaging of the input data, thus providing precise control over the extent of spatiotemporalaveraging we wish to use for decoding. We also found the HDC classifier to have accuracy comparable toor better than those of random forest, SVM, and neural networks. To further validate the proposed HDCclassifier, we compared its accuracy using spiking data from various subsets of brain regions. Interestingly, wefound classifiers using only V1 spike counts to demonstrate the highest accuracy in classifying Gabor patterns,while the classifier using data from the primary, lateral, and posteriomedial visual areas (homologous to theventral visual pathway in primates) achieved the highest accuracy in classifying natural scenes.We then studied the optimal spatial and temporal resolution that maximized the classification accuracyof the HDC classifier. Temporally, we parametrically varied the bin size used to capture neuron spike countsand found an optimal bin size of 2*ms* giving the highest accuracy. Spatially, we averaged neuronal spikecounts at progressively coarser levels, and found the classifier that averaged the spike counts of all neurons ineach region yielding the most accurate classification. These results are indeed limited by the sparse samplingof neurons across brain regions and the few number of tasks performed by each mouse; nevertheless, theyopen a new perspective to understanding the neural code as well as optimal pipelines for processing multi-unitspiking data.1

Disclosures: T. samiei: None. **Z. Zou:** None. **M. Imani:** None. **E. Nozari:** None.

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.23/XX57

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

Support: NIMH (R01MH126531)

Title: Evaluating the effects of active digital devices on dyadic attraction using eye contact detection machine learning

Authors: *J. Q. MANNING[1], A. LAVALLEE[2], M. KYLE[2], M. HUSSAIN[2], M. REIMERS[3], M. MOORE[4], D. DUMITRIU[2];
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Abstract: *Objective*: Several studies have examined the effects of modern digital technology or screen time on long-term child and parent behavior, but emotional health in short-term interactions between child, parent, and digital device have not been explored. Dyadic interactions between mother and infant in the presence of an active digital device (ADD) were analyzed to study the effects of infant-device oriented visual attention on mutual attraction and emotional connection.
Methods: Two phases of dyadic interactions were observed and recorded remotely through a cloud-based videoconferencing service where participants utilized their own ADD (e.g. a web-camera enabled smartphone) to connect: (1) the lap test, a three minute interaction with the infant on the mother's lap, and (2) the carseat test, a two minute interaction with the infant in a car seat. Interactions were conducted such that audio and visual stimuli were delivered through the ADD by a connected instructor before the start of each interaction phase. During the interaction, the instructor ceased audio and visual communication. Infant-device oriented visual attention was measured by the machine learning library, OpenFace. Specifically, to analyze oriented visual attention, we compared horizontal gaze angle parameter outputs of the carseat test videos. Eye-contact detection was reported per each video frame. Mutual attraction was measured by manually coded mutual eye gaze between mother and infant on a millisecond-to-millisecond scale. Emotional connection was measured by the Welch Emotional Connection Screen (WECS) on a second-to-second scale.
Results: In our preliminary dataset, out of 20 analyzed videos, 18 were considered due to lack of gaze angle confidence in two of the interactions. When gaze angle parameters were confined to ±0.1 radians, the average percentage of frames where eye contact with the ADD was detected against the total number of processed frames was 1.48%. The correlation of the carseat phase WECS scores and lap test phase mutual eye gaze scores against the percentage of eye contact detected frames was r=0.18 and r=-0.26 respectively. Mutual eye gaze correlations suggest that babies who shared greater mutual eye contact with their mothers during the lap test exhibited lower rates of eye contact with the ADD during the carseat test.

Disclosures: **J.Q. Manning:** A. Employment/Salary (full or part-time):; Columbia University Medical Center. **A. Lavallee:** 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.; NIMH (R01MH126531). **M. Kyle:** 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.; NIMH (R01MH126531). **M. Hussain:** 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.; NIMH (R01MH126531). **M. Reimers:** 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.; NIMH (R01MH126531). **M. Moore:** 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.; NIMH (R01MH126531). **D. Dumitriu:** 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.; NIMH (R01MH126531).

Poster

PSTR244. Computational Neuroscience: Analytical

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR244.24/XX58

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

Support: R01AG064066
R01AG064066-S1

Title: Global remapping in spatiotemporal coding using the Earth Mover's Distance

Authors: *A. AOUN, O. SHETLER, S. HUSSAINI;
Columbia Univ., New York, NY

Abstract: The neurons of the entorhinal cortex (EC) and hippocampus (HC) coordinate to encode a spatial representation that underlies navigation, memory and other key cognitive aspects. The field maps that emerge from the firing activity of these neurons are a direct readout of memory representation of a particular space and their stability across sessions can be assessed. Remapping of field maps is a crucial aspect that contributes to the flexibility of neural representations encoding our environment and is intricately involved in, and affected by, both functional and disease states. With neurodegenerative disorders, particularly Alzheimer's disease (AD), representational instability across these maps confounds Pearson's R, disrupting our ability to discriminate remapping types. While binary categorization of remapping into ‘rate’ and ‘global’ using Pearson's R can capture the underlying changes in rate, it lacks specificity to spatiotemporal changes, is unable to characterize complex remapping and cannot effectively quantify remapping resulting in non-overlap. We demonstrate that extending our description of remapping by also applying the Earth Mover’s Distance (EMD) on unnormalized distributions, and Wasserstein metric on normalized distributions, allows for a more granular description of remapping that is multifaceted, more stable to noise degeneration and still rate robust. We show how properties of the EMD can be manipulated to identify object cells, trace cells and other stimulus or location driven remapping. We also show how EMD estimators can serve as a practical substitute or supplement for Pearson’s R. Thus we propose the EMD as a metric to describe the differences in global remapping and to identify specific cell types found in the EC-HC circuit.

Disclosures: A. Aoun: None. **O. Shetler:** None. **S. Hussaini:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.01/XX59

Topic: I.07. Data Analysis and Statistics

Title: Schizophrenia's Impact on Spatially Dynamic Network Integration and its Connection to Genetics

Authors: *A. IRAJI[1], P. CAMAZÓN[2], J. CHEN[1], A. FAGHIRI[1], N. LEWIS[3], V. CALHOUN[4]; [1]Georgia State Univ., Atlanta, GA; [2]Inst. of Psychiatry and Mental Health, Hosp. Gen. Universitario Gregorio Marañón, IiSGM, Madrid, Spain; [3]TReNDS, Sandy Springs, GA; [4]Georgia Inst. of Technol., Decatur, GA

Abstract: Introduction We propose a new method, time-resolved spatially constrained independent component analysis (tr-scICA), to capture brain spatial dynamics, i.e. spatial variations of brain networks over time. We then calculate time-resolved spatial functional network connectivity (tr-spFNC) to quantify dynamic spatial integration and segregation using spatial similarity between networks. We use tr-spFNC to investigate changes in schizophrenia (SZ) and their associations with an SZ polygenic risk score (PRS). **Methods** The analysis pipeline includes (1) identifying group-level networks using group-level ICA; (2) estimating corresponding time-resolved networks using our tr-scICA; (3) estimating tr-spFNC using spatial Pearson correlation networks; (4) applying *k*-means clustering on the tr-spFNC matrices to identify spatial dynamic states. We next study the SZ effect using "spFNC feature ~ 1 + diagnosis: sex + age + motion + site". We also evaluate the association between aberrant dynamic spFNC features and the SZ-PRS. Finally, we perform saliency analysis to identify the spatial distribution of disrupted regions. **Results Fig. 1** summarizes the results. We identified 14 brain networks and 4 dynamic states. State 1 has the highest level of integration, and State 4 has the lowest. We found sex-common schizophrenia effects across all 4 states, but sex-specific effects were only observed in State 1. Two spFNC pairs, including "default mode-salience" States 1, significantly correlate with SZ-PRS. Saliency analysis reveals regions with low network contributions are linked to high informational content and are affected by schizophrenia. These regions are typically masked out in standard analyses. **Conclusions** Our method detects spatially dynamic networks and quantify their integration and segregation. Our method can detect sex-specific SZ effects and imaging-genomic associations. SZ alterations are more pronounced in regions with small network contributions. The findings highlight the importance of multifunctionality, network overlapping, and weak connectivity in SZ.

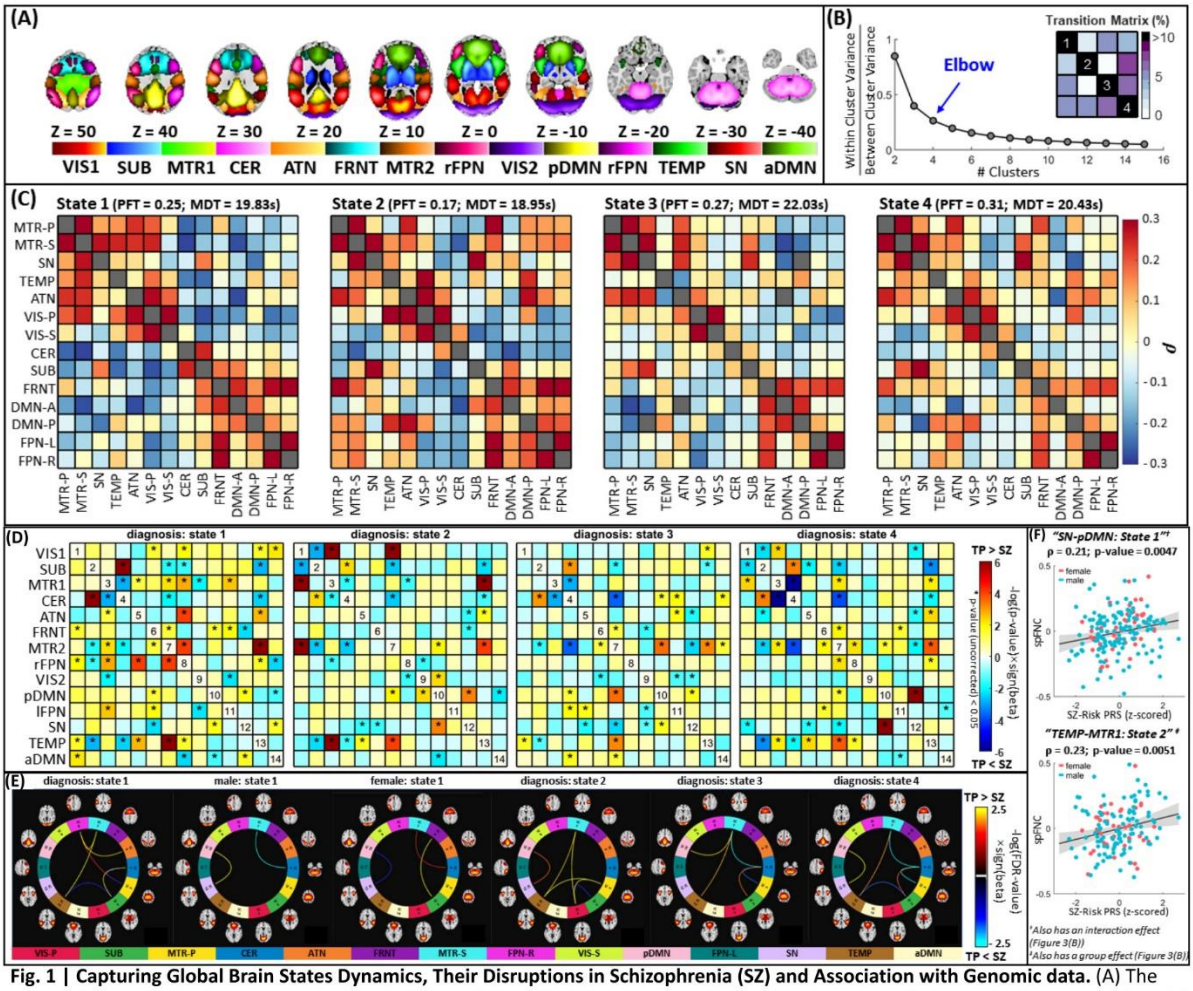

Fig. 1 | Capturing Global Brain States Dynamics, Their Disruptions in Schizophrenia (SZ) and Association with Genomic data. (A) The composite map of the brain networks, thresholded at |Z| > 1.96. (B) Estimation of the optimal number of states using Elbow criterion. (C) The four spatial dynamic states are identified using k-means clustering with L1 distance. PFT: The probability of fraction rate. MDT: mean dwell time. (D) the diagnosis effect (before correcting for multiple comparison), i.e., SZ vs. typical control (TP), Asterisks (*) represents p-value < 0.05. (E) Connectograms of state spFNC pairs with a significant diagnosis or interaction (diagnosis-sex) effects after FDR corrections. Three spFNC pairs from State 1 show significant sex-diagnosis interaction effects, including posterior default mode and salience networks. (F) The association between schizophrenia genetic risk and aberrant dynamic spatial coupling. Among dynamic spFNC pairs with significant schizophrenia effect, two show significant associations with the polygenic risk score (PRS) after FDR correction.

Disclosures: A. Iraji: None. **P. Camazón:** None. **J. Chen:** None. **A. Faghiri:** None. **N. Lewis:** None. **V. Calhoun:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.02/XX60

Topic: I.07. Data Analysis and Statistics

Support: NSF grant NCS-FO 1835268/1834994
NSF STC award CCF-1231216

Title: Using the Allen Brain Observatory data to explore experimental design

Authors: *E. MEYERS, J. PAN;
Yale Univ., New Haven, CT

Abstract: Experimental and data analysis parameters used in neuroscience studies are often chosen in an ad hoc way. For example, the number of stimuli, trials, and neurons recorded are often chosen out of convenience without a clear understanding of how particular choices might affect the conclusions drawn. To gain a better understanding of how such choices affect results, we analyzed the Allen Brain Observatory natural scenes data set, which consists of recordings from over 20,000 neurons from 31 mice, where in each experiment session, 118 stimuli were shown 50 times. By analyzing such a large data set, we could explore how using different experimental parameter choices (e.g., smaller numbers of neurons, stimuli, and trials), as well as data analysis parameters choices, affected the results. We focused on classical statistical analyses, (i.e., using ANOVAs to measure individual neuron selective), and on population decoding analyses. Our classical statistical analyses showed the results were fairly robust to data analysis parameters when estimating information latency of individual neurons as long as information was aggregated over bins greater than 25 ms. Additionally, our findings show that while on aggregate, information flowed through brain regions in a way that has previously been reported (Seigle et at 2021), there was a high degree of heterogeneity between neurons, and a few individual animals showed different information latency patterns in particular brain regions. Our results from decoding analyses show that similar conclusions could be drawn using a significantly smaller number of stimuli and stimulus repetitions, and that using a normalized rank measure allows one to get consistent estimates of decoding accuracy across in experiments where different numbers of stimuli were used. Overall, our findings should help experimental neuroscientists plan future studies, and give insight into which analysis methods to use to gain the clearest results.

Disclosures: E. Meyers: None. **J. Pan:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.03/XX61

Topic: I.07. Data Analysis and Statistics

Title: Transformer models ported from auditory speech recognition excel at scoring sleep

Authors: *W. G. COON, M. OGG;
Intelligent Systems Branch, Neurosci. Group, Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: Accurate sleep assessment is critical to the practice of sleep medicine and sleep research, yet the traditional (gold standard) method of visual scoring by experts is time-consuming and costly. Large quantities of publicly available sleep datasets combined with modern neural network architectures have started to propel automated sleep stage classification into widespread use. Nevertheless, we are still in the early days of automating this process and a variety of optimizations remain to be explored. Here, we adapted a transformer model architecture that achieves state-of-the-art performance on a similar type of sequence learning task (audio speech recognition), and applied it to sleep staging. We leverage several large public sleep datasets for training and internal validation (MESA, MRoS, SHHS, and WSC) and hold out standard benchmark datasets for testing (DREEM, SleepEDF). This transformer model performs comparably to human experts using as little as 10 minutes of input data from a single EEG channel. Using ablation studies, we examined the effect of model size, sleep sequence length, including an EOG channel, and the use of multiple or single nights of sleep from individuals during training. Compared to most recurrent neural network analogues, the attention-based transformer may be better able to distinguish REM sleep from neighboring classes using EEG alone.

Disclosures: W.G. Coon: None. **M. Ogg:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.04/XX62

Topic: I.07. Data Analysis and Statistics

Support: Ford Foundation Postdoctoral Fellowship

Title: Cluster-aware machine learning of neuroimaging transcriptomics for precision neuroscience and psychiatry

Authors: *A. M. BUCH, C. M. LISTON, L. M. GROSENICK;
Psychiatry and Brain and Mind Res. Inst., Weill Cornell Medicine, Cornell Univ., New York, NY

Abstract: Explainable machine learning of complex multimodal data is transforming the landscape of neuroscience research. In many cases, data heterogeneity across samples due to biological clusters is an important component of variation, and revealing these clusters and the biological factors that explain them is an important research approach. For example, in medical diagnoses, interpretable clustering of patients into distinct subtypes improves personalization of

treatment. However, a combination of the well-known “curse of dimensionality” and the clustered structure of biomedical data together present a unique challenge in the high dimensional and limited observation regime common in datasets used in neuroscience. Embedding followed by clustering is popular, but this two-stage process often results in both suboptimal embeddings and degraded cluster separation, motivating a need for joint clustering and embedding approaches that are explainable, robust to technical variability, and generalizable. To overcome both challenges simultaneously we propose a simple and scalable approach to joint clustering and embedding that combines standard embedding methods with a convex clustering penalty in a modular way. Through both numerical experiments and real-world examples, we show that our approach outperforms traditional and contemporary clustering methods on highly underdetermined problems (e.g., with just tens of observations) as well as on large sample datasets. Thus our approach improves significantly on existing methods for identifying patient subgroups in multiomics and neuroimaging data.

Disclosures: **A.M. Buch:** None. **C.M. Liston:** None. **L.M. Grosenick:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.05/XX63

Topic: I.07. Data Analysis and Statistics

Title: Correlation between subcutaneous adipose tissue of the head and body mass index across the lifespan: Implications for functional neuroimaging

Authors: *S. GORNIAK[1], H. MENG[2], S. YAZDEKHASTI[3], L. POLLONINI[3];
[1]Hlth. and Human Performance, [2]Univ. of Houston, [3]Univ. of Houston, Houston, TX

Abstract: High body mass index (BMI) is generally assumed to represent overall amounts of body adipose tissue (fat). Increased adipose tissue amounts in persons with increased BMI has been cited as a barrier to assessment of body tissues such as muscle. Significant increases in the amount of adipose tissue between the dermal layer and the skull may result in high electrical impedance and/or increased light diffusion causing a lower signal to noise ratio during use of neuroimaging tools such as electroencephalography (EEG), transcranial direct current stimulation (tDCS), and functional near infrared spectroscopy (fNIRS). Investigating how subcutaneous adipose tissue in the head region increases with respect to total body fat percentage and BMI across the lifespan is an important step in developing mathematical corrections in neuroimaging measurements as BMI increases, as recommended in other measurement modalities such as electromyography (EMG). We hypothesized that percentage of subcutaneous adipose tissue in the head region would increase with respect to both total body fat percentage and BMI. Our dataset (n = 6,923) included individuals aged 8-89 years of age, with BMI ranging from 12.41 kg/m^2 to 130.21 kg/m^2. A statistically significant increase in subcutaneous head fat

percentage occurred with increased BMI and total body fat percentage. The data investigated in this study indicate that participant age, sex, and BMI are important features to consider in model corrections during data signal processing and analyses for subcutaneous head fat in neuroimaging approaches. The data in this project serve to provide physiological justification for this practice along with regression analyses to be considered for physiologically-based signal to noise correction algorithms.

Disclosures: **S. Gorniak:** None. **H. Meng:** None. **S. Yazdekhasti:** None. **L. Pollonini:** 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.; NSF.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.06/XX64

Topic: I.07. Data Analysis and Statistics

Support: the National Research Foundation of Korea (NRF) grant (No. 2021R1I1A3060828)
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Sabbatical Leave Grant at Handong Global University(HGU-2023)

Title: Evaluation of public motor imagery datasets and low BCI performers

Authors: *Y. LEE[1], D. GWON[1], M. SONG[1], M. AHN[1], C. NAM[2];
[1]Handong Global Univ., Pohang, Korea, Republic of; [2]North Carolina State Univ., North Carolina, NC

Abstract: Motor imagery (MI) is one of the main control paradigms in non-invasive Brain-Computer Interfaces (BCI) and various MI-BCI datasets are publicly available. Consequently, more and more researchers are using those datasets for the study of BCIs. Thus, understanding the datasets, and further assessing the data quality is important issue for reuse of the datasets and implementing an advanced MI-BCI [1]. In this study we evaluated public MI-BCI datasets with various standard algorithms while the previous study [1] only used a single algorithm for objective comparison of the datasets. For this study, we used Cho(2017) (52 subjects, 64 channels, sampling rate 512Hz) and Lee(2019) (54 subjects, 2 sessions, 62 channels, sampling rate 1000Hz,) datasets. These datasets were preprocessed with the same pipeline; epoching (0.5~3.5 sec.), Common average referencing and bandpass filtering with 8–35Hz. Then, the

datasets were evaluated by Common Spatial Pattern and Linear Discriminant Analysis (CSP/LDA), EEGNET, specialized in electroencephalogram and Minimum Distance to Riemannian Mean (MDM). The final accuracy of each case was obtained through 10×10-fold cross-validation technique.The results are presented in Table 1 and 2. EEGNET did not show an interesting result, but MDM yielded the best performance on average. Particularly, this improvement is clearly observed in Cho(2017) showing 64.82% (CSP/LDA) to 78.56% (MDM) and the decreased variance from 1.6% to 0.7% ($p < 0.05$, F-test). We also estimated the portion of low performers showing below 60% accuracy. Interestingly, MDM decreased the Low performer ratio for all datasets. Especially, the ratio was highly dropped from 40.38% (CSP/LDA) to 1.92% (MDM). In conclusion, we demonstrated that classification accuracy is obviously influenced by algorithms, but also the ratio of low performer depends on algorithms. This indicates that a high caution is required to categorize low performers.

[1] Gwon et al., 2023, Review of public motor imagery and execution datasets in brain-computer interfaces. Frontiers in Human Neuroscience.

TABLE 1 | Accuracies of CSP/LDA, EEGNET, and MDM

Accuracy

method / dataset	CSP/LDA	EEGNET	MDM	mean
Lee (2019) (Sess1)	72.25±16	62.42±17	72.01±12	68.89
Lee (2019) (Sess2)	73.07±16	63.67±19	75.21±13	70.65
Cho (2017)	64.82±13	68.39±15	78.56±8	70.59
mean	72.25	70.05	75.26	70.04

(unit : %)

TABLE 2 | Low performer ratio of CSP/LDA, EEGNET, and MDM

Low performer ratio

method / dataset	CSP/LDA	EEGNET	MDM
Lee 2019 (Sess1)	29.63 (16/54)	61.11 (33/54)	14.81 (8/54)
Lee 2019 (Sess2)	27.78 (15/54)	61.11 (33/54)	7.41 (4/54)
Cho 2017	40.38 (21/52)	40.38 (21/52)	1.92 (1/52)

(unit : % (num. of low performers/total num. of subjects))

Disclosures: **Y. lee:** None. **D. Gwon:** None. **M. Song:** None. **M. Ahn:** None. **C. Nam:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.07/XX65

Topic: I.07. Data Analysis and Statistics

Support: VA Merit Review: I01-CX002035-01
VA Merit Review: NURC-007-19S
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VA Merit Review: B1988-I
VA Merit Review: NURC-022-10F
VA Merit Review: NEUC-044-06S

Naval Medical Logistics Command Contract #N62645-11-C-4037
DOD: W81XWH-16-1-0015

Title: Accurate Classification of Post-traumatic Stress Disorder using Heart Rate Variability with Neural Network Pattern Recognition - A Preliminary Study

Authors: E. SONG[1], ***R. LEE**[2], Z. JI[3], D. G. BAKER[4], A. ANGELES QUINTO[3], M. HUANG[3]; [1]Canyon Crest Acad., San Diego, CA; [2]VA Med. Center/Ucsd, San Diego, CA; [3]Radiology, UCSD, San Diego, CA; [4]Psychiatry, VA San Diego Healthcare Syst., San Diego, CA

Abstract: In the past decade, there has been increasing interest in utilizing artificial intelligence, particularly neural network-based deep learning, for identifying patterns in medical datasets and images to help diagnosis and treatment. Heart Rate Variability (HRV) is one of the biomarkers in the diagnosis of psychiatric disorders, including Post-Traumatic Stress Disorder (PTSD), which has been linked to reduced HRV. HRV indexes neuro-cardiac function and is generated by heart-brain interactions. However, for decades, HRV studies in PTSD have focused on its high-frequency (HF) and low-frequency (LF) components, and LF/HF ratio, with limited diagnostic utility. HRV in PTSD has not been studied using advanced machine learning techniques such as deep learning neural networks.In this study, we present a feed-forward Neural Network model, to classify ECGs on 38 PTSD patients and 36 age-matched normal controls. All subjects underwent neuropsychological testing, and the PTSD diagnoses were established by an experienced psychiatrist. The ECGs were placed following lead II configuration. The ECG data were pre-processed in-house, including the following steps: a, filtering, rectifying, and normalization; b, moving-window finding maximum peak; c, artifact detection, and trial rejection. After that, 5 features were extracted from the ECGs:1. SDNN (Standard deviations of the Inter-beat Interval for all sinus beats); 2. NN50 (Number of adjacent NN intervals that differ from each other by more than 50ms); 3. RMSSD (Root mean square of the successive difference between normal heartbeats); 4. ANA (Average of normalized amplitude); and 5. SDNA (Standard deviation of normalized amplitude). The feature matrix is then fed into a 2-layer feedforward Neural Network, which contains 15 Neurons in the hidden layer. Given the limited data size, the training is fast, and performance is good. Among 74 input data, 60 training data classification accuracy was 100%, 7 validation data classification accuracy was 100%, while 6 of 7 test data were classified correctly, which results in an overall testing accuracy distinguishing between normal controls vs. PTSD of 85.7% correct.
In conclusion, this preliminary study demonstrated a simple yet effective supervised learning setup for ECG feature detection and potentially accurate PTSD classification. Future work includes, but is not limited to, additional dimensions in the feature matrix, a larger training database, etc.

Disclosures: E. Song: None. **R. Lee:** None. **Z. Ji:** None. **D.G. Baker:** None. **A. Angeles Quinto:** None. **M. Huang:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.08/XX66

Topic: I.07. Data Analysis and Statistics

Support: National Eye Institute Grant EY032125

Title: Improving Human Retinotopic Mapping with a Gaussian Process Model

Authors: *S. C. WAZ[1], Y. WANG[2], Z.-L. LU[1,3];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Sch. of Computing and Augmented Intelligence, Arizona State Univ., Tempe, AZ; [3]Div. of Arts and Sci., NYU Shanghai, Shanghai, China

Abstract: Human retinotopic mapping has become an important tool in visual neuroscience. The highly influential population receptive field (pRF) model (Dumoulin and Wandell, 2008), which yields estimates of receptive-field center and size for each voxel on the cortical surface, has been widely adopted to derive retinotopic maps from BOLD fMRI retinotopy data. However, there remain a number of challenges. Retinotopic maps *in vivo* preserve the local spatial arrangement of visual input (Wandell et al., 2007), but due to the low spatial and temporal resolutions of fMRI data, pRF-based estimates generally do not. Further, a method to automatically segregate visual areas (including the cortical representation of the fovea) has yet to be established. We address these challenges with three innovations: (1) We parameterize the retinal image using *extended polar angle* (Tu et al., 2021), allowing us to treat dorsal and ventral V1, V2, and V3 - six discrete retinotopic maps - as a single continuous map. (2) We identify a cortical anchor point corresponding to the horizontal meridian at a given retinal eccentricity using the output of the pRF model. Based on this anchor point and a point chosen optimally to represent the fovea, we then set a prior on extended polar angle. (3) We estimate both extended polar angle and eccentricity using a Gaussian process model which provides a distribution over functions rather than individual points, yielding template-free retinotopic map estimates that better preserve local spatial arrangement. We analyzed data from 143 subjects of the Human Connectome Project (Van Essen et al., 2013; Uğurbil et al., 2013). Our Gaussian process model reduced the rate of local spatial disruptions in the estimated map (identified by Beltrami coefficients of magnitude >1; Ta et al, 2021) by 37.1% along the total cortical mesh analyzed. Our model automatically yielded precise boundaries between the six discrete visual field maps, with a mean standard deviation of 0.072 rad (Q1: 0.050 rad, Median: 0.062 rad, Q3: 0.079 rad). Similar levels of precision were achieved for eccentricity contours: among points with an estimated eccentricity of 4°, the mean standard deviation was 0.127° (Q1: 0.090°, Median: 0.104°, Q3: 0.126°). Our functional estimates of the location of the cortical fovea differed systematically from the fovea based on cortical anatomy, being more dorsal and medial for 93.0% of subjects and deviating from the anatomical fovea by 2.32° on average. The newly developed methods can greatly improve the quality and quantification of human retinotopic maps.

Disclosures: S.C. Waz: None. **Y. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent interest. **Z. Lu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent interest.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.09/XX67

Topic: I.07. Data Analysis and Statistics

Title: Investigating Spectral Properties of Spontaneous Brain Activity: A Time-Domain Localization Approach

Authors: *A. FAGHIRI[1], V. CALHOUN[2];
[1]Georgia State Univ., Atlanta, GA; [2]Georgia Inst. of Technol., Atlanta, GA

Abstract: The study of spectral properties of spontaneous brain activity can provide us with new insights into different cognitive functioning in both typical controls and individuals with psychiatric disabilities. Apart from oscillatory patterns, we can also have more broadband signals that are related to the underlying neural activity of the brain. Many studies have investigated both oscillatory and power law patterns in the power spectral density (PSD) of the signal, but most of them assume stationarity. Contrary to this assumption, we have much strong evidence that indicates that the spectral properties of biological signals change with time. In this work, we have introduced a method that mixes the ideas behind time-frequency analysis and the method of moment approach to estimate PSD in a fashion that is temporally localized. We then use k-means to cluster these estimated localized PSD patterns. We applied the proposed approach to a resting state functional magnetic resonance imaging (fMRI) dataset. We identified various short-time PSD patterns, including power law (i.e., 1/f like) patterns, oscillatory patterns, and combinations of the two. These patterns exhibit different spatial characteristics and may provide insights into the underlying brain functioning. For example, we found that visual regions in the brain show scale-free PSD (1/f-like pattern), while the cerebellum showed a combination of both 1/f and oscillatory patterns. This might be the underlying reason why previous studies have found the slope of 1/f patterns in PSD is lower in the cerebellum compared to visual areas. We also found that sub-cortical areas show more of an oscillatory pattern (not a 1/f-like pattern). We used phase randomization to build a non-parametric null model and showed the improbability of our results being random. We also showed that our results are replicable when we use data from different sessions of the same individuals. One additional benefit of the proposed method is that we can explore the short-time spectral properties of other moment orders (e.g., first cross moment or pairwise connectivity). All in all, in this study, we demonstrated the benefits of using an approach that is localized in the time domain to study the spectral properties of spontaneous brain activity.

Disclosures: A. Faghiri: None. **V. Calhoun:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.10/XX68

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01AG054081
Tiny blue dot Foundation

Title: Multilevel State-Space Models Enable High Precision Event Related Potential Analysis

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[1]Dept. of Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp., Boston, MA;
[2]Anesthesia, Critical Care, and Pain Mgmt., Massachusetts Gen. Hosp., Charlestown, MA;
[3]Harvard-MIT Hlth. Sci. and Technol., MIT, Cambridge, MA

Abstract: Event related potentials (ERPs) provide a non-invasive method to study psychophysiological correlates of sensory and cognitive processes with components that are informative of the course of sensory ('exogenous') and cognitive ('endogenous') processes with millisecond temporal resolution. ERPs are tiny ~1μV signals that are embedded in background spontaneous oscillations that may be 10 to 100 times larger. Classically, combining a large number of single-trial waveforms into an averaged ERP waveform is considered to be a "gold standard" for averaging out the event-unrelated activity. However, it is often difficult to obtain sufficient trials that are required to effectively cancel out the background of persistent oscillations. Also, excessive averaging could easily obscure fine structures of the ERPs, since intrinsic factors like mental fatigue and attention that are known to modulate these fine structures, can change over time.
To reduce this reliance on large numbers of trials, we model the spontaneous stimulus-agnostic activity in EEG as a superimposition multiple oscillators, modeled after state-space oscillators, rather considering them as white or otherwise unstructured noise. The response evoked by stimulus presentation manifested in EEG is considered as a convolution between ERP waveforms and an impulse train pertaining to discrete events of stimulus presentation, as done in classical ERP literature. We further impose a temporal continuity constraint in the form of following state-space model: resulting in a multi-level state-space framework. We hereby refer to this ERPs as state-space ERP (SS-EEP). We learn the state-space oscillator parameters and temporal smoothness parameter of ERP using an instance of Expectation-Maximization algorithm, i.e., we use the oscillator models to work in tandem with ERP models to explain the EEG recording, effectively removing the contamination coming from strong oscillations.
Our simulation study demonstrates that the SS-ERP method can remove the effects of oscillatory activity and provide superior performance at capturing the true latencies and amplitudes of the ERP peaks from a small number of trials. We further benchmarked SS-ERP in extracting the P300 response, which showcases an impressive improvement in effect size (cohen's d) of 5.96 folds, and in precision by 12.37 with only 52 trials. Thus, by significantly reducing the number of trials requirements, our proposed method will potentially enable tracking of short-term

changes in ERP due to various intrinsic and extrinsic factors of interest, which would have significant implications for neuroscience studies and clinical applications.

Disclosures: **P. Das:** None. **M. He:** None. **P.L. Purdon:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.11/Web Only

Topic: I.07. Data Analysis and Statistics

Support: EPSRC Engineering and Physical Sciences Research Council

Title: An MRI-free approach for transcranial direct current stimulation (tDCS) electric field magnitude estimation

Authors: ***J. TOTH**[1], M. BROSNAN[3], M. ARVANEH[2];
[2]Dept. of Automatic Control and Systems Engin., [1]Univ. of Sheffield, Sheffield, United Kingdom; [3]Univ. Col. Dublin, Dublin, Ireland

Abstract: Transcranial electrical stimulation (tDCS) is a non-invasive brain stimulation technique that aims to modulate brain activity. However, electric fields generated in the brain vary significantly between subjects undergoing the same stimulation parameters, due to the differences in head and brain morphology across the population [1]. Currently, structural MRI scans are required to account for these inter-individual differences through the use of current flow modelling. tDCS-induced current flow is simulated by segmenting MRI scans into tissue types and performing finite element analysis on these models with assumed conductance values [2]. The requirement of access to MRI scans for current flow modelling makes tDCS simulation more expensive and less accessible [3].
We present a novel method to estimate electric field magnitude during tDCS without the need for an MRI scan. We simulated MRI scans using the "Realistic volumetric-approach to simulate transcranial electrical stimulation" (ROAST) [2] on the CanCAN dataset of cognitively normal adults [4]. After manual cleaning, N=453 T1 and T2 MRI scans were used to simulate tDCS across 10 montages across brain regions in both bipolar and high-definition tDCS (HD-tDCS) montage types. Linear regression models were then applied to the resulting average magnitude of the electric fields to correlate the relationship between electric field magnitude and age, sex, head circumference, head width, head length, and head height information. Across six bipolar montages, our models accounted for 52% ($p<0.01$) and 36% ($p<0.01$) of the variability in the average and peak electric field magnitudes on average. Across four HD-tDCS montages, our models account for 22% ($p<0.01$) and 23% ($p<0.01$) of the variability in the average and peak electric field magnitudes.
[1] I. Laakso et al, "Inter-subject Variability in Electric Fields of Motor Cortical tDCS", 2015

[2] Y. Huang et al, “Realistic volumetric-approach to simulate transcranial electric stimulation—ROAST—a fully automated open-source pipeline”, 2019
[3] J. S. A. Lee et al, “A Future of Current Flow Modelling for Transcranial Electrical Stimulation?”, 2021
[4] J.R Taylor, “The Cambridge Centre for Ageing and Neuroscience (Cam-CAN) data repository: Structural and functional MRI, MEG, and cognitive data from a cross-sectional adult lifespan sample”, 2017. Dataset available at: http://www.mrc-cbu.cam.ac.uk/datasets/camcan

Disclosures: **J. Toth:** None. **M. Brosnan:** None. **M. Arvaneh:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.12/XX69

Topic: I.07. Data Analysis and Statistics

Support: DOD Grant PR140252

Title: Exploring the Complex Relationships between Tinnitus Severity, Hearing Status, and White Matter Microstructure: Insights from a Multisite Study

Authors: *A. BANERJEE[1], R. A. KHAN[2], Y. TAI[3], F. T. HUSAIN[4];
[1]Neurosci., Univ. of Illinois at Urbana-Champaign, Urbana, IL; [2]Neurosci., Univ. of Illinois At Urbana-Champaign, Urbana, IL; [3]Speech Pathology and Audiol., Ball State Univ., Muncie, IN; [4]Neurosci., Univ. Illinois, Champaign, IL

Abstract: Tinnitus, often experienced as a chronic condition is characterized by the perception of sound in the absence of external stimulation, poses a significant challenge in understanding its neural mechanisms. This study builds upon the analysis of previous civilian research population (n= 96) of Khan et.al. (2021) to investigate the relationship between tinnitus and hearing loss using diffusion tensor imaging (DTI) in military population (n=60). We hypothesized the presence of distinct patterns of white matter alterations attributable to tinnitus, hearing loss, or their cooccurrence in the military group based on prior findings in the civilian group. Contrary to our expectations, analysis of DTI data revealed no significant differences in white matter alterations between control and tinnitus groups, and subsequent subgroup analysis did not reveal significant differences in fractional anisotropy (FA) due to either presence of tinnitus or hearing loss. In the civilian dataset, we further investigated the association between pure tone average (PTA) hearing loss and FA in white matter tracts, alongside examining the relationship between tinnitus severity (measured by Tinnitus Functional Index or TFI) and FA in individuals with normal hearing and hearing loss based on the obtained significant clusters covering various brain regions of interest in the Khan et.al. study. Our results revealed significant ($p<0.05$) correlation patterns: hearing loss was positively associated with FA and PTA in the corpus callosum (r =

0.17), while normal hearing showed a negative correlation (r = -0.06). Among individuals with normal hearing, tinnitus severity positively correlated with PTA in the left anterior corona radiata (r = 0.20), suggesting a potential link between tinnitus intensity and hearing sensitivity. In the tinnitus with normal hearing group, positive correlations were observed between TFI scores and FA values in specific tracts such as the right superior corona radiata (r = 0.45) and the genu of the corpus callosum (r = 0.22). Conversely, in the tinnitus with hearing loss group, negative correlations were found in the left anterior corona radiata (r = -0.11) and the left inferior longitudinal fasciculus (r = -0.01). Subsequent investigations will explore the associations between PTA, TFI and FA values, within the identified significant clusters of the civilian dataset, specifically in the military group. These findings offer valuable insights into the interplay among tinnitus severity, hearing status, and white matter microstructure, warranting further research to uncover underlying mechanisms and explore potential implications for tinnitus management.

Disclosures: A. Banerjee: None. **R.A. Khan:** None. **Y. Tai:** None. **F.T. Husain:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.13/XX70

Topic: I.07. Data Analysis and Statistics

Title: Identifying sources of electroencephalographic (EEG) electrode placement variability in brain-machine interface devices

Authors: *E. L. FRANTZ[1,2], A. GERAGHTY[1,3], M. KEY[2,1], N. BRIDGES[1], W. AUE[1];
[1]Air Force Res. Lab., Fairborn, OH; [2]Infoscitex Corp, Dayton, OH; [3]Oak Ridge Inst. for Sci. and Educ., Oakridge, TN

Abstract: Electroencephalography (EEG) technology has burgeoned in recent years, resulting in new neural recording devices with diverse use applications. For EEG readings to be interpretable, electrode placement needs to be accurate. To date, little work has been done to determine how EEG device design characteristics impact the accuracy of electrode placement. We outline a 3D scanner-based method for investigating electrode placement variability within and between six experienced EEG technicians for three different devices: BioSemi, Neuroelectrics Starstim 32, and Emotiv Epoc+. Three-dimensional scanning digitized the placement of the device on a single mannequin head. Accuracy of the individual electrode placement, relative to the target location, and placement variability (i.e., standard deviation [SD]) were calculated for both intra and inter-researcher differences. The mean intra-researcher SD was reasonably consistent across all electrodes. However, the intra-researcher accuracy varied more, depending on electrode location within a headset and electrode location. For example, both Biosemi and Neuroelectrics had higher placement accuracy at the top of the head and lower accuracy near the ears. The Emotiv headset had greater placement accuracy for anterior

electrodes, with the placement of posterior electrodes being less accurate. Inter-researcher placement accuracy differed for all three headsets, with the Biosemi headset electrodes having the highest inter-researcher placement accuracy. The Emotiv headset tended to be the most susceptible to position errors. Models were created to predict electrode placement accuracy and SD correlated well with recorded values. We concluded that electrode placement accuracy and SD varying across devices and researchers could be attributed to headset design with more elastics caps having the best overall performance. Future studies would benefit from using human subjects, and a larger sample size of researchers.

Disclosures: E.L. Frantz: None. **A. Geraghty:** None. **M. Key:** None. **N. Bridges:** None. **W. Aue:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.14/Web Only

Topic: I.07. Data Analysis and Statistics

Support: Support Center for Advanced Telecommunications Technology Research Foundation
Toyama First Bank Scholarship Foundation
AMED: JP18dm0307008

Title: Persistent firing and periodic patterns of working memory retention in cortical currents estimated from electroencephalography data

Authors: S. YOSHIIWA[1], H. TAKANO[2], K. IDO[2], M. KAWATO[3], *K.-I. MORISHIGE[2,4]; [1]Grad. Sch. of Engin., [2]Toyama Prefectural Univ., Toyama, Japan; [3]ATR BICR, Kyoto, Japan; [4]ATR NIA, Kyoto, Japan

Abstract: Electroencephalography (EEG) studies of working memory have demonstrated cortical activities and neural oscillations, but it wasn't clarified how the stored information is retained in the brain. To address this gap, we measured the scalp EEG data while participants (11 men and 3 women aged 21-51 years) performed an n-back working memory task. This task consists of three periods. (1) In the encoding period, seven arrow stimuli, chosen from four types (left, right, up, down), were presented and replaced sequentially on a monitor. One stimulus was randomly presented as a red arrow. Participants were instructed to memorize the direction of the arrow that appeared two steps before the red arrow. (2) In the retention period, this information was maintained for three seconds. (3) In the retrieval period, the participants judged whether the probe arrow direction matched the retained direction. Although the measured EEG data were contaminated by eye artifacts, we simultaneously estimated cortical and eye currents and separated the effects of artifacts by extra-dipole method with a statistical map generated from

Neurosynth as prior information. If memory retention is achieved by sustained firing patterns of neurons, there should be differences in the intensity of the estimated current at each dipole. However, if the function is implemented in periodic patterns, the spectral features of the estimated currents should differ. We split the encoding and retention periods into twelve sub-periods, then examined whether there were differences in the magnitude of the estimated currents in response to different memory loads and found significant differences in the encoding and retention sub-periods ([0.2-1.0 s]: p=0.001; [1.0-1.8 s]: p=0.002; [1.8-2.6 s]: p=0.01; [4.2-5.0 s]: p=0.04; [5.0-5.5 s]: p<0.0001; [5.5-6.0 s]: p=0.004, FDR-corrected, paired t-test). Additionally, spectral features of beta and gamma waves had significant differences in several cortical regions ([1.8-2.6 s]: (high beta) p=0.001, (gamma) p=0.04; [2.6-3.4 s]: (low beta) p=0.02, (high beta) p=0.02, (gamma) p=0.04; [3.4-4.2 s]: (low beta) p=0.02, (high beta) p=0.01, (gamma) p=0.02; [4.2-5.0 s]: (high beta) p=0.04, (gamma) p=0.03; [5.0-5.5 s]: (high beta) p=0.01, (gamma) p=0.03; [6.0-6.5 s]: (gamma) p<0.0001; [7.0-7.5 s]: (gamma) p=0.04, FDR-corrected, paired t-test). Our results indicate that both current amplitudes and oscillatory representations in the beta and gamma bands over multiple cortical regions may contribute to visual working memory functions.

Disclosures: **S. Yoshiiwa:** None. **H. Takano:** None. **K. Ido:** None. **M. Kawato:** None. **K. Morishige:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.15/XX71

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01 NS112252
NIH Grant T32 EB021937

Title: Reproducible state-dependent functional connectivity patterns of arousal and autonomic centers in the brainstem

Authors: *H. POURMOTABBED[1], C. G. MARTIN[2], S. E. GOODALE[1], D. J. DOSS[1], S. WANG[1], H. KANG[3], V. L. MORGAN[4], D. J. ENGLOT[5], C. CHANG[2];
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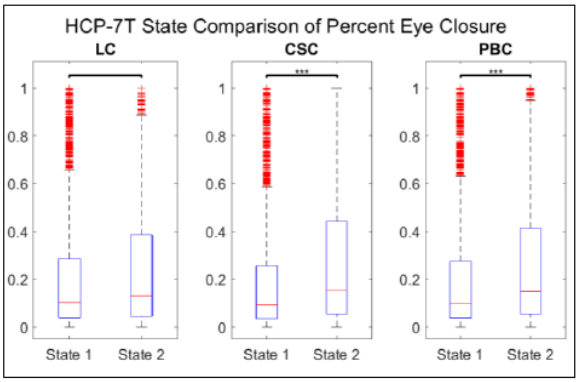
Abstract: Neuromodulatory centers of the brainstem mediate intrinsic fluctuations in brain activity across different arousal and autonomic states. Previous fMRI studies have investigated the functional connectivity (FC) of these structures in healthy and diseased populations, but it is not clear how their interactions are altered across vigilance (e.g., alert/drowsy) states. This study

aimed to leverage multimodal fMRI to examine the vigilance-dependent FC of brainstem nuclei. Due to SNR and localization challenges of these structures, we also evaluated the reproducibility across three datasets with different acquisition parameters. This study included pupillometry-fMRI (7T single-echo) from the Human Connectome Project (HCP; n = 176) and EEG-fMRI (3T multi-echo) from Vanderbilt University (VU; n = 30) and NIH (n = 9). For the VU and NIH datasets, VIGALL software was used to identify alert/drowsy epochs from the EEG, and the whole-brain FC of 9 brainstem regions was compared between alert and drowsy states. For the HCP, states were derived via *k*-means clustering on the windowed whole-brain FC of the nuclei, and the FC and percent eye closure were compared between states. The spatial overlap of the state-dependent FC difference maps across datasets was estimated with the Dice coefficient (DC). The locus coeruleus (LC), cuneiform/subcuneiform nucleus (CSC), and parabrachial nuclear complex (PBC) had the greatest vigilance-related FC alterations ($p < 0.05$) for the VU (**Fig. 1**). Two putative drowsy/alert states were identified for the HCP. State 2 had a significantly greater percent eye closure ($p <$ 1e-3) than state 1 for the CSC and PBC and a non-significantly greater percent eye closure ($p = 0.07$) for the LC. The nuclei had stronger FC to the thalamus and visual and somatomotor cortex in drowsy versus alert for the VU, and, likewise, in state 2 versus 1 for the HCP. The spatial overlap of the FC difference maps was moderate to strong (DC > 0.4) for most nuclei. Our results indicate that FC of brainstem nuclei is highly state dependent and that the state-dependence is reproducible across fMRI modalities.

Spatial Overlap of State-Dependent FC *t*-maps	Dice coefficient		
	LC	CSC	PBC
VU vs NIH	0.32	0.55	0.53
VU vs HCP-7T	0.55	0.62	0.62
NIH vs HCP-7T	0.38	0.62	0.57



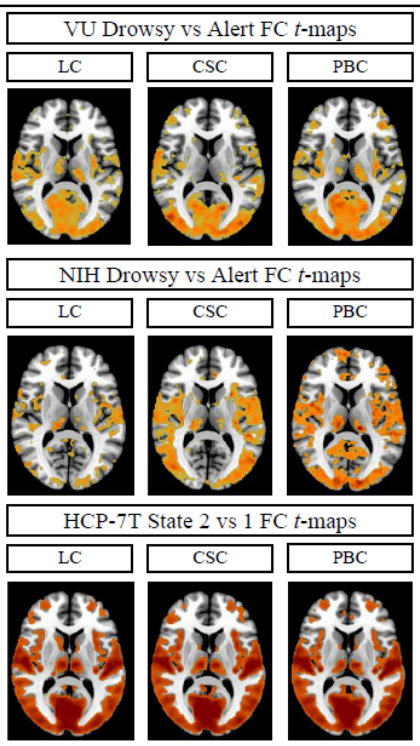


Fig. 1. [Right] State-dependent functional connectivity (FC) difference *t*-maps of the locus coeruleus (LC), cuneiform/subcuneiform nucleus (CSC), and parabrachial nuclear complex (PBC) (linear mixed-effects models; thresholded at 40% of the greatest *t*-values; minimum threshold of $p < 0.05$, whole-brain FDR-corrected). Positive values indicate greater FC in the drowsy compared to the alert state. [Top Left] Spatial overlap (Dice coefficient) of the FC difference *t*-maps of the LC, CSC, and PBC across the four datasets. [Bottom Left] Comparison of the percent eye closure between states for the HCP 7T dataset (linear mixed-effects model; $p < 0.05$, FDR-corrected).

Disclosures: **H. Pourmotabbed:** None. **C.G. Martin:** None. **S.E. Goodale:** None. **D.J. Doss:** None. **S. Wang:** None. **H. Kang:** None. **V.L. Morgan:** None. **D.J. Englot:** None. **C. Chang:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.16/XX72

Topic: I.07. Data Analysis and Statistics

Title: Unsupervised Decoding of Brain-States in fMRI Studies

Authors: *J. MISRA, L. PESSOA;
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Abstract: Traditional neuroscience research has been conducted under tight experimental control, which can limit the ecological validity of findings. Recent research has shifted towards more unconstrained experiments that allow for the exploration of freely unfolding behavioral patterns. However, establishing brain-behavior mappings in these types of experiments can be challenging, as it requires the manual or automatic annotation of behavioral/stimulus events that repeat across time and subjects. Switching Linear Dynamical Systems (SLDS) is a statistical framework that combines discrete states and linear dynamics, and has gained significant traction for the analysis of multivariate timeseries datasets with complex patterns and changing behaviors. In this work, we propose an unsupervised approach that directly leverages brain recordings to infer brain-states in time using the SLDS framework. Specifically, the model transforms brain-recordings into low-dimensional latent factors, whose evolution at each time step is governed by linear dynamics specific to the brain-state active at that instant. We used whole-brain BOLD fMRI activity recorded from 114 humans during a study involving dynamic threat processing in healthy individuals. We demonstrate that the sequence of brain-states contains information about the stimuli and the order of their presentation. We do this by finding associations between brain-states and different stimulus scenarios. We find that brain-states exhibit distinct distributed patterns of activity as well as functional coupling. In addition, upon visualizing the latent factors as trajectories, we found them to be separable across brain-states, suggesting that brain-states may be characterized by different neural mechanisms. Together, our results reveal generalizable, functionally relevant brain-states that reflect the stimulus structure in an fMRI study, thereby providing promise for applications in naturalistic neuroscience.

Disclosures: **J. Misra:** None. **L. Pessoa:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.17/XX73

Topic: I.07. Data Analysis and Statistics

Support: Agency of Defense Development (ADD) in Korea grant 915061201

Title: Generating and Translating Brain Signals for Motor Control Using Generative Adversarial Network

Authors: *H. KIM[1], J. KIM[2], C. CHUNG[3];
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Abstract: Understanding how the human brain processes these motor components is one of the significant interests in neuroscience. For this interest, current findings suggested that the cortical processing of motor kinematics appears differently depending on its behavioral characteristics (Kim, Kim, and Chung, 2023). Such findings had a practical implication to improve the decoding performance of brain-computer interfaces (BCI) predicting limb movement. However, constraints on neural data acquisition limit the utilization of this implication. For example, acquisition methods such as intracranial EEG (iEEG) have a high spatial resolution but limited coverage (Sejnowski, Churchland, & Movshon, 2014), making it difficult to obtain signals in brain areas essential for decoding behaviors. Furthermore, collecting enough data to train the complex artificial intelligence models is difficult. Here, we show a method for translating and generating the cortical signals of the motor area essential for producing motor kinematics, such as acceleration, from the other cortical signals that participate in motor processing but do not in producing. We developed the generative adversarial neural network (GAN) model based on the MelGAN (Kumar et al., 2019), which was introduced to translate signal data. We trained the model to learn the spectrotemporal features of the motor cortex's signal waveforms. When source signals of a motor-related area, such as the intraparietal sulcus (IPS), entered, the model inferred the signal waveforms of the motor cortex (M1) by translating the spectrotemporal characteristics of IPS into those of M1. The spectrotemporal signal features of generated signals contain the unique characteristics of motor areas. Furthermore, the acceleration trajectories of hand-reaching movement could be decoded from the generated signals. We conjecture that these findings may help to address the limitation by translating signals or augmenting neural datasets.

Disclosures: H. Kim: None. **J. Kim:** None. **C. Chung:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.18/XX74

Topic: I.07. Data Analysis and Statistics

Title: A spatially-resolved, single-cell analysis of human olfactory mucosa highlights transcriptional dysregulation in sustentacular cells with SARS-CoV-2 viral load

Authors: *E. METZGER[1], J. W. REEVES[1], E. KILLINGBECK[1], Y. LIANG[1], M. KHAN[2], J. BEECHEM[3], P. MOMBAERTS[4], L. VAN GERVEN[5];
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Abstract: Anosmia is a common symptom of COVID-19 and often persists well after the acute phase of the disease. The loss of smell is thought to be the result of the effects of SARS-CoV-2 on cell types that underly olfactory function. These cell types—which include sustentacular cells and olfactory sensory neurons—exist in the olfactory mucosa, which consists of an archipelago of islands surrounded by respiratory mucosa. Our earlier work using the GeoMx® Digital Spatial Profiler characterized whole-transcriptome effects of SARS-CoV-2 in the olfactory epithelium of a postmortem case at the spatial resolution of hundreds of cells. The transcriptional dysregulation of sustentacular cells we identified raises the question of which genes in sustentacular cells are altered as a result of viral load. We have now addressed this spatial single-cell question using the CosMx™ Spatial Molecular Imager. We built a spatially informed atlas from healthy and disease states. Our panel consisted of 984 host targets and 9 probes for SARS-CoV-2. In total, we measured 63,589,058 spatial transcripts in 401,233 cells. We classified cells into known cell types using a combination of seed profiles from publicly available scRNA-seq data, semi-supervised clustering, and visual inspection of cell classifications and comparison with histological expectations. The semi-supervised clustering algorithm allowed us to discern more nuanced cell types of the epithelium (olfactory vs. respiratory horizontal basal cells) and to identify cell types that were not classified in the reference data (such as suprabasal cells). Since SARS-CoV-2 infection results in degradation of host mRNAs in the host cells, our approach was flexible enough to capture heavily infected cell types not adequately reflected in the reference (*e.g.*, infected secretory cells and ciliated cells). Following classification, we focused on 725 sustentacular cells, grouped them into virus negative (535), low viral load (121), and high load (69), and found ~120 differentially expressed genes (DEGs). Differences between these groups contain “classic” DEGs such as TMPRSS2 and genes related to inflammatory or myeloid signaling. We conclude by discussing the role of sustentacular dysregulation in anosmia. While our atlas was able to answer the specific biological question herein, we are addressing a myriad of other scientific inquires related to viral infection and loss of smell. In total, our results underscore the importance of building spatially enriched transcriptomic atlases of both healthy and diseased states.

Disclosures: E. Metzger: A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stockholder. **J.W. Reeves:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

Stockholder. **E. Killingbeck:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stockholder. **Y. Liang:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stockholder. **M. Khan:** None. **J. Beechem:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stockholder. **P. Mombaerts:** None. **L. Van Gerven:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.19/XX75

Topic: I.07. Data Analysis and Statistics

Title: Whole-brain causal discovery using fMRI: improving scalability and accuracy

Authors: *F. ARAB[1], A. GHASSAMI[2], H. JAMALABADI[3], M. A. PETERS[4], E. NOZARI[1]; [1]Univ. of California, Riverside, Riverside, CA; [2]Boston Univ., Boston, MA; [3]Phillips Univ. of Marburg, Marburg, Germany; [4]Univ. of California, Irvine, Irvine, CA

Abstract: The understanding of causal relationships within the human brain is challenging due to several factors, including a lack of easily accessible and mechanistically understood interventional data. Functional MRI (fMRI) provides valuable observational data for causal discovery but also presents challenges due to high dimensionality, low temporal resolution, and indirect measurements of neural processes. Previous research in the field primarily relies on causal discovery techniques such as Granger causality and Dynamic Causal Modeling (DCM) to address the temporal structure within fMRI data. However, when confronted with complexities such as contemporaneous effects or latent common causes, the results obtained from these methods may lack causal interpretability. In contrast, majority of the modern alternative causal discovery approaches either do not cater specifically to time series data or possess limitations in their ability to: (1) handle cycles in the network, (2) scale to large networks, and/or (3) handle the presence of latent nodes. The absence of ground-truth connectivity has further impeded clear comparisons among existing methods for applications over neuroimaging data. In this study, we first provide a comprehensive comparison of existing causal discovery techniques suitable for whole-brain fMRI based on each method's theoretical properties and numerical outcomes on a wide range of simulated fMRI. This motivates the introduction of a new method that improves upon the PCMCI algorithm (Runge et al, Sci. Adv., 2019) in terms of computational efficiency, directionality information, and correction for temporal multiple comparisons. We demonstrate the accuracy of this method against several state-of-the-art approaches using simulated fMRI. We then use the proposed method to estimate causal connectomes from resting state Human

Connectome Project fMRI and demonstrate its ability to learn causal graphs that (1) are highly consistent across subjects, (2) are consistent with known resting state dynamics, (3) are sparser and more consistent across subjects compared to functional connectivity, (4) properly reflect the temporal structure of fMRI data and the importance of contemporaneous effects, (5) capture Euclidean distance-dependence in causal interactions, and (6) demonstrate statistically significant laterality and gender differences in degree distributions (but not causal flows). Overall, our results validate the power of the proposed method in detecting causal interactions from resting state fMRI and open myriad avenues for future investigations of causal discovery from task and stimulation-induced neuroimaging data.

Disclosures: F. Arab: None. **A. Ghassami:** None. **H. Jamalabadi:** None. **M.A. Peters:** None. **E. Nozari:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.20/XX76

Topic: I.07. Data Analysis and Statistics

Support: R01MH118239
R01MH132806

Title: Using cell fractions to enhance elastic net models for gene expression imputation in the amygdala, with implications for network analysis

Authors: *J. DRAKE, Jr.[1,2], Z. TAYLOR[2], A. DENHAM[1], S.-A. BACANU[3], J. SHIN[4], T. HYDE[4], V. I. VLADIMIROV[2];
[1]Texas A&M Univ., College Station, TX; [2]Univ. of Arizona, Phoenix, AZ; [3]Virginia Inst. for Psychiatric and Behavioral Genet., Richmond, VA; [4]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Background: GE imputation methods have gained increasing popularity as an alternative methods of measuring brain gene expression (GE). PrediXcan is one such method; however, a limited number of genes are imputed accurately, which limits its application in network analysis. To address this limitation, we have expanded on PrediXcan's elastic net methodology by incorporating neuronal cell fraction estimates to impute additional genes with higher accuracy. Weighted gene co-expression network analysis (WGCNA) was used to evaluate the utility of the imputed GE in network analysis, and gene set enrichment analysis (GSEA) was used to assess the gene ontology (GO) terms associated with the imputed GE modules.
Methods: For our GE imputation, we used a postmortem brain sample containing genotype and RNA-Seq data generated in the amygdala from 180 neurotypical, 234 major depressive disorder (MDD), and 122 bipolar disorder (BIP) subjects, with a total N=536. To minimize overfitting,

we split the sample into 70% training and 30% testing datasets, with both the training and testing sets containing proportionate phenotype distribution. We used glmnet to create 19,948 mRNA elastic net models with 10-fold cross-validation. Considering the importance of cell-specific gene expression, we used a single-cell reference dataset to deconvolute the bulk GE into 17 cell fractions, which were then incorporated into our models. We retained only models with an average coefficient of determination (R^2) > 0, Z-score p-value < 0.05, and average correlation between the imputed and measured expression > 0.10.
Results: At an average R^2 of 0.134, 14,058 gene models survived, which was used to impute GE in the testing dataset. In WGCNA, scale-free topology was reached with 38 imputed modules in the MDD subjects and 39 modules in the BIP subjects. For the MDD analysis, 12 modules were associated with MDD at a nominal p-value ≤ 0.05, and 8 showed preservation, which included 1 of the disease-associated modules (i.e., bisque4). For the BIP analysis, 4 modules were associated with BIP at a nominal p-value, and 8 modules showed preservation, which included 2 of the disease-associated modules (i.e., green and midnightblue). Enrichment for major depressive disorder (FDR = 0.002) and brain aging (FDR = 0.003) was observed in the green and midnightblue modules, respectfully.
Conclusion: The availability of expression data in the target sample is needed for deconvolution and subsequent imputation, requiring alternative approaches for cell fraction deconvolution. Nevertheless, here we show that incorporating cell fraction into the GE imputation greatly improves the accuracy and number of imputed genes.

Disclosures: **J. Drake:** None. **Z. Taylor:** None. **A. Denham:** None. **S. Bacanu:** None. **J. Shin:** None. **T. Hyde:** None. **V.I. Vladimirov:** None.

Poster

PSTR245. Data Analysis and Computation: Network Models, Machine Learning, Omics, Decoding

Location: WCC Halls A-C

Time: Monday, November 13, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR245.21/XX77

Topic: I.07. Data Analysis and Statistics

Support: K01DA043615
T32AG04968808

Title: School Protective Factors, Religiosity, Screentime, and Neighborhood Conditions Predict Alcohol Initiation in pre-Adolescents, an eXposome Wide Association Study (XWAS) in the Adolescent Brain Cognitive Development (ABCD) Study

Authors: *F. ADAMS[1], Y. HE[2], I. IVANOV[3], C. J. PATEL[2], M. PARVAZ[3];
[1]Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [2]Harvard Med. Sch., Boston, MA; [3]Dept. of Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Youth who initiate drinking before age 15 are seven times more likely to develop alcohol use dependence. Despite growing concerns regarding adolescent alcohol use onset, our understanding of the nature of alcohol use during this vulnerable period is not fully elucidated. The exposome concept comprehensively assesses non-genetic factors linked to adolescent alcohol initiation. We performed an eXposome-Wide Association Study (XWAS) using the Adolescent Brain Cognitive Development (ABCD) Study (n = 11,876; ages 9-10 years old). We identified 322 exposomic features that summarize youth's lifestyle-, home-, culture-, neighborhood-, and school-related environments. We performed univariate logistic regressions between individual exposures and alcohol initiation (alcohol naive; n = 8,749, alcohol initiators; n = 2,017) while adjusting for age, sex, race/ethnicity, and data collection site and were corrected for false-discovery rate (FDR). Of the 322 exposomic variables, 17 significantly differentiated alcohol initiators and alcohol naïve youth at ages 9-10 years old ($p<0.05$). Notably, higher school involvement ($b=-0.0142$, $p<0.001$), school engagement ($b=-0.0098$, $p<0.001$), religiosity ($b=-0.0204$, $p<0.001$), and parental acceptance ($b=-0.0385$, $p<0.001$) were negatively associated with early alcohol initiation. Whereas more screen time exposure on weekends to R-rated movies ($b=0.0517$, $p<0.001$), and higher levels of high college enrollment ($b=0.0226$, $p<0.001$) and high skill employment ($b=0.0234$, $p<0.001$) in one's neighborhood were positively associated with early alcohol initiation in adolescents. The observed associations, particularly between alcohol initiation and school engagement and involvement, highlight the importance of improving school programs to keep youth actively engaged. The influence of culture and religion on alcohol initiation emphasizes the significance of considering these factors in prevention efforts. The results underscore the need for interventions targeting safe household practices, as higher income and more educated families are more likely to see alcohol initiation among youth. Encouragingly, these predictors present as modifiable targets and offer valuable guidance for policy interventions and initiatives aimed at reducing early alcohol initiation and promoting healthier outcomes for youth.

Disclosures: **F. Adams:** None. **Y. He:** None. **I. Ivanov:** None. **C.J. Patel:** None. **M. Parvaz:** None.