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Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: BX002452
NS113763

Title: Identifying key regulators in the direct conversion of human skin fibroblasts to neurons by gene regulatory network analysis

Authors: *L. LI[1], B. ZHU[1,2], J. FENG[1,2];
[1]State Univ. of New York, Buffalo, Buffalo, NY; [2]Veterans Affairs Western New York Healthcare Syst., Buffalo, NY

Abstract: Transdifferentiation between cell types has relied on knowledge-based search for optimal reprogramming factors. Our recent study found that the overexpression of ASCL1, miR9/9*-124, nPTB shRNA, and p53 shRNA efficiently converted human skin fibroblasts to neurons. By analyzing longitudinal RNA-seq data of human skin fibroblasts being converted with various combinations of these reprogramming factors, we constructed gene regulatory network (GRN) models capturing the high order information important for neuronal conversion. Examination of gene communities and transcription factors (TFs) in the GRNs identified OTX2 and LMX1A as the key regulators of conversion to neurons, as they had strongest connections to genes functionally associated with neuronal development and differentiation. We confirmed the critical roles of OTX2 and LMX1A experimentally as their knockdown markedly impaired the conversion. The study shows that GRN models are effective in augmenting empirical discovery of optimal reprogramming factors in the transdifferentiation of human skin fibroblasts to neurons. Further improvements in this approach may identify a generally applicable principle for direct cell-fate conversion.

Disclosures: L. Li: None. **B. Zhu:** None. **J. Feng:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.02/A2

Topic: A.08. Development of Neural Systems

Title: Optimization of a 3D “brain-in-a-dish” tri-culture model using isogenic iPSC-derived neurons, astrocytes, and microglia

Authors: *C. CARLSON, R. K. FIENE, C. A. SAVIC, M. E. DONEGAN, M. K. LIVINGSTON, S. SCHACHTELE;
FUJIFILM Cell. Dynamics, Madison, WI

Abstract: The use of 3D neural spheroids (or “neurospheres”) is a rapidly developing technology with great potential for increased understanding of neurodegenerative diseases and neuroinflammation. However, creation of an in vitro 3D cell culture system that incorporates microglia, the resident immune cell type in the brain, has been restricted by various technical limitations. While some protocols to generate brain organoids have shown *de novo* microglia differentiation, these methods are lengthy, challenging, and not always consistent. Recently, techniques to assemble neurospheres using terminally differentiated induced pluripotent stem cell (iPSC)-derived neural cells have emerged, enabling a shortened and modular approach to create 3D “brain-in-a-dish” models. In this study, we first demonstrate how human iPSC-derived neurons and astrocytes can be mixed together in defined ratios in ultra-low attachment (ULA) plates to generate 3D neurospheres that are functional in high-throughput calcium oscillation assays. Next, we developed a human iPSC-derived microglia cell that constitutively expresses green fluorescent protein (GFP). This cell type can be utilized to visualize microglia incorporation into the 3D neurospheres and aids in the optimization of culture conditions that support survival and function of all three cell types. Specifically, we were able to investigate numerous variables, including timing of microglia addition, ratios of individual cell types, identity of different neuronal cell types, formulation of media and supplements, addition of extracellular matrix, ULA plate types, and calcium assay reagents. Live-cell imaging (Incucyte SX5) and functional testing (FDSS/µCell) provided rapid screening for conditions favorable to GFP-microglia infiltration and other factors that influence the success or failure of such an experiment. Microglia incorporation and distribution within neurospheres was characterized further using immunostaining. In addition, we validated this model as a model for neuroinflammation by treating microglia-containing neurospheres with inflammatory stimuli (LPS, IFN-gamma) and evaluating multiple parameters indicative of microglia activation, including cytokine release (HTRF assays). Overall, we present the extensive optimization of conditions that facilitate iPSC-derived microglia incorporation into iPSC-derived 3D tri-culture model, providing an advanced isogenic “brain-in-a-dish” 3D model of neuroinflammation that is useful in various applications and is amenable to high-throughput screening workflows.

Disclosures: C. Carlson: A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **R.K. Fiene:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **C.A. Savic:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **M.E. Donegan:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **M.K. Livingston:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **S. Schachtele:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.03/A3

Topic: A.08. Development of Neural Systems

Support: Work supported by #NEXTGENERATIONEU (NGEU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – (DN. 1553 11.10.2022)

Title: Chemical modulation on in vitro cortical-hippocampal models

Authors: *F. POGGIO, M. BROFIGA, F. CALLEGARI, M. TEDESCO, P. MASSOBRIO; Univ. of Genoa, Genova, Italy

Abstract: The brain is the most complex system of our body. One of the main challenges of neuroengineering is developing an *in vitro* model that allows studying the human brain in its complexity. It is characterized by different neuronal populations (e.g., cortical, hippocampal, thalamic neurons) that interact following well-defined principles of connectivity. *In vitro* models, where dissociated neurons are chronically coupled to Micro-Electrode Arrays (MEAs), allow to explore many neuronal network properties including the pharmacological and toxicological effects on the spontaneous activity of excitable cells like neuronal networks. Nowadays, *in vitro* pre-clinical drug tests are performed on homogenous and non-modular *in vitro* models that did not recreate heterogeneity and modularity features of the *in vivo* microenvironment. On the present work we investigated whether the introduction of modularity and heterogeneity would determine different results (with respect to the conventional homogeneous models) when applying a chemical stimulation. In particular, we evaluated the effects of bicuculline (BIC), a competitive antagonist of $GABA_A$ receptors, on the firing activity of modular heterogeneous, modular homogeneous, and non-modular homogeneous networks. We computed a dose-response curve for each configuration, and then we extracted their IC_{50} value. IC_{50} values of the heterogeneous configuration were statistically lower than the ones of homogenous modular networks, both in the case of cortical and hippocampal assemblies. A statistical difference in terms of cortical IC_{50} emerged also among homogeneous non-modular and heterogeneous cortical configurations (which had the lowest values, indicating an anticipation of the effect). Instead, in the hippocampal case, another difference was highlighted by the addition of the modularity: modular IC_{50} resulted to be significantly higher than non-modular controls. These results showed clear differences between modular, homogeneous, and heterogeneous cultures, suggesting that ignoring modularity and heterogeneity could lead to underestimate or overestimate the effect of a drug and therefore they should be included in *in vitro* neuronal model adopted to perform pharmacological studies.

Disclosures: F. Poggio: None. **M. Brofiga:** None. **F. Callegari:** None. **M. Tedesco:** None. **P. Massobrio:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.04/Web Only

Topic: A.01. Neurogenesis and Gliogenesis

Title: Taurine analogs promoteneuronal progenitor cells´neuronal differentiation from subventricular zone

Authors: *E. SOLARES;
Ctr. de Investigación y Estudios Avanzados, Ciudad de México, Mexico

Abstract: Taurine analogs promotes neural progenitor cells' neuronal differentiation from the subventricular zone.
AuthorsEva M. Solares-Rioja, Emilio J. Galván, Lenin D. Ochoa-de la Paz, Nadia Estefanía Gutiérrez Castañeda, Luis Roberto Olvera.Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Universidad Nacional Autónoma de México.
Given the relevance to physiological processes such as senescence, neurogenesis continues to garner considerable interest, and the possibility of regulating the neurogenic process with compounds relevant to neurodegeneration and aging is an engaging endeavor for neuroscience. Taurine is a is a non-essential amino sulfonic acid widely-distributed in the central nervous system, involved in multiple physiological processes, including neuronal differentiation. However, the role of the taurine homologs, including homotaurine and hypotaurine in the process of neuronal differentiation and neurogenesis is virtually unknown. Here we show the effects of homotaurine and hypotaurine on the neuronal differentiation process. 6-day-old CD1 mice were used to obtain neuronal progenitor cells (NPC) from the subventricular zone (SVZ). Neurosphere cultures were obtained from NPC-SVZ and complemented with homotaurine (2.5 mM) or hypotaurine (10 mM). Immunofluorescence assays and morphometric analyses were used to evaluate neuronal differentiation. Immunofluorescence assays revealed that homotaurine and hypotaurine increases the number of doublecortin-positive cells (immature neurons), compared to the control group. A morphometric analysis revealed that both analogs promote neuronal complexity, increasing the number of dendritic ramifications. Patch-clamp recordings revealed spikes with kinetic properties similar to the action potentials of functional neurons. This work provides experimental evidence that taurine analogs are promoters of neuronal differentiation.

Disclosures: E. Solares: None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.05/A4

Topic: A.01. Neurogenesis and Gliogenesis

Title: Neurotoxicity and Drug Screening Assay Characterization in Healthy and Progranulin R493X HZ KO iPSC-derived Induced Excitatory Neurons

Authors: J. MA[1], R. A. BRADLEY[1], R. K. FIENE[1], C. HOGAN[1], C. B. CARLSON[1], S. A. HILCOVE[1], S. SCHACHTELE[1], *E. JONES[2], J. LIU[1];
[1]FUJIFILM Cell. Dynamics, Madison, WI; [2]FUJIFILM Cell. Dynamics, Laguna Hills, CA

Abstract: Neurogenin-2 (NGN2) forward programming of human induced pluripotent stem cells (iPSCs) offers a robust method for generating scalable quantities of neurons with low lot-to-lot variability. Using this methodology, we generated highly pure excitatory glutamatergic neurons (iCell Induced Excitatory Neurons) at commercial scale from iPSC lines with an apparently healthy normal (AHN) background or a heterozygous (HZ) and pathogenic R493X nonsense mutation in the progranulin gene (GRN) to model frontotemporal dementia (FTD). These induced cells are highly pure neurons (>90% βIII-Tubulin-positive) and express excitatory glutamatergic genes, including vesicular glutamate transporters (VGLUT) and AMPA receptor subunits (GRIA). We verified that these characteristic markers are expressed consistently across lots and confirmed that a reduction in granulin monomers in the GRN R493X cell line was observed. In the current study, we evaluated the suitability of these induced excitatory neurons for high-throughput neurotoxicity and drug screening experiments, including neurite outgrowth (Incucyte), multielectrode array (MEA), calcium imaging, and cell survival assays. Within each assay we established a baseline comparison between the AHN and GRN R493X HZ KO induced excitatory neurons to identify and characterize differences in phenotypes. Notably, differences in MEA activity development were detected, with GRN R493X HZ KO displaying aberrant network synchrony compared to AHN neurons. These baseline metrics of survival, neurite outgrowth, and activity were then challenged via treatment with a panel of neurotoxic compounds or chemotherapeutic agents to determine dose responses across high throughput assays. These studies demonstrate the high-throughput utility and biological relevance of induced excitatory neurons across numerous neurotoxicity assays, suggesting these cells offer a platform for early drug screening and disease modeling.

Disclosures: **J. Ma:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **R.A. Bradley:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **R.K. Fiene:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **C. Hogan:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **C.B. Carlson:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **S.A. Hilcove:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **S. Schachtele:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **E. Jones:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **J. Liu:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.06/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: Intramural program of NIAAA

Title: Neurodifferentiation impaired by ethanol and reversed by GPR110 activation in mouse- and human-neural stem cell culture models and in vivo.

Authors: *G. K. PARRA-MERCADO, J. BENCZE, Y. JOO, H.-Y. KIM;
NIH, Natl. Inst. on Alcohol Abuse & Alcohol NIAAA, Rockville, MD

Abstract: Ethanol (EtOH) consumption during pregnancy can adversely affect developing fetus, causing craniofacial and neuro-behavioral abnormalities often presented in fetal alcohol spectrum disorders (FASDs) in humans. The severity of the effects may depend on the disruption of fetal brain development including proliferation, differentiation, migration, and maturation of neural cells. EtOH intake was shown to inhibit the proliferation of neural precursor or stem cells in developing brains, as well as adult neurogenesis in part due to the reduction of cAMP signaling cascade. Previously, we have found that *N*-docosahexaenoylethanolamine (synaptamide, Syn), an endogenous metabolite of docosahexaenoic acid, promotes neurogenesis, neuritogenesis, and synaptogenesis at low nanomolar concentrations by activating its target receptor GPR110 and cAMP/PKA signaling in developing mouse neurons. In this study, we investigated the relevance of GPR110 activation in ethanol-induced developmental abnormalities in cultured primary neural cells as well as *in vivo*. Mouse neural stem cells (mNSC) were prepared from GPR110 WT and KO fetal mouse brains at E14.5, and human neural progenitor cells (hNPCs) were derived from WT iPSC and a mutant iPSC line devoid of GPR110 activity. Effects of EtOH and Syn on *in vivo* neurogenesis were examined by FACS analysis of 3-day old offspring brain samples after BrdU (100 mg/kg) labeling on E13, followed by EtOH gavage (1.5 g/kg) and Syn treatment (20 mg/kg) twice on E14 and E16. We found that both mNSCs and hNPCs express GPR110, and synaptamide dose- and GPR110-dependently increases cAMP levels. Immunolabeling showed that daily exposure of mNSCs or hNPCs to 25-50 mM EtOH for 4 days (DIV1-4) resulted in a significant reduction of neurodifferentiation markers such as MAP2 and beta-III tubulin (Tuj1) and less neurite ramification compared to control cultures. This EtOH effect was reversed by 10 nM Syn treatment, increasing the Tuj1-expressing neurons in WT cultures. *In vivo* neurogenesis experiment also indicated that EtOH significantly decreases the percentage of NeuN+/BrdU+ nuclei in pup brains while Syn treatment restores ethanol-induced impairment of neurogenesis. Taken together, the exposure to EtOH in an early stage of neurodevelopment impairs neurogenic differentiation and maturation in mouse and human cell culture models as well as *in vivo*. Importantly, GPR110 activation by synaptamide reverted the neurodevelopmental deficit caused by ethanol, presenting a potential strategy to ameliorate the adverse impact of perinatal exposure to ethanol.

Disclosures: G.K. Parra-Mercado: None. **J. Bencze:** None. **Y. Joo:** None. **H. Kim:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.07/A6

Topic: A.08. Development of Neural Systems

Support: Work supported by #NEXTGENERATIONEU (NGEU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – (DN. 1553 11.10.2022)

Title: Chitosan: an alternative adhesion factor for the neuronal differentiation of hiPSCs

Authors: *S. GRASSELLI, D. DI LISA, A. ANDOLFI, P. FERRARI, S. MARTINOIA, L. PASTORINO;
Univ. of Genoa, Genoa, Italy

Abstract: The use of human induced pluripotent stem cells (hiPSCs) derived neurons is paving the way for the study of patient-specific conditions. Typically, hiPSCs are expanded and differentiated onto Matrigel coated substrates. However, to obtain a functional neural network, differentiated cells need to be transferred to laminin or polyornithine coated substrates, undergoing great stress. For this reason, finding an adhesion factor that supports both the differentiation and maturation of the neural network is of crucial importance. Natural polymers are suitable alternatives thanks to their intrinsic biocompatibility and affinity to the extracellular matrix. Among these, chitosan holds great promise, being abundant and cost-effective. Moreover, chitosan has been used in different studies to support the formation of neuronal networks *in vitro*.
A comparison between Matrigel and chitosan was carried out. Cell survival and differentiation was monitored *via* immunocytochemistry and western blot analysis at different time points over 14 days of culture. Cells were stained with SOX2 or Nestin (stemness markers) and MAP2 or β-III Tubulin (neuronal markers).
Chitosan coated micro-electrode arrays (MEAs) were used to evaluate the electrophysiological activity of neural networks after neuronal induction. Mean firing rate, number of active electrodes and mean bursting rate were evaluated.
No discrepancy between cells differentiated on Matrigel and chitosan was evidenced by immunofluorescence, proving chitosan is a suitable adhesion factor for hiPSCs differentiation. Images showed a gradual decrease in SOX2 and Nestin expression, with an increase in the expression of neuronal markers over time. Results were confirmed by western blot. Moreover, it was possible to record electrophysiological activity of hiPSCs differentiated on chitosan coated MEAs at different time points (until DIV 45), highlighting the progressive maturation of a functional neural network.
Considering our results, chitosan holds great promise as an alternative to Matrigel. The advantages are several: chitosan is a far cost-effective material compared to Matrigel, its composition is not as variable and hiPSCs can differentiate and form a network on the same substrate. Moreover, chitosan can be processed to obtain a 3D cell-laden hydrogel allowing an easy translation from 2D to 3D cell studies.

Disclosures: **S. Grasselli:** None. **D. Di Lisa:** None. **A. Andolfi:** None. **P. Ferrari:** None. **S. Martinoia:** None. **L. Pastorino:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.08/A7

Topic: A.08. Development of Neural Systems

Support: Work supported by #NEXTGENERATIONEU (NGEU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – (DN. 1553 11.10.2022)

Title: Investigating the impact of excitation/inhibition balance in human iPSCs-derived neuronal networks during long-term development on MEAs

Authors: *G. PARODI[1], G. ZANINI[2], M. BROFIGA[2], V. PASTORE[2], M. CHIAPPALONE[2], S. MARTINOIA[2];
[1]DIBRIS, [2]Univ. of Genoa, Genova, Italy

Abstract: In the brain cortex, the neuronal activity is controlled by a well-defined interplay between excitation (E) and inhibition (I), defined as 'E/I balance'. The alteration of the physiological E/I balance can lead to the insurgence of neurodevelopmental disorder, such as autism spectrum disorders, epilepsy, and schizophrenia. The incidence of such genetic diseases has become extraordinarily high, involving approximately 1 in 150 people, making the E/I balance investigation a fundamental topic in modern neuroscience. The aim of this work is to explore how the E/I balance impacts the spontaneous activity of *in vitro* human-derived neural networks. To reach the goal, we performed long-term (~100 days) recordings using Micro-Electrode Arrays (MEAs) of both homogeneous (only excitatory or inhibitory neurons) and heterogeneous (mixed neurons) cultures with controlled E/I ratios. In particular, we successfully realized five different configurations by modifying the glutamatergic and GABAergic percentages (i.e., E:I 0:100, 25:75, 50:50, 75:25, 100:0), proving that we are able to control the E/I ratios and to maintain them for more than 3 months. By computing descriptive features of the electrophysiological activity, we showed that the homogeneous cultures act differently from the heterogeneous configurations. In particular, the inhibitory networks exhibit a pronounced tonic firing, lacking in the organization into bursts, while the excitatory cultures showed parameters representative of spiking, bursting and network bursting activity that deviate from the heterogeneous networks' behaviour. On the other hand, we proved that decreased inhibition in the heterogeneous networks affects the duration and the organization of the bursting and network bursting activity. In our work we presented a reliable *in vitro* system based on h-iPSC-derived neurons, in which, by controlling the ratio between excitation and inhibition, we could investigate the role of the E/I balance in the network dynamics. Our model is suitable for

translational studies and precision medicine applications in the field of neurodevelopmental disorders where the physiological E/I ratio is disrupted or impaired.

Disclosures: **G. Parodi:** None. **G. Zanini:** None. **M. Brofiga:** None. **V. Pastore:** None. **M. Chiappalone:** None. **S. Martinoia:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.09/A8

Topic: A.08. Development of Neural Systems

Support: Work supported by #NEXTGENERATIONEU (NGEU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – (DN. 1553 11.10.2022)

Title: Toward the brain-on-chip models: role of modularity, heterogeneity and three dimensionality on the electrophysiological activity

Authors: *M. BROFIGA, F. CALLEGARI, F. POGGIO, I. DONATI DELLA LUNGA, L. CERUTTI, M. TEDESCO, P. MASSOBRIO;
Univ. of Genoa, Genova, Italy

Abstract: The human brain comprises about 86 billion neurons that originate rich and intricate networks. Neurons are organized in well-defined spatial locations that define clusters of neurons (modules). Each cognitive and motor function is possible thanks to the correct interaction among the different modules. The disruption, loss, or alteration of these connections can produce pathological conditions. To understand how the information is transmitted and computed, we need to investigate the communication among the different modules. Another essential feature to comprehend how the electrophysiological signals are transmitted is the three-dimensional spatial organization. There are different approaches to investigating the transmission of the information: many studies use in vivo approach but, in this case, it is very difficult to study a specific circuit. Thus, the module activity we observe is always influenced by all its inputs. In this perspective, in vitro engineered models could be a powerful tool. They reduce the complexity of the system, keeping the key features of the in vivo environment of the brain, thus ensuring consistent results. In this work, we exploited polymeric masks coupled to Micro-Electrode Arrays to investigate the role of the key features of the human brain in the electrophysiological activity of in vitro neuronal networks. Studying the cortical-hippocampal circuit, we observed a more scattered signal propagation when the hippocampal population was present. We explained such behaviour by investigating the functional connectivity: in the cortical-hippocampal configuration, a higher segregation was observed. Moreover, the inhibitory hippocampal connections strongly modulated the cortical activity. Finally, we introduced the third feature, three-dimensionality,

recreating a wider pattern of activity with respect bidimensional networks. In perspective, we moved one more step towards recreating the mechanical properties of the extracellular matrix by building cortical and hippocampal spheroids, which are able to better reproduce the intrinsic characteristics of the native tissues, and which can be combined to create different interacting neuronal modules. This new model can be exploited as platform for precision medicine.

Disclosures: **M. Brofiga:** None. **F. Callegari:** None. **F. Poggio:** None. **I. Donati della Lunga:** None. **L. Cerutti:** None. **M. Tedesco:** None. **P. Massobrio:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.10/A9

Topic: A.08. Development of Neural Systems

Support: Work supported by #NEXTGENERATIONEU (NGEU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – (DN. 1553 11.10.2022)

Title: Exploring the impact of electrical stimulation on glutamatergic neuronal networks derived from h-iPSCs

Authors: *G. ZANINI, G. PARODI, M. CHIAPPALONE, S. MARTINOIA;
Univ. of Genoa, Genova (GE), Italy

Abstract: *In vitro* neuronal network models provide a valuable tool for gaining deeper insights into the complex processes of the human brain. Specifically, the use of human induced pluripotent stem cells (h-iPSCs) coupled to Micro-Electrode Arrays (MEAs) offers the possibility to replicate and analyze some of the structural and functional characteristics of the human brain on an engineered *in vitro* platform. Since neurons communicate through electrical and chemical stimuli, electrical stimulation can be used to better understand the electrophysiological dynamics of neuronal networks. However, there is currently a lack of standardized stimulation protocols for neuronal cultures derived from h-iPSCs, resulting in the absence of clearly identified effective parameters to evoke an electrophysiological response. In this work, we used cortical glutamatergic neurons derived from h-iPSCs and embryonic rat astrocytes coupled to MEAs to characterize the network response with respect to different voltage amplitudes applied to single microelectrodes. Specifically, the cultures were stimulated with biphasic voltage pulse at different amplitudes (1.5 V, 2 V, 2.5 V, 3 V) with a duration of 200 μs and a low-frequency stimulation (i.e., 0.2 Hz). Analyzing the evoked neuronal activity, we found that bursts were aligned with stimuli when the neuronal network was stimulated with pulses of amplitude higher than 2 V. At lower stimulation amplitudes, the bursts occurred independently of the timing of the stimuli. We also found that the Network Burst Duration

(NBD) decreased when the voltage amplitude stimulation increased, while the Network Burst Rate (NBR) increased when the voltage amplitude stimulation increased. The achieved results suggest that stimulation is more effective as stimulation amplitude increases, with the most significant impact observed at an amplitude of 3 V. This study constituted a starting point for determining other effective parameters for stimulation and deepening the electrophysiological activity characterization of neural networks derived from h-iPSCs.

Disclosures: **G. Zanini:** None. **G. Parodi:** None. **M. Chiappalone:** None. **S. Martinoia:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.11/Web Only

Topic: A.01. Neurogenesis and Gliogenesis

Title: Impact of Zika virus infection on the differentiation of a human neural stem cell line

Authors: *E. RUBIO[1], M. COMAS[2], C. CASTILLO[3];
[1]Unidad de Investigación en Virología y Cáncer, Hosp. Infantil de Mexico, Ciudad de México, Mexico; [2]Facultad de Ciencias, [3]Facultad de Medicina-CIACYT, Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico

Abstract: Perinatal viral infections can cause neurodevelopmental disorders with varying degrees of cognitive and mental sequelae. One virus that affects neurodevelopment is the Zika Virus (ZIKV), which can cause microcephaly and other congenital malformations during pregnancy. Despite the severity of neurodevelopmental impairments associated with ZIKV infection during pregnancy, little is known about the neuropathogenic mechanisms responsible for central nervous system malformations and dysfunctions. In this study, our main objective was to investigate a ZIKV infection model using human neural progenitor cells, specifically the hNS-1 cell line, which can differentiate into neurons, astrocytes, and oligodendrocytes. After infecting the cells with ZIKV, we found that oxidative stress and metabolic activity increased in the infected immature cells. However, no changes were observed in cells at a certain degree of maturity (3, 5, and 7 days of differentiation). Furthermore, we measured the viral production kinetics for five days. We observed an increase in viral production over time in the immature cells, and cells differentiated for three days but not for five and seven days. We also evaluated neural differentiation by measuring the expression of GFAP (astrocyte marker) and β-Tubulin III (neuron marker) in undifferentiated hNS-1 cells infected with ZIKV. We found increased GFAP messenger RNA expression in infected cells at 9 and 15 days of differentiation, suggesting changes in the astrocyte population. Based on these changes in GFAP expression, we isolated the astrocyte population from a 21-day differentiated hNS-1 cell culture and demonstrated that ZIKV infection reduces cell viability, increases the production of reactive oxygen species (ROS), and results in high viral titers. Additionally, we observed changes in the expression of genes involved in viral entry into cells and genes related to glutamatergic system homeostasis. Our findings

provide new evidence on how ZIKV infects neural progenitor cells and its dependence on cell differentiation. We also highlight the modification of GFAP expression and the potential functionality of astrocytes. These results contribute to a better understanding of the pathophysiology of congenital ZIKV-associated disease.

Disclosures: **E. Rubio:** None. **M. Comas:** None. **C. Castillo:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.12/A10

Topic: A.01. Neurogenesis and Gliogenesis

Support: Phase II SBIR grant from NIH/NIMH R44MH119621

Title: Anti-retroviral therapy is toxic to iPSC-derived NPCs in vitro and alters neuronal calcium transients in differentiated NPCs.

Authors: *C. G. RINES[1], K. L. GORDON[1], N. SUAREZ[1], A. S. SMITH[1], K. L. JORDAN-SCIUTTO[2], J. H. PRICE[1], P. M. MCDONOUGH[1];
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Abstract: HIV-positive (HIV+) pregnant women are given combination anti-retroviral therapy (cART) throughout pregnancy for maternal health and prevention of perinatal transmission of HIV. While treatment with cART has improved the life expectancy of many HIV+ individuals, there is growing concern that these drugs are neurotoxic and contribute to the development of HIV-associated neurocognitive disorders (HAND). Furthermore, little is known about the effect of cART on the developing brain. Previous research has linked the HIV integrase inhibitor Dolutegravir to fetal neural tube defects, and there may be other anti-retrovirals (ARVs) that impact fetal neurodevelopment. It is critical to optimize assays to test for ARV effects on neurodevelopment. We describe a suite of 384-well screening assays that we have developed for this purpose. The first assay tests ARV treatment on human induced pluripotent stem cell (hiPSC)-derived neural progenitor cells (NPCs) for effects on cell viability and self-renewal. Using this assay we have demonstrated toxicity from Elvitegravir and Dolutegravir alone or in combination with Emtricitabine. Additionally, we investigated Biktarvy, a combination drug consisting of Bictegravir, Tenofovir Alafenamide and Emtricitabine given to HIV+ pregnant women. We found that a 3-day C_{max} treatment of Biktarvy was toxic to two separate NPC lines (made in-house and Elixirgen Scientific). These results suggest that cART can affect neural progenitor survival in vitro and may also affect pathways associated with neurogenesis. The second assay investigates how ARV treatment impacts the differentiation and fate of NPCs testing for changes in calcium activity and neuron to glia cell ratios. Elixirgen NPCs were differentiated in the presence of Biktarvy for 3 days (acute treatment) or for the entirety of the 6-week differentiation (continuous treatment). Using Vala Sciences' IC200 imaging platform, we

identified differences in neuronal calcium transients between acute and continuously treated cultures. We also found that both acute and continuous treatment of Biktarvy at C_{max} were toxic to the differentiating NPCs as they did not survive the differentiation and maturation protocol.Finally, our third assay seeks to determine the effect of ARV treatment on matured tricultures containing hiPSC-derived neurons, astrocytes, and microglia. Elixirgen NPCs and iPSC-derived microglia (made in house) were differentiated for 5 weeks and received a 3-day or 7-day treatment of Biktarvy at $C_{max.}$ In contrast with what we observed in the NPC differentiation assay, the treatment did not cause alarming toxicity to the morphology and quantity of cells in culture.

Disclosures: C.G. Rines: None. **K.L. Gordon:** None. **N. Suarez:** None. **A.S. Smith:** None. **K.L. Jordan-Sciutto:** None. **J.H. Price:** None. **P.M. McDonough:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.13/Web Only

Topic: A.01. Neurogenesis and Gliogenesis

Support: ANPCyT-FONCYT PICT 2018-1744
ANPCyT-FONCYT PICT 2019-0284
ANPCyT-FONCYT PICT-2021-I-INVI-00751
CONICET PIP 11220210100198CO

Title: Selective inhibition of class I or class IIa HDACs differentially induces neuronal differentiation on murine neuroblastoma N2a cells.

Authors: S. JUNGE[1], A. BERNARDI[1], M. S. VILLALBA[1], F. J. URBANO[2], *V. BISAGNO[1]; [1]Austral Univ., Pilar, Argentina; [2]IFIBYNE-CONICET, Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract: Histone deacetylases (HDACs) are enzymes involved in regulating chromatin functions. They are responsible for removing acetyl groups thus allowing histones to wrap the DNA more tightly. HDACs are clustered into different groups; the goal of this study was to study *in vitro* the use of selective inhibitors of class I/IIa HDACs. HDACs inhibitors (HDACi) are upcoming interesting targets for their role in epigenetic/non-epigenetic regulation, and their potential use as anti-cancer agents. We used N2a cell cultures, a fast-growing mouse neuroblastoma cell line, capable of differentiating into neurons. N2a cells were cultured for 4 or 7 days *in vitro* (d.i.v.) using a 24-well plate in a DMEM with low serum (0.5%) condition and treated with HDACi. MS275 and MC1568 were used to inhibit class I and IIa HDACs respectively, at 500nM (high) or 50nM (low) concentrations. Equivalent DMSO concentrations were used as control. Whole cell patch-clamp recordings were performed to study neuron-like characteristics. All statistical differences were tested using ANOVAs. Results showed a severe

decrease in cell viability 4 d.i.v with MS275 (high), further corroborated by dapi staining. None of the inhibitors affected cell viability at 7 d.i.v. We then proceeded to analyze their morphological-induced changes in N2a cells. We quantified the absence or presence of three different neuron-like outgrowths: dendrites, axons, and filopodia. We found that HDACi induced differential morphology compared to DMSO. While Class I HDACi increased axons at high concentrations, dendrites were reduced. On the contrary, under low concentrations we saw an increase in dendrites and a decrease in axons. Class IIa HDACi presented a reduction of axons and dendrites at high concentrations, and an increase in filopodia. At low concentrations, the number of dendrites increased yet filopodia decreased. Moreover, at 4 d.i.v., patch-clamp recording showed an increase in voltage-gated ionic channel expression; 70% of the cells treated with Class I HDACi at low concentration showed positive voltage-gated ionic current versus only a 30% increase in DMSO. Membrane capacitance, a parameter related to membrane area, was recorded for all treatments. Both groups of HDACi at high concentrations showed lower levels of capacitance compared to DMSO. In summary, treatment with HDACi increased N2a differentiation at 4 days *in vitro*. Survival, morphology and voltage gated ionic channel were affected. These results suggest that HDACi are arresting tumoral growth, leading to a N2a neuron-like differentiation and a specific role of class I HDACs on maintaining a tumor-like conformation.

Disclosures: S. Junge: None. **A. Bernardi:** None. **M.S. Villalba:** None. **F.J. Urbano:** None. **V. Bisagno:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

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Program #/Poster #: PSTR514.14/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF Korea Grant 2021R1I1A3060435
Chonnam National University Hospital Biomedical Research Institute BCRI22051
he Jeollanam-do Science and Technology R&D Project (Development of Stem Cell-Derived New Drug)
Korea Institute for Advancement of Technology (KIAT) funded by the Korean Government

Title: Effects of neurogenic differentiation of BML-281 on neuroblastoma SH-SY5Y cells via the Wnt signaling pathway

Authors: J. CHOI[1], S. GANG[2], R. MAHESH[1], J. HWANG[1], H. JEONG[1,5], J. YOO[6], H.-H. CHO[3], B. C. KIM[4], G. JANG[7], H.-S. JEONG[1,5], *S. JANG[1,5];
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Korea, Republic of; [5]StemCell Bio Inc., Jellanamdo, Korea, Republic of; [6]Physiological Educ., [7]Sch. of Biol. Sci. and Technol., Chonnam Natl. Univ., Gwangju, Korea, Republic of

Abstract: Histone deacetylase (HDAC) inhibitors are known to promote differentiation through post-translational modifications of the histones of chromatin. BML-281, which is the HDAC6 inhibitor, has been known to prevent tumors, acute dextran sodium sulfate, and lung injury. The neurogenic differentiation effect of BML-281 was poorly understood yet. In this study, we investigated the effect of BML-281 on neuroblastoma SH-SY5Y cell differentiation into mature neurons by immunocytochemistry (ICC), reverse transcriptase PCR (RT-PCR), quantitative PCR (qPCR), and western blotting analysis. We found that the cells showed neurite outgrowth and morphological change into mature neurons with BML-281 under a microscope. It was confirmed that the gene expression of neuronal markers (NEFL, MAP2, Tuj1, NEFH, and NEFM) was increased on certain concentrations of BML-281. Similarly, the protein expression of neuronal markers (NeuN, Synaptophysin, Tuj1, and NFH) was upregulated with BML-281 compared to the control. Following treatment of BML-281, the expression of Wnt 5α was increased and the downstream pathways were also activated. Interestingly, both Wnt/Ca2+ and Wnt/PCP pathways activated and regulated PKC, Cdc42, and p-JNK, and highly expressed c-Jun, finally. Therefore, BML-281 induced the differentiation of SH-SY5Y cells into mature neurons by activating the non-canonical Wnt signaling pathway.

Disclosures: J. Choi: None. **S. Gang:** None. **R. Mahesh:** None. **J. Hwang:** None. **H. Jeong:** None. **J. Yoo:** None. **H. Cho:** None. **B.C. Kim:** None. **G. Jang:** None. **H. Jeong:** None. **S. Jang:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.15/A12

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

Support: NIH Grant R35 HG010718
BBRF YI Grant 30506

Title: A novel splice variant of TET1 localizes to nuclear speckles in neural cells

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Abstract: Dynamic regulation of 5-methyl cytosine (5mC) epigenetic marks is required for many nervous system functions, including learning and memory. TET enzymes (TET1-3) contribute to this regulation through their ability to serially oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC), thereby initiating demethylation. Recent reports have shown each of the three mammalian Tet genes expresses multiple isoforms, complicating interpretation of previous findings. To begin to

address this issue, and focusing on Tet1, we first amplified full length cDNAs for *Tet1 full length* (*Tet1^{FL})* and *Tet1 short* (*Tet1^{S}*) from mouse neural cell lines Neuro2a and HT-22. Unexpectedly, we detected two novel splice variants, one each expressed from the *Tet1^{FL} and Tet1^{S}* promoters: both the result of large internal exon skipping. Further RT-PCR analysis revealed both splice variants are also expressed in the brain. The alternative splice variant from the *Tet1^{FL}* promoter, we term *Tet1 full length delta (Tet1$^{FL\Delta}$*), encodes for the same protein as the *Tet1^{S}* transcript, albeit with an alternative untranslated region (UTR). In contrast, the splice variant of *Tet1^{S}*, termed *Tet1 short delta* (*Tet1$^{S\Delta}$*), encodes for a 563 amino acid protein that co-localizes with the nuclear speckle marker SC35, hinting that it many play a novel role in RNA processing. While in early stages, our data suggest some Tet isoforms may carry out novel cellular functions in the nervous system beyond regulating DNA methylation.

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Poster

PSTR514. Models of Neuronal Differentiation

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.16/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R35GM146883

Title: Fibrillarin-like 1 regulates ribosomal RNA 2'O methylation in neurons

Authors: *S. A. ALSHAWI[1], S. CRUCIANI[2], E. M. NOVOA[2], J.-D. BEAUDOIN[1];
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Abstract: Chemical modifications of RNA have been seen to impact neuronal function, development, and neurological diseases. Ribosomal RNA (rRNA) is heavily modified by 2'O methylation (2'O-Me). There is increasing evidence that regulation of rRNA modification impacts developmental programs including neuronal differentiation. However, how these modifications may be regulated to control translation for the specific needs of neurons is unclear. Fibrillarin (FBL) is known to be the canonical 2'O methyltransferase that applies 2'O-Me to ribosomal RNA. While total loss of FBL is lethal as it is critical to ribosome biogenesis, modulation of FBL expression can regulate pluripotency and neuronal differentiation. Additionally, altering FBL expression can cause changes in 2'O-Me of rRNA. Changes in 2'O-Me of rRNA have been seen to alter translational levels of select mRNAs and modulate cap-independent translation. The role of additional methyltransferases in shaping ribosomal function in neurogenesis is unknown.
FBL has a mammalian-specific paralog fibrillarin-like 1 (FBLL1). The function of FBLL1 is unknown, though its structural similarity to FBL suggests that it is also a methyltransferase. Interestingly, unlike FBL which is expressed throughout all adult tissue types, FBLL1 is

specifically expressed in the brain and testes. Within the brain, FBLL1 is expressed exclusively in neurons. Additionally, while FBL expression decreases, we have seen that FBLL1 expression increases through neurogenin 2-induced neuronal differentiation of H9 human embryonic stem cells. We hypothesize that FBLL1 may act as an additional RNA methyltransferase that applies distinct 2'O-Me to ribosomes to shape translation through neuronal differentiation. Our preliminary results show that, when ectopically expressed in HEK293 cells, FBLL1 binds 18S rRNA and localizes to the nucleolus, the site of rRNA modification. Next, to understand FBLL1's contribution to methylation of rRNA in neurons, we used direct RNA sequencing by Nanopore to identify sites of 2'O-Me in wildtype and FBLL1 knockout H9 stem cell-derived neurons. We validated these findings with biochemical methods (RiboMethSeq and RibOxi-seq). We observed changes in 2'O-Me sites on 18S rRNA with genetic loss of FBLL1 in neurons. These results suggest that FBLL1 acts as a neuronal 2'O-methyltransferase that shapes rRNA modification in a cell-specific manner. Ultimately, this work may reveal a novel mechanism of translation regulation in neuronal development.

Disclosures: S.A. Alshawi: None. **S. Cruciani:** None. **E.M. Novoa:** None. **J. Beaudoin:** None.

Poster

PSTR514. Models of Neuronal Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR514.17/A14

Topic: A.01. Neurogenesis and Gliogenesis

Title: The effect of redox signalling dysregulation on neuronal development in a mouse model of lysosomal storage disease

Authors: *J. TIBERI[1,2], S. CAMUSO[1,2], R. STEFANELLI[1], S. CANTERINI[1,3], P. LA ROSA[1,3], M. T. FIORENZA[1,3];
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Abstract: Although considered just as harmful by-products of cellular metabolism, representing one of the main drivers of oxidative stress (OS), during recent years, ROS have been shown to regulate several cellular processes, including neurogenesis and stemness/differentiation balance, thereby influencing the physiology and neuronal differentiation of neural stem cells (NSCs). Consequently, their activity is subject to a strict regulation by the cellular antioxidant system, which mainly relies on the transcription factor NF-E2- related factor 2 (Nrf2). Indeed, Nrf2 The latter is the master regulator of antioxidant defense and , which is emerging as a key modulator in the regulation of neural differentiation. Niemann-Pick type C1 disease (NPCD), is a neurodegenerative lysosomal storage disorder, characterized by the accumulation of cholesterol and glycosphingolipids in late endosomal/lysosomal compartments. This, causes leading, among others, to alterations in the antioxidant defense and increased sensitivity to ROS and OS.

Pursuing the hypothesis that the derangement of redox signaling mechanisms in NPCD may affects the complex sequence of events leading to neuronal differentiation, we have established two *in vitro* cell models, using *Npc1 knockout* (KO) mid-gestation embryos as donors: i) neural precursor cells (NSCs), which rapidly grow and form neurospheres *in vitro* when maintained under stemness conditions, whereas, undergo differentiation following appropriate stimuli; and, ii) mouse embryonic fibroblasts (MEFs), which can be easily expanded, obtaining large cell amounts required for biochemical approaches assays. Our present evidence, obtained by morphological and molecular analyses of stemness (Pax6) and differentiation markers (β3 Tubulin), indicate that NSCs derived from *Npc1*-mice exit proliferation and initiate differentiation program significantly earlier as compared to *wt* littermates. Similar features have been observed analyzing in *in vivo* cortices.Then, studying Nrf2 functional activity in MEFs, we have observed that its nuclear translocation and target genes' activation is severely compromised in *Npc1* mice-derived MEFs. This finding suggests that the impaired Nrf2 nuclear trafficking contributes to the stemness/differentiation imbalance in NPCD, identifying the Nrf2- redox signaling axis as a therapeutic option for this disease.

Disclosures: J. Tiberi: None. **S. Camuso:** None. **R. Stefanelli:** None. **S. Canterini:** None. **P. La Rosa:** None. **M.T. Fiorenza:** None.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.01/A15

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01NS121339

Title: Vascular-guided cortical organization

Authors: L. K. AHIRWAR[1], S. THOMAS[2], D. W. MCBRIDE[3], S. L. BLACKBURN[4], *P. THANKAMANI PANDIT[5];
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Abstract: The cerebral cortex, as the outermost layer of the brain, plays a crucial role in various cognitive functions, encompassing consciousness, perception, memory, language, and decision-making. Neurovascular interactions are vital for the proper development of both blood vessels and neurons in the brain. However, our understanding of how vascular components contribute to neuronal patterning in the cortex remains limited. In our study, we have made a significant discovery regarding the role of the epigenetic regulator histone deacetylase2 (HDAC2) in

regulating the expression of genes specific to central nervous system endothelial cells (ECs) that are essential for angiogenesis. By deleting HDAC2, we observed an increased angiogenesis in the embryonic brain. To examine the impact on neuronal development, we employed immunohistochemical markers for neuronal proliferation, projection neurons, and inhibitory neurons. Our findings revealed that prenatal deletion of HDAC2 in ECs led to enhanced neuronal proliferation (indicated by Ki67 staining) and disrupted patterning of both projection neurons (marked by TBR1) and inhibitory neurons (identified by calbindin staining). Furthermore, postnatal deletion of HDAC2 from ECs resulted in impaired growth and reduced brain size. These brains exhibited abnormal neuronal patterns and elevated expression of the transcription factor Auts2. Importantly, Auts2 has been implicated in neurodevelopment and is considered a potential candidate gene for several neurological disorders, including autism spectrum disorders, intellectual disability, and developmental delay. Collectively, our findings suggest that blood vessels or CNS ECs can emit critical signals for proper neuronal patterning in the cortex. Alterations in these signals may have profound effects on neurodevelopmental disorders.

Disclosures: L.K. Ahirwar: None. **S. Thomas:** None. **D.W. McBride:** None. **S.L. Blackburn:** None. **P. Thankamani Pandit:** None.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.02/A16

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant RF1 MH124605-01

Title: A multimodal 3D developing mouse brain common coordinate framework for quantitative cell census mapping

Authors: *F. KRONMAN[1], J. K. LIWANG[1], R. BETTY[2], Y.-T. WU[3], S. B. MANJILA[1], J. MINTEER[1], D. SHIN[1], L. PUELLES[4], J. C. GEE[5], J. ZHANG[6], L. NG[7], Y. KIM[1]; [1]Penn State Col. of Med., Hershey, PA; [2]Millersville Univ., Millersville, PA; [3]Cedars-Sinai Med. Ctr., Los Angele, CA; [4]Univ. Murcia Fac of Med., Murcia, Spain; [5]Dept. of Radiology, Univ. of Pennsylvania, Philadelphia, PA; [6]Radiology, New York Univ., New York, NY; [7]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Lack of standard 3D reference atlases in developing mouse brains limits data integration of 3D brain cell type imaging to understand brain development. Existing developmental mouse brain reference atlases rely on 2D distorted male histology samples to delineate anatomical regions, failing to meet the standards of modern 3D imaging. The Allen Common Coordinate Framework (CCFv3) is an excellent atlas example for the adult mouse brain but fails to account for developmental anatomy. Here, we present multimodal 3D developmental common coordinate frameworks (DevCCFs) that accounts for differential

morphology during mouse brain development. We generated DevCCFs composed of a morphological average of male and female mouse brains at embryonic day (E)11.5, E13.5, E15.5, E18.5, postnatal day (P)4, P14, and P56. We used light sheet fluorescent microscopy (LSFM) and magnetic resonance imaging (MRI) to create undistorted morphologically averaged templates at 10um isotropic resolution. Moreover, established 3D anatomical parcellations at each age defined by a developmental ontology. Iterative parcellations allow modifications in the DevCCFs as anatomical standards continue to be understood based on community knowledge of the developing brain. Thus, the DevCCFs can be used to analyze and integrate signals from various imaging modalities at different scales to promote open, collaborative, and inclusive neuroscience.

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Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.03/A17

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS098804
SFARI-T-Convergence 1018944

Title: Dynamics in 3D regulatory chromatin topology configure gene-regulatory networks that underlie postmitotic neuronal maturation

Authors: V. RAMESH[1], X. WEI[2], J. OU[2], Y. DIAO[2], ***A. E. WEST**[1];
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Abstract: Neurons are remarkably long-lived cells that are born early in development and maintained over the lifespan of an organism. Neurogenesis is followed by the transcriptional maturation of these cells into functional neurons that can participate in CNS circuitry. This is mediated at least in part by regulators of chromatin biology, and mutations in chromatin regulators are implicated in the advent of neurodevelopmental disorders. We and others have shown that cis-regulatory interactions between promoters and enhancers are dynamically modulated in maturing neurons. How these interactions are established and regulated to permit transcriptional maturation, remains poorly understood. To discover how transcriptional regulatory networks evolve over neuronal maturation, we used a novel low-input sequencing method called ‘HiCAR’ or (HiC on accessible regulatory DNA) to profile dynamics in regulatory chromatin loop formation in maturing cerebellar granule neurons (CGNs) as a function of time and in response regulatory perturbations. We observe that as CGNs mature, they show bidirectional changes in cis-regulatory loop formation at genes that turn on early versus

late in differentiation. By 7 days-in-vitro (DIV7), CGNs gain about four-times as many regulatory loops compared to granule neuron precursors (GNPs), over half of which are anchored at least at one promoter and concentrated within topologically associating domains (TADs). We also find these interactions to be potentially poised by an architectural role for repressive chromatin modification H3K27me3 using a small-molecule inhibitor of H3K27me3-writer EZH2. Taken together, we show that the transcriptional maturation of CGNs occurs in concert with the maturation of their gene-regulatory topology.

Disclosures: V. Ramesh: None. **X. Wei:** None. **J. Ou:** None. **Y. Diao:** None. **A.E. West:** None.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

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Program #/Poster #: PSTR515.04/A18

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS098804
SFARI-T-Convergence 1018944

Title: Autism-associated mutations in Kdm6b affect its role as a chromatin regulator in brain development

Authors: *R. LI[1,2], U. CHAN[2], V. RAMESH[2], A. WEST[2];
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Abstract: The regulation of gene expression in the developing brain is coordinated by changes in chromatin regulation. Chromatin regulation involves the deposition and removal of molecular modifications on the histone proteins that give structure to and control the function of genomic DNA. The trimethylation of histone H3 at lysine 27 (H3K27me3) is a chromatin mark that recruits transcriptional repressors, silencing gene transcription. The JmjC family lysine demethylase KDM6B has been identified as an enzyme that removes H3K27me3, allowing for expression of genes critical for brain development. Genetic variants in human *KDM6B* have been found to be associated with intellectual disability and autism spectrum disorder (ASD); however, the molecular mechanisms by which these genetic variants affect KDM6B function remain unknown. We mapped these variants into the structure of KDM6B, and computationally assessed their likely effects on KDM6B protein function. To experimentally determine how ASD-associated sequence variants in KDM6B affect the function of this protein in cells, we generated ASD-associated mutations into a KDM6B expression construct and assessed their effects on the enzymatic function of this protein as well as its stability and nuclear localization. We found that several ASD-associated mutations occur in the H3K27me3 binding pocket of KDM6B and impair the ability of this enzyme to demethylate histones. In parallel, through RNA sequencing experiments in cerebellar granule neurons from mouse, we found that expression of the

enzymatically active form but not the enzymatically dead form of KDM6B was sufficient to rescue synaptic gene expression following knockdown of endogenous KDM6B. Together, these findings elucidate a novel role of KDM6B in controlling gene expression in maturing neurons, and they expand our knowledge on how chromatin dysregulation can lead to neurodevelopmental disorders like ASD.

Disclosures: **R. Li:** None. **U. Chan:** None. **V. Ramesh:** None. **A. West:** None.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

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Program #/Poster #: PSTR515.05/A19

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01MH124870

Title: Satb2 expression in CA1 hippocampal pyramidal neurons is necessary for the development of feedforward inhibitory circuits

Authors: *M. A. HANSON, N. BIBI, A. SAFA, D. NAGARAJAN, A. C. JOHANTGES, E. K. PAYNE, A. H. MARSHALL, J. C. WESTER;
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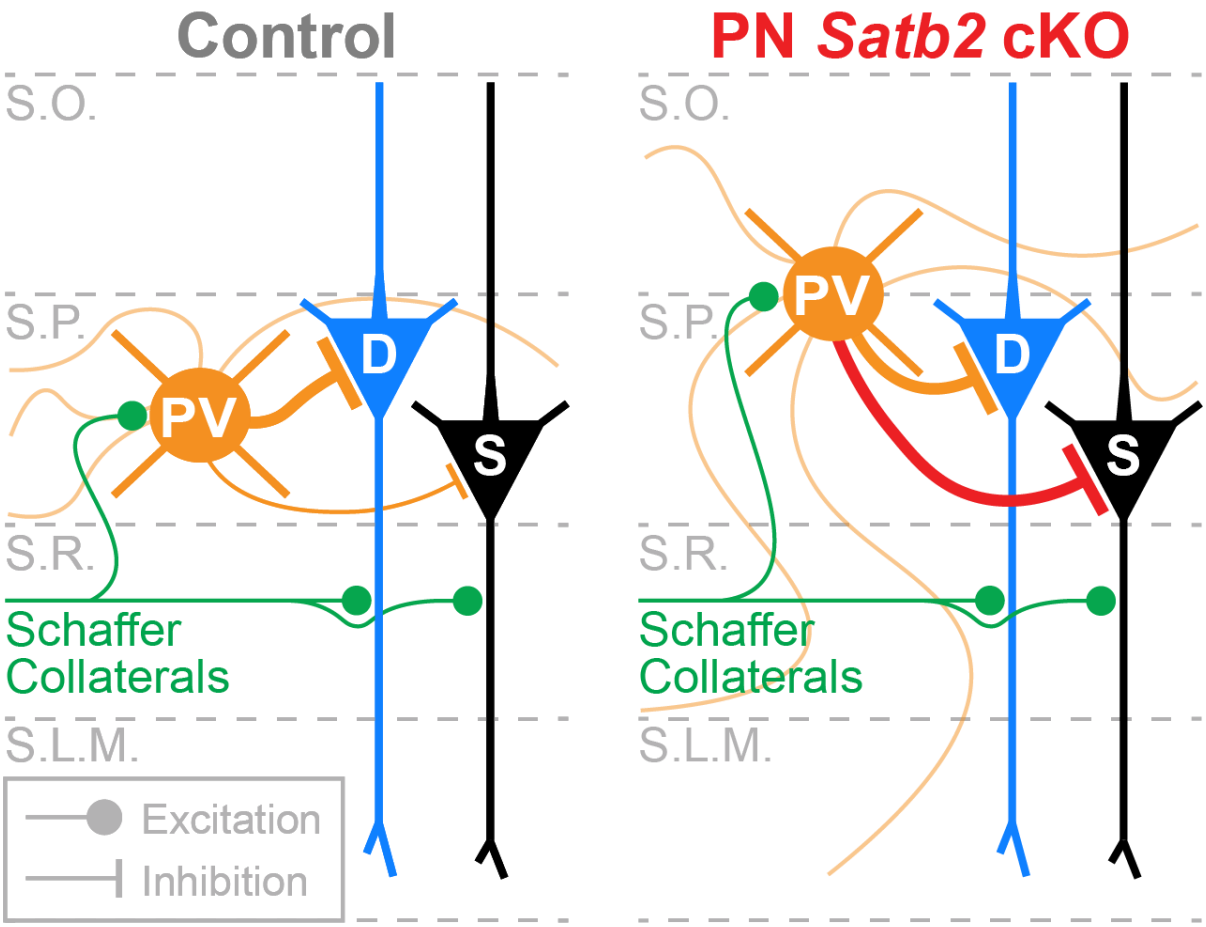
Abstract: Pyramidal neurons (PNs) in CA1 provide the output of hippocampal computations to the rest of the brain. They can be parsed based on radial position into deep and superficial subtypes that form biased circuits with local inhibitory interneurons. These biased circuits suggest that deep and superficial PNs may process parallel information streams. Using mice, we show that the transcriptional regulator Satb2 is necessary for superficial PN differentiation and the development of biased inhibitory circuits with parvalbumin basket cells (PVBCs). First, we found that SATB2 expression is restricted to superficial PNs in neonates. To investigate Satb2's function, we conditionally knocked out (cKO) both alleles from PNs using the Emx1-Cre line. In juveniles, this altered the intrinsic membrane properties of superficial neurons to resemble deep neurons. Next, we investigated the impact of Satb2 cKO on synaptic connectivity and physiology. In control mice, we replicated previous work showing that superficial PNs demonstrate weaker feedforward inhibition from CA3 Schaffer collateral input compared to deep PNs due to smaller unitary inhibitory synaptic currents from PVBCs observed in paired whole cell recordings. Strikingly, in Satb2 cKO mice, feedforward inhibition and unitary inhibitory synaptic currents from PVBCs increased selectively onto superficial PNs to match deep PNs. The change in synapse strength was specific to connections from PVBCs to superficial PNs, as excitatory afferent synapses from Schaffer collaterals and the perforant path were unaffected. Connectivity and physiology of PN to PVBC synapses and PVBC to deep PN synapses were also unaffected. Finally, we found that PN Satb2 cKO also causes mislamination of PV cell bodies and their axons. This correlates with ectopic expression of the cytokine CXCL12 in Satb2 cKO

PNs outside the stratum pyramidale. We conclude that Satb2 expression in superficial PNs maintains differential feedforward inhibitory circuitry between deep and superficial layers in CA1 via regulation of PVBC circuitry in a non-cell-autonomous manner.



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Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.06/A20

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01MH124870

Title: Transcriptional regulation of hippocampal CA1 pyramidal cells by Satb2 throughout development

Authors: *E. PAYNE, M. HANSON, N. BIBI, A. SAFA, H. EL-HODIRI, A. FISCHER, J. WESTER;
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Abstract: Title: Transcriptional regulation of hippocampal CA1 pyramidal cells by Satb2 throughout development**Theme and Topic:** A.01.d. Neuronal differentiation**Keywords:** NEURAL CIRCUIT; CA1; TRANSCRIPTOMICS **Authors:** *E.K. Payne, M.A. Hanson, N. Bibi, A. Safa, H. El-Hodiri, A.J. Fischer, J.C. Wester - Dept. of Neuroscience, The Ohio State University, Columbus, OH**Funding:** NIH R01MH124870
Pyramidal cells (PCs) within the CA1 region of the hippocampus are projection neurons that communicate the output of hippocampal computations to diverse brain regions. It is now clear these PCs are heterogeneous and may serve unique roles in processing information for learning and memory. They can be broadly parsed by their radial position within the stratum pyramidale as deep or superficial. Deep and superficial PCs have unique electrophysiological and transcriptomic profiles and form biased synaptic connections with inhibitory interneurons. There is also heterogeneity among deep and superficial PCs: they can have unique projection targets and recent single-cell transcriptomic data suggest there are multiple PC subtypes within CA1. The mechanisms that regulate CA1 PC differentiation to produce these diverse types, which are crucial to understand hippocampal function, are unknown. The chromatin remodeler and transcription factor Satb2 is a promising candidate. In the neocortex, Satb2 is a master regulator of pyramidal cell identity. In the hippocampus, it is preferentially expressed in PCs in CA1, but its function in their development is unknown. Strikingly, we found that Satb2 protein expression is restricted to immature superficial PCs and absent from deep PCs at birth in mice. This suggests Satb2 may have a conserved role in controlling neuronal differentiation in CA1. Here, we used a multiomics approach to investigate the epigenetic regulatory mechanisms by which Satb2 guides PC differentiation during development. We used Emx1-Cre mice crossed to mice with a floxed Satb2 allele to conditionally knockout both copies of Satb2 from PCs. We then harvested hippocampi at two developmental time points: 1) whole hippocampi at postnatal day 2, and 2) microdissected CA1 at postnatal day 21. We then dissociated the tissue for simultaneous RNA and ATAC-seq of single nuclei using the 10X Genomics Multiomics platform. We performed analyses of differential gene expression and chromatin access in control and conditional knockout mice. These data provide a comprehensive readout of genetic mechanisms by which Satb2 controls PC differentiation with cell-type resolution.

Disclosures: **E. Payne:** A. Employment/Salary (full or part-time):; The Ohio State University. **M. Hanson:** A. Employment/Salary (full or part-time):; The Ohio State University. **N. Bibi:** None. **A. Safa:** A. Employment/Salary (full or part-time):; The Ohio State University. **H. El-Hodiri:** A. Employment/Salary (full or part-time):; The Ohio State University. **A. Fischer:** A. Employment/Salary (full or part-time):; The Ohio State University. **J. Wester:** A. Employment/Salary (full or part-time):; The Ohio State University.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.07/A21

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01MH124870

Title: Superficial pyramidal neurons preferentially target oriens-lacunosum moleculare interneurons in CA1 hippocampus

Authors: *A. C. JOHANTGES, A. SAFA, E. K. PAYNE, M. A. HANSON, A. H. MARSHALL, N. BIBI, J. C. WESTER;
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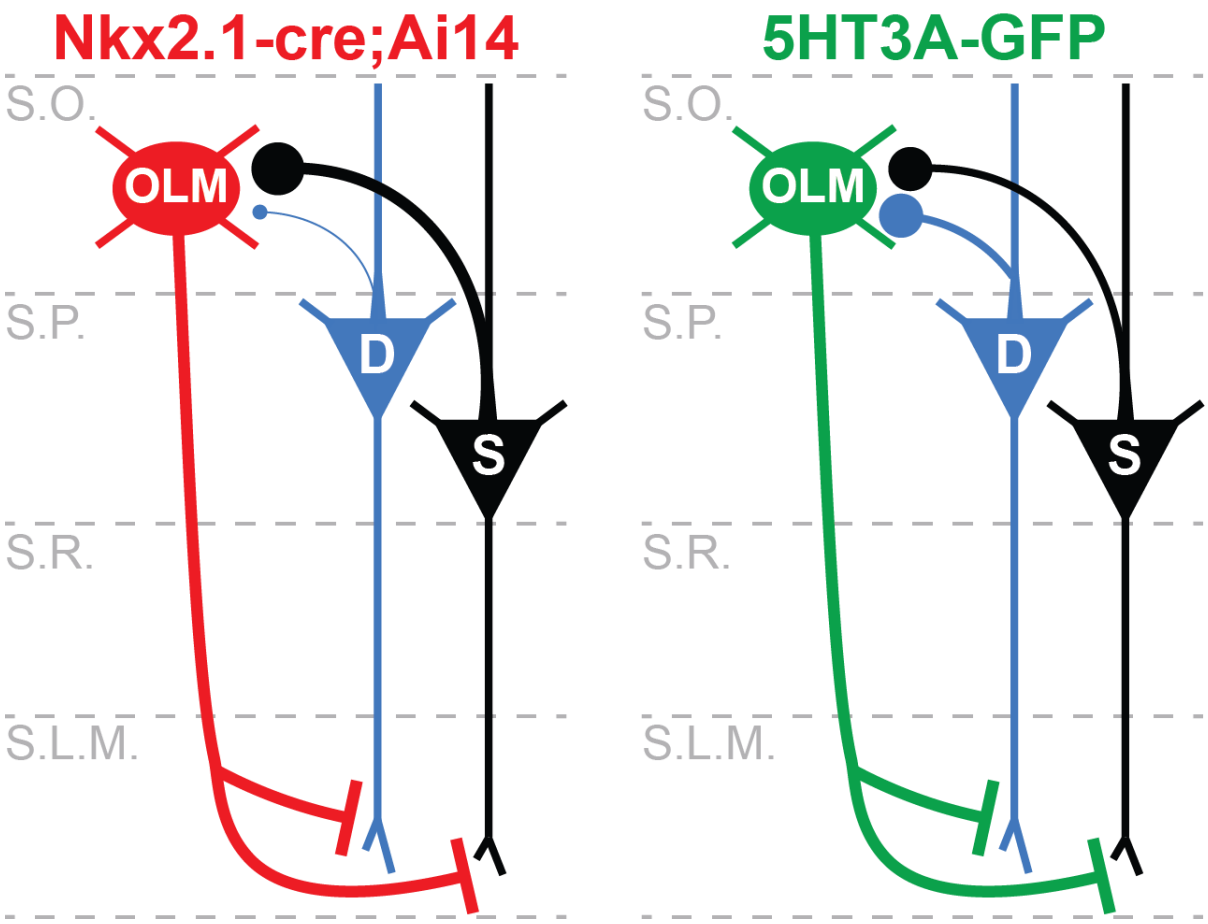
Abstract: Oriens-Lacunosum Moleculare (OLM) cells are hippocampal inhibitory interneurons that target the apical dendrites of pyramidal neurons (PNs) to regulate their excitability and plasticity. PNs provide facilitating synaptic input to OLM cells to form a feedback inhibitory circuit. In CA1, recent work found that PNs are not homogeneous and can be divided into deep and superficial subgroups based on their radial position within the stratum pyramidale. Deep and superficial PNs form biased circuits with PV- and CCK-expressing basket cells, but it is unknown if they also form subtype-specific circuits with OLM cells. Here, we used dual whole-cell patch clamp recordings in mouse hippocampal slices to investigate the connectivity and physiology of synapses from deep and superficial PNs to OLM cells in CA1. Strikingly, we found that superficial PNs preferentially target OLM cells compared to deep PNs. These findings extend previous work that suggests deep and superficial PNs engage in parallel microcircuits in CA1. Next, we investigated if this connectivity bias extends to different populations of OLM cells. Previous studies found that the Nkx2.1-Cre and 5HT3A-GFP mouse lines label mostly non-overlapping subsets of OLM cells with Cre or GFP expression, respectively. However, it remains unclear if they are functionally distinct subtypes as their electrophysiological and morphological features are the same. Furthermore, while there are some transcriptomic differences between them, their molecular expression profiles are similar. Surprisingly, we found that superficial PNs preferentially target OLM cells in the Nkx2.1-Cre but not 5HT3A-GFP transgenic lines. This suggests OLM cells reported by the Nkx2.1-Cre and 5HT3A-GFP mouse lines form unique local circuits with PNs. Specifically, Nkx2.1-Cre OLM cells participate in separate deep and superficial PN microcircuits in CA1 but 5HT3A-GFP do not and may integrate information between them.



Disclosures: **A.C. Johantges:** None. **A. Safa:** None. **E.K. Payne:** None. **M.A. Hanson:** None. **A.H. Marshall:** None. **N. Bibi:** None. **J.C. Wester:** None.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.08/A22

Topic: A.01. Neurogenesis and Gliogenesis

Support: KAKENHI, Grant/Award Numbers: 16H06316, 16H06463
National Center of Neurology and Psychiatry, Grant/Award Number: N/A
Smoking Research Foundation; Takeda Science Foundation

Title: Serotonin Transporter Expression in Glutamatergic Neurons during Critical Developmental Period: Implications for Neurodevelopmental Disorders

Authors: *J. R. AWASTHI[1,2], K. TAMADA[3,2], T. TAKUMI[3,2];
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Abstract: Neurodevelopmental disorders, such as autism spectrum disorders (ASD), exhibit a wide range of behavioral abnormalities, such as deficits in social interaction, impaired communication, repetitive behavior or restricted interest, low IQ, language delay, auditory and visual hypersensitivity, aggressiveness, motor clumsiness, epilepsy, disturbed sleep, and others. This suggests altered neuronal activity and connectivity across multiple brain areas. This study aims to identify common underlying mechanisms contributing to these diverse behavioral abnormalities.

We mapped the serotonin (5-HT)-utilizing neurons across whole brain areas during different developmental stages using SERT (serotonin transporter)-EGFP (enhanced green fluorescence protein) bacterial artificial chromosome (BAC) transgenic mice. Coupled with the GFP antibody and confocal microscopy, this transgenic mouse allows us to visualize the expression of SERT much better than earlier techniques. SERT is an integral membrane protein responsible for 5-HT reuptake into presynaptic terminals.

Our findings reveal that SERT expression is observed in both 5-HT and non-5-HT neurons. However, SERT expression in non-5-HT neurons is restricted to specific brain areas for a transient period between embryonic day 15 and postnatal week 3, with varying expression timings among different regions, and expression is lost thereafter. Interestingly, the transiently SERT-expressing non-5-HT neurons are glutamatergic (glut) neurons in brain areas such as the cortex, thalamic nuclei, hippocampus, lateral septal nuclei, nucleus accumbens, bed nuclei of stria terminalis, striatum, globus pallidus, preoptic hypothalamic nuclei, midbrain colliculi, olfactory bulb, anterior olfactory nuclei, retina, optic nerve, and skin.

These findings suggest that glutamatergic neurons in these brain areas utilize 5-HT during development. Disruption in the 5-HT level or its absorption by these neurons during development may impair normal circuitry formation, leading to autistic features. Furthermore, this may explain the observed imbalanced excitatory/inhibitory ratio and increased susceptibility to epileptic seizures in individuals with autism.

It is plausible that environmental factors such as prenatal infections or medication usage could interfere with SERT-Glut neuron development during this critical developmental period, thereby triggering the onset of autism. Further investigation of it using the maternal immune activation model holds promising potential for unraveling the etiology of neurodevelopmental disorders, including ASD.

Disclosures: J.R. Awasthi: None. **K. Tamada:** None. **T. Takumi:** None.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.09/A23

Topic: A.01. Neurogenesis and Gliogenesis

Support: R35NS097370
R35NS116843

Title: Dysregulation of m^{6}A signaling leads to deficits in hypothalamic neurogenesis and function of energy balance

Authors: *Y. SHEN[1], S. WONG[4], T. MA[5], H. SONG[2], G.-L. MING[3];
[2]Neurosci., [3]Dept Neurosci, [1]Univ. of Pennsylvania, Philadelphia, PA; [4]Perelman Sch. of Med. at the Univ. of P, Philadelphia, PA; [5]Inst. of Brain Science, Fudan Univ., Shanghai, China

Abstract: m^{6}A is the most prevalent internal modification in mRNAs of eukaryotic cells, and impact on modified transcripts is mediated by m^{6}A-specific effector proteins. m^{6}A plays important roles in the developing and adult mammalian nervous system, including cortical neurogenesis, cerebellar development, adult neurogenesis, memory formation and consolidation, and axonal regeneration. The hypothalamus plays a crucial role in controlling appetite and energy intake, while the mechanisms through which epi-transcriptomic modification of m^{6}A regulates gene expression to control hypothalamic neurogenesis and the function of energy balance have not been explored. We found that m^{6}A depletion by conditional knockout of m6A writer Mettl14 in the embryonic hypothalamus in mice using Nkx2.1-Cre caused excessive obesity in adulthood with impaired glucose-insulin homoeostasis by increasing energy intake. Deletion of Mettl14 led to increased proliferation of hypothalamic neural progenitor cells (NPCs) and defective generation of feeding-related neurons at the embryonic stages. Deletion of another m^{6}A writer Mettl3 in mice led to similar phenotype. Gene expression and epitranscriptomic profiling of m^{6}A sites on hypothalamic NPCs identified changes in the TGF-β and Wnt pathways. Lastly, we optimized a feeder-free method to generate arcuate nucleus (Arc) organoids from human iPSCs to investigate the role of m6A signaling in human Arc neurogenesis. Our studies identify essential role of m^{6}A signaling in the generation of hypothalamic neurons and show that deficits in m6A signaling leads to obesity.

Disclosures: **Y. Shen:** None. **S. Wong:** None. **T. Ma:** None. **H. Song:** None. **G. Ming:** None.

Poster

PSTR515. Neuronal Cell Fate and Differentiation

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.10/B1

Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS Grant K08NS099502
Project ALS award

Title: Developing hindbrain motor neurons show spatial and temporal transcriptomic diversity mapping to wiring decisions

Authors: *M. F. ROSE[1], T. RAY[2], N. SHIROONI[1], S. SANTOS[1], A. TENNEY[2], D. CREIGHTON[2], A. GELBER[2], A. BARROGA[1], K. CHEN[1], H. GUARDIANO[1], J. LI[1], L. NGUYEN[1], V. NGUYEN[1], J. PADILLA-TRIGO[1], B. PHAM[1], Y. TRINH[1], X. YANG[1], M. TISCHFIELD[2], E. MURRAY[3], T. COLLINS[2], A. A. NUGENT[2], P. F. ANG[2], S. C. IZEN[2], M. F. BAUER[2], W. HUANG[2], R. SATIJA[4], E. MACOSKO[5], F. CHEN[3], A. REGEV[3], E. ENGLE[6]; [1]Univ. of California Irvine, Irvine, CA; [2]Boston Children's Hosp., Boston, MA; [3]The Broad Inst., Cambridge, MA; [4]New York Genome Ctr., New York, NY; [5]Broad Inst. of MIT and Harvard, Cambridge, MA; [6]Boston Children's Hosptial, Boston, MA

Abstract: The brainstem ocular motor neurons (OMNs) mediate eye movements and are differentially affected in some disorders, compared with other motor neurons (MNs). In congenital cranial dysinnervation disorders (CCDDs) such as Duane Syndrome, OMN subpopulations show disrupted or aberrant innervation, while in Amyotrophic Lateral Sclerosis (ALS), OMNs continue to function while other MNs degenerate. Here we define unique gene expression patterns among developing MNs, and generate a toolbox of protocols and genetic markers to help study these disorders. We combine various mouse genetic reporter lines with intersectional temporal and spatial transcriptomics (bulk-, single cell-, and single nuclei RNA-seq, and Slide-seq) to isolate and compare eight distinct mouse MN populations from embryonic days E9.5-E18.5: the three ocular motor nuclei (CN3, CN4, CN6) and the other primary MN types (CN5, CN7, CN9/10, CN12 in brainstem, and spinal MNs). Gene expression was validated with database analysis, *in situ* hybridization, antibodies, and genetic axonal labeling. We correlate gene expression differences with cell age by both EdU labeling and tamoxifen-mediated temporal CreER induction, and visualize iDISCO- and EyeDISCO-cleared whole embryos by light sheet microscopy. Each MN population shows a unique genetic fingerprint, including novel markers of spatially- and temporally-distinct OMN subpopulations. Some OMN nerve branches correspond with cell birthdate and selectively contribute to specific aberrant branches in the *Mafb*-knockout mouse model of Duane Syndrome. Overall, this MN transcriptomic atlas uncovers distinct developmental gene expression patterns and markers of the various cranial motor neurons, and provides new tools to study their differential vulnerability in the CCDDs and other motor neuron disorders.

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Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.11/B2

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant EY031690

Title: Isoform specific role for Protocadherin γC4 in neuronal survival in the mouse retina

Authors: *C. M. MCLEOD[1], H. G. LANTHIER[1], J. A. WEINER[2], R. W. BURGESS[3], A. M. GARRETT[1];
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Abstract: The γ-Protocadherins (γ-Pcdhs) are a family of 22 cell adhesion molecules that are widely expressed throughout the central nervous system from the *Pcdhg* gene cluster. Each isoform has a distinct extracellular, transmembrane, and membrane proximal cytoplasmic domain, but shares a common C-terminal cytoplasmic domain, suggesting that each isoform may have a unique as well as a shared function. As a whole γ-Pcdhs are of interest due to their association with a variety of essential processes during CNS development from neuronal survival, synapse formation, and dendritic self-avoidance to the formation of dendritic arbors. Strikingly, the loss of γ-Pcdhs results in neonatal lethality in mice via massive apoptosis of many neuronal types throughout the CNS resulting from an exacerbation of normal developmental apoptosis. The mechanism by which γ-Pcdhs promote survival during development is not known. It was recently found that expression of the γC4 isoform alone is sufficient for postnatal viability and near normal neuronal density in the spinal cord. Here, we seek to understand the unique role of γC4 in the process of neuronal survival using the mouse retina as a model. We hypothesize that the γC4 isoform is the only necessary and sufficient γ-Pcdh isoform to promote neuronal survival in the retina and binds specific protein partners which promote that survival. To investigate these hypotheses, we are using mouse lines with reduced γ-Pcdh diversity to ask whether γC4's role in neuronal survival is truly unique among isoforms, and how that role is mediated at the protein level. We also use transgenic animals to ask which unique binding partners of γC4 are involved in the apoptotic pathway. Our results show that γC4 is the only γ-Pcdh isoform necessary for neuronal survival in mouse retina, and that survival may be driven by the variable cytoplasmic domain of the protein (VCD). Using transgenic γC4 mice we have identified several candidate γC4 interacting proteins which may play a role in modulating apoptosis. We are confident our approach will answer the question of how and through which pathway γC4 is able to promote neuronal survival in the CNS.

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Poster

PSTR515. Neuronal Cell Fate and Differentiation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR515.12/B3

Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS IRP

Title: Neuregulin1 nuclear back signaling regulates dentate gyrus granule cell subtype specification.

Authors: *P. RAJEBHOSALE[1], L. JIANG[2], K. R. JOHNSON[3], N. S. DESAI[3], L. W. ROLE[3], D. A. TALMAGE[3];
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Abstract: The dentate gyrus granule cells (GC) gate information coming into the hippocampus. Most GCs have a single primary dendrite extending vertically into the molecular layer. A second population of GCs, known as semi-lunar granule cells (SLGCs), have multiple splaying primary dendrites. SLGC are born embryonically, account for <10% of all GCs but are predicted to have a strong influence on DG activity. Little is known regarding how these two GC subtypes are specified. To understand the diversity of GC subtypes, we performed morpho-electric profiling and single nucleus RNASeq (snRNASeq) to define the molecular makeup of GC subtypes. ~10% of the cells profiled had a SLGC-like morphology and were all located at the dorsal edge of the granule cell layer (GCL). Cells with SLGC-like morphology differed in several electrical properties compared to typical GCs. snRNASeq also identified *penk* expressing GCs, which have been shown to have SLGC-like morphology. Marker gene discovery for *prox1+penk+* cells revealed several other genetic markers for *penk* expressing GCs. Among genes highly expressed in *penk+* cells is *nrg1*. Nrg1 interacts with its receptor ErbB4 at synapses, upon binding ErbB4, Nrg1 can undergo proteolysis by gamma secretase, releasing a cytosolic intracellular domain (ICD), which can traffic to the nucleus and regulate gene expression (nuclear back signaling). A psychosis-associated missense mutation in *NRG1* ($V_{321}L$) impairs cleavage by gamma-secretase, implicating nuclear back signaling in schizophrenia pathology. Given that GCs with multiple splaying primary dendrites have been observed in several animal models of schizophrenia, we looked for alterations to GC properties in the $V_{321}L$ mutant DG. Over 70% of profiled GCs in the $V_{321}L$ DG had SL-like morphologies. GCs from mutant mice showed alterations to their electrical properties and showed loss of distinction between typical and SL-like GCs. Additionally, snRNASeq of $V_{321}L$ DG showed significantly higher numbers of *prox1+penk+* cells. Thus, loss of Nrg1 nuclear back signaling resulted in: 1. more GCs with multiple splaying primary dendrites not restricted to the superficial layer of the GCL, 2. Loss of distinction in electrical properties between GCs with typical vs. SLGC-like morphologies, and 3. more cells expressing SLGC marker genes. We propose that Nrg1 nuclear back signaling suppresses SLGC-like fate postnatally in the DG and that aberrant postnatal production of this cell type might be a common feature of schizophrenia models.

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Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.01/B4

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant DP1 NS10665-04
NIH Grant R01 NS045523-16

Title: Identification of subcellular growth cone proteins regulating development of interhemispheric cortical projection neuron connectivity

Authors: *D. E. TILLMAN[1,2,3], E. CHAI[2,1], O. DURAK[1,2], J.-Y. KIM[1,2,3], P. VEERARGHAVAN[1,2], J. E. FROBERG[1,2], B. BUDNIK[4], J. D. MACKLIS[1,2];
[2]Stem Cell and Regenerative Biology, Ctr. for Brain Sci., [3]Mol. and Cell. Biol., [4]Wyss Inst., [1]Harvard Univ., Cambridge, MA

Abstract: Cortical projection neurons (PN) have critical roles in sensory, motor, cognitive, and behavioral circuits. During development, PN build intricate circuitry by "projecting" their axons through diverse extracellular environments, then innervating specific targets located at great distances (10^3 - 10^5 cell body diameters) from their nucleus-containing somata. This precise navigation is regulated by growth cones (GCs): semi-autonomous, subcellular compartments at tips of growing axons that rapidly integrate extracellular signals to control axonal pathfinding, then mature into synapses. Callosal projection neurons (CPN) are a relatively recently evolved PN subtype that primarily reside in layers II/III, and project to contralateral cortex. Prior work has identified molecules in cell bodies that regulate CPN development, including the key transcription factor *Bcl11a/Ctip1*, which is required for precise targeting of CPN, and is a highly penetrant, monogenic gene for autism spectrum disorders (ASD) and intellectual disability (ID). However, mechanisms linking nuclear controls to developmental regulation of GC protein abundances are poorly understood, and even less is known about how dysregulation of GC proteomes in disease causes aberrant connectivity and adult behavior. Our lab developed experimental and analytical approaches to purify GCs and their parent somata from specific PN subtypes in developing mouse cortex, then quantitatively "map" RNAs and proteins between these subcellular compartments (*Nature*, 2019; *Nat. Prot.*, 2022). We recently identified that GC transcriptomes change with deletion of *Bcl11a* (*bioRxiv*, 2022), and as CPN cross the midline (*bioRxiv*, 2023). Here, we expand on our lab's RNA-focused findings by using advances in ultra-low-input mass spectrometry to identify differentially abundant proteins between $Bcl11a^{-/-}$ and $Bcl11a^{+/+}$ CPN GCs that were purified before or after midline crossing. We investigate select candidates to identify proteins that function in precise circuit formation and/or CPN midline crossing. This work identifies key proteins that likely regulate development, function, and maintenance of cortical circuitry, and enables future investigations into how proteomic regulation in distinct subtypes, stages, and/or subcellular compartments controls normal circuit development, and how its dysregulation might lead to a variety of neurodevelopmental and neuropsychiatric disorders.

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Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.02/B5

Topic: A.05. Axon and Dendrite Development

Title: The role of the murine cell adhesion molecule Nectin 3 in laminar-specific neocortical connectivity

Authors: *L. E. GUZMÁN CLAVEL, S. SUDARSANAM, A. L. KOLODKIN;
Kavli Neurosci. Discovery Institute, Solomon H. Snyder Neurosci. Dept., The Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Neurons form precise connections, leading to the formation of complex neuronal circuits that enable the diverse functionality of the brain. Laminar organization of neural connections facilitates the precise matching of pre-and post-synaptic partners. This is particularly evident in the neocortex, where neurons in six main layers exhibit stereotyped laminar-specific connectivity patterns. For example, layer II/III (L2/3) cortical projection neurons (CPNs) extend interstitial axon branches in layers II/III and V, but not in layer IV. While the cell dynamics of this process have been described, the molecular mechanisms underlying layer-specific interstitial, or collateral, axon branching remain elusive. The expression of nectins, a family of cell adhesion molecules, is highly enriched in upper cortical layers. Cell adhesion molecules have the potential to facilitate the initiation and maturation of neuronal connections. In particular, nectins mediate the initiation of adhesive contacts through heterophilic interactions and recruit cadherins to synaptic junctions. Nectin 3 (N3) promotes synapse formation in the mouse visual cortex, and so N3 is a candidate for regulating the elaboration of laminar-specific CPN interstitial axon branches. Using temporally controlled in-utero electroporation and the sparse-labeling vector Supernova, we overexpressed full-length N3 in L2/3 neurons at embryonic day 15.5 (E15.5) and found increased levels of ectopic axonal collaterals in layer 4, with significantly larger numbers of axon branches and protrusions as compared to control L2/3 neurons. Similarly, overexpression of Nectin 3 lacking its afadin (AFD)-binding domain phenocopied the full-length N3 overexpression ectopic CPN layer 4 axon branching, suggesting that Nectin-AFD interactions are dispensable for this effect. Using in-situ hybridization and immunohistochemistry, we contrast mRNA and protein expression of N3 and N1, observing layer-specific differences in expression across development. N3 mRNA is expressed in both L2/3 and L4 at P4-P7, but N3 protein expression is specific to L4 at these time points, suggesting that post-translational modifications of N3 may be at play. Ongoing efforts are also aimed toward elucidating the role of alternative splicing of N3 mRNA and its contribution to layer specific N3 protein expression and function. Our data suggest that tight temporal and spatial regulation of N3 levels in different cortical layers underlies the development of laminar-specific neuronal circuitry. N3 loss of function studies in L2/3 and L4 neurons are in progress to define N3 roles in regulating upper CPN morphology and circuit assembly.

Disclosures: L.E. Guzmán Clavel: None. **S. Sudarsanam:** None. **A.L. Kolodkin:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

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Program #/Poster #: PSTR516.03/B6

Topic: A.05. Axon and Dendrite Development

Support: BBSRC Grant: BB/G007632/1
NASA/INSGC grant

Title: Signaling mechanisms of Nell2-mediated retinal axon guidance in the layer-specific visual projections

Authors: C. M. NAKAMOTO, M. DUNSON, C. MEYERS, A. CARNEY, S. HAFNER, T. RAGLE, ***M. NAKAMOTO**;
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Abstract: During development of the vertebrate visual system, retinal axons not only reach the topographically correct positions within their targets in the brain but also confine their terminal arbors and synapses to specific layers or laminae. In the chicken retinotectal system, retinal axons terminate in superficial layers of the optic tectum (retinorecipient laminae) and do not normally invade deeper non-retinorecipient laminae. In the mammalian retinogeniculate system, axons from the ipsilateral and contralateral eyes project to distinct layers (or domains) in the dorsal lateral geniculate nucleus (dLGN). Nell2 (also known as Nel) is a multi-modular extracellular glycoprotein that is predominantly expressed in the nervous system. We have previously demonstrated that Nell2 is expressed in specific layers of the chicken optic tectum and the murine dLGN during development. In the developing chicken optic tectum, Nell2 is expressed in the layer that is located at the boundary between the superficial retinorecipient laminae and the deeper non-retinorecipient laminae (lamina g of the *stratum griseum et fibrosum superficiale*). In the developing murine dLGN, Nell2 is expressed in the layer (domain) where ipsilateral retinal axons project. *In vitro*, Nell2 exerts inhibitory effects (inhibition of axon outgrowth, induction of growth cone collapse) on all retinal axons in the chick and on contralaterally-projecting retinal axons in the mouse. In addition, Nell2 null mice show defects in the eye-specific retinogeniculate projection. Our results indicate that Nell2 acts as an inhibitory guidance cue in the layer-specific visual projections. In the present study, we examined ligand-receptor interactions in Nell2-medicated retinal axon guidance. Firstly, by using a series of expression constructs for different domains of the Nell2 protein, we performed binding assays and axon behavior assays. We found that cysteine-rich domains of Nell2 bind to and inhibit murine retinal axons *in vitro*. Secondly, we detected expression Ros-1, a receptor tyrosine kinase that binds to Nell2, in developing retinal ganglion cells by immunohistochemistry. Taken together, these results suggest that Nell2 exerts its effects through interactions between its cysteine-rich domains and the Ros1 receptor and acts as an inhibitory cue that guides retinal axons to appropriate layers in the brain targets.

Disclosures: **C.M. Nakamoto:** None. **M. Dunson:** None. **C. Meyers:** None. **A. Carney:** None. **S. Hafner:** None. **T. Ragle:** None. **M. Nakamoto:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.04/B7

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01NS117701

Title: The function of Nox2 in retinal ganglion cells for retinotectal pathfinding

Authors: *P. VEGA-RODRIGUEZ, C. J. WEAVER, A. TERZI, T. S. PIKES, Y. LEUNG, Q. DENG, D. M. SUTER;
Biol. Sci., Purdue Univ., WEST LAFAYETTE, IN

Abstract: Reactive oxygen species (ROS) play an important signaling role in cell proliferation, differentiation, adhesion, motility, and immune response. Appropriate intermediate levels of ROS are critical for normal cell development and physiology. Here, we provide evidence for NADPH oxidase 2 (Nox2)-derived ROS in neuronal development. Pharmacological inhibition of Nox enzymes in zebrafish larvae by Celastrol resulted in increased ganglion cell layer (GCL) width as well as decreased optic nerve thickness and tectal innervation by retinal ganglion cell (RGC) axons. These morphological changes could be partially rescued with the addition of hydrogen peroxide (H_2O_2). We have established Nox isoform-specific deletions by CRISPR/Cas9 in order to identify which specific isoform is required for axonal growth and guidance along the retinotectal pathway. From this analysis we found that Nox2-deficiency caused increased GCL width and mistargeted RGC axons in the optic tectum (OT) as well as aberrant axonal projections in other parts of the central nervous system. However, the details of how Nox2 regulates the formation of retinotectal connections are still unclear. RGC-specific knockout and rescue of Nox2 will address the question of whether Nox2 in RGCs is required and sufficient, respectively, for establishment of proper retinotectal connections in developing zebrafish larvae. We hope that our findings will contribute to a better understanding of the role of Nox2-derived ROS in nervous system physiology and disease.

Disclosures: **P. Vega-Rodriguez:** None. **C.J. Weaver:** None. **A. Terzi:** None. **T.S. Pikes:** None. **Y. Leung:** None. **Q. Deng:** None. **D.M. Suter:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.05/B8

Topic: A.05. Axon and Dendrite Development

Support: NIMH Grant F31MH129079
NINDS Grant R01NS050835

Title: Repeated use of teneurin-3 and latrophilin-2 in circuit-wide topographic target selection of the extended hippocampal network

Authors: *E. C. GINGRICH[1,2], D. T. PEDERICK[3], L. LUO[3];
[2]Neurosciences Interdepartmental Program, [1]Stanford Univ., Stanford, CA; [3]Biol., Stanford University/HHMI, Stanford, CA

Abstract: Precise circuit assembly is critical for nervous system function and is accomplished, in part, through cell-surface proteins (CSPs) that mediate cell-cell interaction during target selection. Contact-dependent attraction and repulsion are two mechanisms mediated by these CSPs that can be used to select synaptic partners, but the relatively low number of CSPs compared to the vast number of synapses that must be specified presents a biological challenge to developing axons. A possible strategy to overcome this challenge is repeated use of the same receptor-ligand pair to specify multiple connections across a network. One such receptor-ligand pair, Teneurin-3 (Ten3) and Latrophilin-2 (Lphn2), has complementary expression across multiple interconnected regions of the extended hippocampal network: Ten3 is expressed only in the medial sub-network (MHN) and Lphn2 only in the lateral sub-network (LHN), following a "Ten3 to Ten3 and Lphn2 to Lphn2" connectivity rule. Within one of these projections, CA1 to subiculum (Sub), Ten3-expressing CA1 axons from MHN are attracted to subicular Ten3 and repelled by subicular Lphn2 while Lphn2-expressing CA1 axons from LHN are repelled by subicular Ten3 (Berns et al., *Nature* 554:328-333.2018; Pederick et al., *Science* 372:1068–1073, 2021). The stereotyped, topographical connections and the circuit-wide complementary expression of Ten3 and Lphn2 make this system ideal to test whether the same mechanisms of Ten3/Lphn2-mediated repulsion and homophilic Ten3/Ten3-mediated attraction are re-used at each anatomical node of the circuit. Using a conditional knockout approach in mice, we have found that Ten3 is required in entorhinal cortex (EC) axons to correctly target proximal CA1 and distal Sub. Furthermore, EC axons mistarget when Ten3 and Lphn2 are conditionally deleted from Sub, suggesting these mechanisms generalize to other local connections. We are currently examining the role of Ten3 and Lphn2 in extended connections within these networks. To our knowledge, this study is the first to examine if a single receptor-ligand pair can instruct wiring specificity across multiple nodes of a functional network using a conditional knockout approach.

Disclosures: E.C. Gingrich: None. **D.T. Pederick:** None. **L. Luo:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.06/B9

Topic: A.05. Axon and Dendrite Development

Support: Japan Society for the Promotion of Science 23K06328

Title: Plxna3 deficient mice exhibit the midline crossing defect of callosal pioneer axons and decreased cell density of astrocytes in the region of indusium griseum

Authors: *K. YUKAWA[1], M. HOSSAIN[1], T. TSUZUKI[1], I. TAKAHASHI[1], T. KAWASAKI[2], T. NEGISHI[1];
[1]Meijo Univ., Nagoya, Japan; [2]Natl. Inst. of Genet., MIshima, Japan

Abstract: <META NAME="author" CONTENT="名城大学 教員">The corpus callosum (CC) is the largest commissure that shapes the major interhemispheric connection. Plexin-A3 (PlxnA3) is a transmembrane receptor for semaphorins, a family of axon guidance molecules playing key roles during nervous system development. *PlxnA3* is expressed in the cingulate cortex from which pioneer axons project toward the midline for the initiation of CC development. However, the role of PlxnA3 in CC development remains unclear. In the immunohistochemistry (IHC) to examine the role of PlxnA3, the midline crossing of neuropilin1-expressing callosal pioneer axons at embryonic day 17.5 (E17.5) was significantly impaired in *PlxnA3* KO mice under a genetic background of BALB/cAJ mice as compared with wild type (WT) mice (χ^2 test, $P < 0.05$). Agenesis of corpus callosum in the rostral and medial part of the CC was confirmed in *PlxnA3* KO mice at postnatal day 0.5. IHC to examine PlxnA3 expression in glial cells in the cortical midline at E17.5 revealed the expression of PlxnA3 in GFAP-positive (GFAP+) mature astrocytes residing in indusium griseum (IG) and midline zipper (MZ), and in GLAST+ cells. To examine the formation of guidepost structures in *PlxnA3* KO mice, we performed IHC in both WT and *PlxnA3* KO brains at E17.5 using antibodies against GFAP and Sox9, a glial nuclear marker expressed in radial glia, glial progenitors and mature glia. As a result, both GFAP+ cells and Sox9+ cells in the IG region of *PlxnA3* KO brains were significantly fewer than those of WT ($P < 0.05$, Student's *t* test). Thus, both GFAP+ cells and Sox9+ cells in the IG region of *PlxnA3* KO brains were significantly less than WT, but not in the MZ region. Mature GFAP+ astrocytes in the IG produce and secrete axon guidance molecules. To examine the expression pattern of *Slit2* mRNAs in the IG of *PlxnA3* KO brains at E17.5, we performed *in situ* hybridization of *Slit2* mRNAs followed by IHC with anti-GFAP antibody. *Slit2* mRNAs were localized to GFAP+ cells in IG and MZ in WT brains at E17.5. In contrast, *Slit2* mRNAs were hardly expressed in IG, and rather diffusely expressed in the cortical midline in *PlxnA3* KO brains at E17.5. Taken together, the results indicate that PlxnA3 is necessary for the complete formation of IG in which mature astrocytes gather and secrete axon guidance cues like Slit2 to guide the pioneer axons for CC development.

Disclosures: K. Yukawa: None. **M. Hossain:** None. **T. Tsuzuki:** None. **I. Takahashi:** None. **T. Kawasaki:** None. **T. Negishi:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.07/B10

Topic: A.05. Axon and Dendrite Development

Support: NIH Pioneer Award DP1 NS106665
NIH Grant R01 NS045523
Simmons Awards at the Harvard Center for Biological Imaging (RRID:SCR_018673)

Title: Discovery and functional characterization of growth cone-localized ribosome-centric translational controls involved in neuronal subtype-specific axon guidance

Authors: *T. TRAN[1], J. FROBERG[1], O. DURAK[1], B. BUDNIK[4], J. D. MACKLIS[2,3]; [2]Dept. of Stem Cell and Regenerative Biol., [3]Ctr. for Brain Sci., [1]Harvard Univ., Cambridge, MA; [4]Wyss Inst., Boston, MA

Abstract: In the mammalian cerebral cortex, diverse subtypes of projection neurons (PNs) form extremely long-range axonal connections to their distinct targets, and are responsible for motor, sensory, cognitive, and behavioral functions. During development, PNs form growth cones (GCs), subcellular specializations that locally integrate environmental cues and direct axonal pathfinding and synapse formation semi-autonomously. These processes are likely controlled at least partly by local subcellular translational regulation. Increasing evidence implicates the role of ribosomes in neuronal biology; while other studies highlight the vast potential for specialization of ribosomes' function via modifying their compositions of structural ribosomal proteins (RPs) and associated proteins (RAPs), motivating investigations of ribosome machinery as powerful translational control mechanisms. These observations lead us to hypothesize that during development ribosomes might exhibit GC subcellular and/or subtype-specific protein compositions in cortical PN that might regulate brain circuit development. We used RP L22-3xHA^{fl} mice to first address the hypothesis of PN subtype-specific ribosomal protein compositions. We combined Emx1-driven Cre induction and retrograde labeling to target ribosomes in callosal PN (CPN; send their axons via the corpus callosum axonal tract linking the two cortical hemispheres). We pulled down ribosomes with high specificity from FACS-purified somata, and proteomic analysis confirmed enrichment of RPs. We are working to enhance the method's power to enable comparisons between PN subtypes. To target CPN axonal GC ribosomes, we injected plasmids inducing HA-tagged ribosome expression into one lateral ventricle and performed *in utero* electroporation (IUE) at E14.5, aiming to collect axonal GCs in the contralateral cortex at P3 for ribosome pulldown. We found no evidence for HA-tagged ribosomes in axons or GCs following E14.5 IUE of Cre into RP L22-3xHA^{fl} mice. In contrast, IUE of a plasmid targeting RP L31, RPL31-3xHA-IRES-myrTdTomato, leads to robust HA immunolabeling in axons and GCs of CPN, motivating us to prioritize this approach for CPN axonal/GC ribosome pulldown experiments. Combining *in vivo* subtype-specific biology, proteomics, projection analysis, subcellular molecular mapping, this project aims to substantially increase understanding of GC dynamics, circuit-specific formation, and later maintenance and function of physiologically relevant systems. Furthermore, it will contribute to understanding the subcellular properties of the ribosomal complex in a cellular model of extreme polarization.

Disclosures: **T. Tran:** None. **J. Froberg:** None. **O. Durak:** None. **B. Budnik:** None. **J.D. Macklis:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.08/B11

Topic: A.05. Axon and Dendrite Development

Support: MOE2018-T2-2-103
MOH-000212
MOH-000207

Title: Mutation in IVNS1ABP Dysregulates Drebrin Phosphorylation and Actin-Microtubule Interaction in Growth Cones

Authors: *S.-M. CHOU[1], F. YUAN[1], S.-C. ZHANG[2,1];
[1]Duke-NUS Med. Sch., Singapore, Singapore; [2]Univ. of Wisconsin - Madison, Univ. of Wisconsin, Madison, WI

Abstract: We discovered an undiagnosed disease in which children display premature aging and severe neuropathy. Whole exome-sequencing revealed a point mutation, c.758T>G, in the IVNS1ABP gene. What does IVNSIABP do and how does the IVNS1ABP mutation result in neuropathy are unknown. By generating induced pluripotent stem cells (iPSCs) and correcting the mutation and then differentiating them to cortical neurons, we found a reduced growth cone size, especially the peripheral domain (P-domain), in the mutant cells, which is restored by correcting the mutation. There is also a decreased contact between actin bundles and tyrosinated microtubules, along which there is an increased aggregation and reduced phosphorylation of Drebrin, a protein involved in the regulation of actin-microtubule interactions as well as microtubule bundling in the central domain (C-domain) of the growth cone. Indeed, there is an increase in non-bundling microtubules, along which is a reduced transport of IVNS1ABP to the C-domain and transition zone (T-zone). These results suggest a role of IVNS1ABP in growth cone size and dynamics via regulation of drebrin phosphorylation, explaining the contribution of IVNS1ABP mutation to neuropathy.Keywords: IVNS1ABP; kelch protein; growth cone; actin; microtubule; Drebrin

Disclosures: **S. Chou:** None. **F. Yuan:** None. **S. Zhang:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.09/B12

Topic: A.05. Axon and Dendrite Development

Support: NIH RO1 5R01NS113314-04
NIH R21 1R21NS125419-01

Title: Determination of mTOR-Independent TSC2 targets in human cortical growth cones

Authors: *A. PIER[1], T. S. CATLETT[2], T. M. GOMEZ[3];
[1]Neurosci., Univ. of Wisconsin - Madison Neurosci. Training Program, Madison, WI; [2]Dept. of Neurosci., Univ. of Wisconsin-Madison, Madison, WI; [3]Dept of Neurosci., Univ. of Wisconsin - Madison, Madison, WI

Abstract: Tuberous Sclerosis Complex (TSC) is an autosomal dominant neurodevelopmental disorder caused by a monogenic mutation to either *TSC1* or *TSC2*. Nearly one-half of TSC patients have mild to profound intellectual disabilities and autism, with the majority developing seizures. The TSC1 and TSC2 proteins are known to interact and form a protein complex (TSC1-TSC2), which negatively regulates mTORC1-mediated protein synthesis and activates mTORC2-mediated cytoskeletal rearrangements. Regulation of local protein synthesis and the cytoskeleton are vital for proper axon guidance and neural network formation. In a previous study, we found that human forebrain (hFB) neurons differentiated from $TSC2^{+/-}$ induced pluripotent stem cells (iPSCs) exhibited dramatic defects in axon outgrowth and sensitivity toward several canonical axon guidance cues. Surprisingly, these defects were found to be *independent* of both mTORC pathways, while basal and cue-activated RhoA signaling was diminished. While mis-regulation of the actin cytoskeleton in $TSC2^{+/-}$ growth cones is clearly involved in observed defects, we suspected that microtubule (MT) dynamics may also be abnormal. Therefore, we examined the expression levels of key MT regulators within growth cones of TSC patient derived ($TSC2^{+/-}$) and corrected ($TSC2^{+/+}$) hFB neuron cultures. We find that HDAC6 expression is significantly increased in mutant $TSC2^{+/-}$ neuronal growth cones compared to corrected $TSC2^{+/+}$ controls. As HDAC6 is known to deacetylate MTs, we examined microtubule acetylation levels normalized to α-tubulin for both groups. We discovered that $TSC2^{+/-}$ growth cones have reduced microtubule acetylation compared $TSC2^{+/+}$. Currently, we are examining MT dynamics in live hFB neuronal growth cones expressing GFP-EB3 to determine whether MT dynamics are altered between TSC2 genotypes. In addition, we are testing whether increased HDAC6 is responsible for observed changes in axon extension and cue responses. In a related approach, we are conducting rescue experiments using a lentivirus (LV) to express a Tet-inducible Halo-tagged TSC2 construct we generated. This LV will allow us to acutely restore the normal expression levels of TSC2 in $TSC2^{+/-}$ *and* $TSC2^{-/-}$ neurons. In addition, we will localize TSC2 within live growth cones during stimulation with guidance cues and assess whether this LV restores HDAC6 expression levels. Modifications to the LV construct will allow us to analyze the relevant domains of TSC2 that are necessary for the proper regulation of RhoA activity and HDAC6 expression levels, as well as regulation of axon extension and cue responses.

Disclosures: A. Pier: None. **T.S. Catlett:** None. **T.M. Gomez:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.10/B13

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant 1ZIANS003140-08

Title: The Rho-GEF Trio interacts with CRMP2 downstream of Semaphorin3A to regulate cytoskeletal dynamics

Authors: *A. LOMBARDO, E. FINGLETON, Y. LI, K. W. ROCHE;
NIH, Bethesda, MD

Abstract: Trio is a neuronal Rho-Guanine nucleotide Exchange Factor (GEF) critical for Rac1 and RhoA activation and thereby modulates actin polymerization, cytoskeleton remodeling, and neuronal development. Previously, we identified Collapsin Response Mediator Proteins (CRMPs 1-4), as Trio interactors via affinity purification-mass spectrometry. CRMP2 is the best characterized member of the CRMP family and shares functional characteristics with Trio. Both Trio and CRMP2 play a bidirectional role in axon outgrowth and repulsion, are involved in the Rac1 and RhoA signaling pathways, and are implicated in Autism Spectrum Disorder. CRMP2 is a key mediator of Semaphorin3A-induced axon retraction and growth cone collapse, which are necessary events to modulate cytoskeletal dynamics during regenerative and developmental processes. Given that Trio is essential for RhoA activation, which promotes actomyosin contractility, and interacts with CRMP2, Trio may also be a critical mediator of neuronal migration within the Semaphorin3A pathway. We characterized the Trio and CRMP2 protein interaction using co-immunoprecipitation assays from rat whole brain homogenate. Additionally, we evaluated the distribution of Trio and CRMP2 expressed in heterologous COS7 cells and observed colocalization. To evaluate the effect of Trio on Semaphorin3A-induced axon retraction, we used short hairpin RNA to knock-down Trio in primary hippocampal neurons. We then tested signaling pathways by bath applying Semaphorin3A. We observe that Semaphorin3A serves as an axon guidance repulsive cue, which is in keeping with the literature. However, knocking down Trio abrogates Semaphorin3A-induced axon retraction, suggesting Trio functions as a mediator of the Semaphorin3A pathway. Future studies will help elucidate the exact molecular mechanism of Trio as a CRMP2 interactor and a mediator within the Semaphorin3A pathway. A more complete understanding of the functional importance of Trio will provide insight as to how Trio's disruption contributes to the pathogenesis of several neurodevelopmental disorders and may illuminate novel therapeutics for individuals possessing Trio mutations.

Disclosures: A. Lombardo: None. **E. Fingleton:** None. **Y. Li:** None. **K.W. Roche:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.11/B14

Topic: A.05. Axon and Dendrite Development

Support: DP2MH122398

Title: Ganon-1, a novel ncrna, binds to mTOR in developing axons and impacts axonal growth.

Authors: *G. CRUTCHER[1], S. MALAIYA[2], R. WHITTEN[2], C. BRANDENBURG[3], A. ROMANOWSKI[4], A. POULOPOULOS[5];
[1]Univ. of Maryland: Baltimore, Baltimore, MD; [2]Univ. of Maryland, Baltimore, Baltimore, MD; [3]Pharmacol., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [4]Univ. of Maryland Baltimore, Baltimore, MD; [5]Pharmacol., Univ. Maryland, Baltimore, MD

Abstract: During the early phases of development, neurons extend a hand-like structure known as the growth cone, which guides the axon to its projection site in the brain. Transcriptomic analyses of growth cones have revealed a non-coding RNA produced by a gene, we named Ganon-1. This RNA is highly enriched during neural development. According to NCBI and other databases, Ganon-1 is considered "PREDICTED," which means it has been identified in large-scale sequencing projects, but no gene-specific approaches have been undertaken to validate its presence. To investigate Ganon-1, we performed 5'/3' rapid amplification of cDNA ends (RACE) experiments in developing neurons. These experiments revealed that Ganon-1 is present in the cytosol with at least three distinct 3' ends. 5' RACE results showed that Ganon-1 may produce a non-coding RNA that shares sequence homology with the 5' terminal oligopyrimidine (TOP) motif commonly associated with mTOR-dependent mRNAs. To examine the functionality of Ganon-1's 5' TOP motif, we conducted co-immunoprecipitation experiments to isolate the mTOR complex machinery and used PCR to identify the presence of Ganon-1. Purified mTOR fractions were found to be enriched with Ganon-1, suggesting that the 5' TOP motif is functional. Additionally, we overexpressed Ganon-1 in cultured cells, which resulted in longer axonal outgrowth compared to control transfected cells. We also performed RNAscope with a probe designed to target Ganon-1 in order to map its expression profile throughout development. Our findings reveal a novel target for mTOR pathologies and highlight the need for further investigation into the interactions between non-coding RNAs and the mTOR machinery.

Disclosures: G. Crutcher: None. **S. Malaiya:** None. **R. Whitten:** None. **C. Brandenburg:** None. **A. Romanowski:** None. **A. Poulopoulos:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.12/B15

Topic: A.05. Axon and Dendrite Development

Support: EMBO Postdoctoral Fellowship 364-2021

Title: Gsk3β Regulates MAP1B to promote cortical interstitial axon branching through modulation of the tubulin code

Authors: *J. ZIAK[1,2], S. SUDARSANAM[1,2], B. TRIGG[1,2], J. DORSKIND[1,2], Y. XU[1,2], S. BARAO[1,2], U. MUELLER[1,2], A. KOLODKIN[1,2];
[1]Johns Hopkins Med. Institutions, Baltimore, MD; [2]Kavli Neurosci. Discovery Inst., Solomon H. Snyder Dept. of Neurosci., Baltimore, MD

Abstract: The cerebral cortex is a laminated structure that plays a central role in cognition, motor skills and sensory processing. The establishment of functional cortical connectivity requires that neurons elaborate complex axon branching patterns. However, the intracellular signaling pathways that regulate laminar-specific interstitial axon branching are poorly understood. Strikingly, experimental approaches that allow for studying mouse neuronal connectivity in a quantitative fashion at the single cell level are limited. Thus, identifying molecular components underlying interstitial axon branching has been a long-standing question in the field.
We are investigating the unique patterns of interstitial axon branching and overall morphology of layer 2/3 callosal projection neurons (CPNs). We have developed in utero electroporation strategies that allow for temporally controlled, sparse and robust labeling of select excitatory cortical CPNs. Combining these approaches with brain clearing and lightsheet imaging allows for precise visualization of complete axonal arbors.
Using these techniques, we analyzed over 2000 individually *in vivo* labelled excitatory callosal projection neurons, with, in total, more than 15.000 interstitial axon branches. We show that activation of the serine/threonine kinase GSK3β promotes interstitial axon branching in layer 2/3 CPNs by releasing MAP1B-mediated inhibition of axon branching. MAP1B mutagenesis experiments show that naïve MAP1B restricts interstitial branching while phosphorylated MAP1B promotes it. In addition, we find that GSK3β/MAP1B signaling regulates the tyrosination/detyrosination cycle of α-tubulin, which in turn increases or decreases the probability of generating interstitial axon branches. Increasing the ratio of tyrosinated to detyrosinated α-tubulin promotes interstitial axon branching and targeted expression of a fluorescent sensor in layer 2/3 CPNs reveals high levels of tyrosinated α-tubulin in axonal segments enriched for interstitial axon branches. We propose a model whereby a MAP1B brake restricts interstitial axon branching until it is released by GSK3β phosphorylation, allowing for regulated generation of a pool of tyrosinated microtubules in the axonal shaft. These observations are an important first step in understanding the generation of stereotypical laminar-specific CPN axon branches and may be generally applicable to multiple populations of cortical excitatory projection neurons. Together, we describe here one of the first intracellular signaling pathways that cell-autonomously regulates interstitial axon branching in the developing neocortex.

Disclosures: J. Ziak: None. **S. Sudarsanam:** None. **B. Trigg:** None. **J. Dorskind:** A. Employment/Salary (full or part-time):; Novartis. **Y. Xu:** None. **S. Barao:** None. **U. Mueller:** None. **A. Kolodkin:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.13/B16

Topic: A.05. Axon and Dendrite Development

Support: FSU 8474000395

Title: Septin 5 and 6, dual regulators of stability and maintenance of the axon initial segment

Authors: *H. HAMDAN[1,2], T. TORII[3,4], M. N. RASBAND[5], M. N. RASBAND[5];
[1]Physiol. and Immunol., Khalifa Univ., Abu Dhabi, United Arab Emirates; [2]Neurosci., Baylor Col. of Med., Houston, TX; [3]Neurosci., Baylor Col. of Med., Houston, TX; [4]Lab. of Ion Channel Pathophysiology, Grad. Sch. of Brain Sci., Doshisha Univ., Kyotanabe-shi, Kyoto, Japan; [5]Neurosci., Baylor Col. of Med. Dept. of Neurosci., Houston, TX

Abstract: Hamdan Hamdan [1, 3], Tomohiro Torii[2, 3], Matthew N. Rasband[3]
1 Department of Physiology and Immunology, College of Medicine and Health Sciences, and Biotechnology Center, Khalifa University, Abu Dhabi, UAE.2 Laboratory of Ion Channel Pathophysiology, Graduate School of Brain Science, Doshisha University, Kyotanabe-shi, Kyoto, Japan.3 Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA.
Septin 5 and 6, dual regulators of stability and maintenance of the axon initial segment
The axon initial segment (AIS) is a unique neuronal domain that generates action potentials and regulates neuronal polarity. Increasing numbers of studies report AIS proteomes. Previously, we performed advanced proteomics using the BioID system and found AIS septins. Septins are a family of GTP-binding proteins that associate with actin and microtubules. In particular, both of Septin5 (Sept5) and Septin6 (Sept6) interact with AnkyrinG (AnkG) and control AIS assembly and maintenance in neurons during development in vitro, however the molecular mechanisms involving septins are still largely unknown.Here, we show Sept6 regulates Trim46 levels to control AIS maintenance, since the expression levels of Trim46 are significantly decreased in Sept6 shRNA-expressing primary hippocampal neurons. Interestingly, silencing of Sept5 did not affect Trim46 immunoactivity. Moreover, silencing of Sept5 or Sept6 reduced EB3 (end-binding protein 3) specific immunoactivity in AIS, suggesting these septins may also regulate EB3-mediated interactions between AnkG and microtubules. Collectively, Sept5 and/or Sept6 are components of the AIS and may regulate microtubule and actin dynamics in the AIS since septin-mediated actin-microtubule crosstalk is essential for maintenance of cell morphology.

Disclosures: H. Hamdan: None. **T. Torii:** None. **M.N. Rasband:** None. **M.N. Rasband:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.14/B17

Topic: A.05. Axon and Dendrite Development

Support: DFG/SFB1286

Title: Paralemmin-1 is a new modulator of the Membrane-associated Periodic Skeleton in neurons

Authors: V. MACARRON PALACIOS[1], J. HUBRICH[1], M. DO REGO BARROS FERNANDES LIMA[1], C. ACUNA[2], M. TRAPP[3], A. PATRIZI[3], *E. D'ESTE[1], M. KILIMANN[4];
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Abstract: The Membrane-associated Periodic Skeleton (MPS) is a specialized cytoskeletal lattice found in virtually all neuronal cells. As the name indicates, this structure is located underneath the plasma membrane and features a ~190 nm periodic organization. The main MPS components are actin and adducin, which build ring-like structures that are horizontally crosslinked by spectrin tetramers. Recent proteomic studies identified hundreds of proteins interacting with the MPS, including motor proteins, cell adhesion molecules, and receptors. Although remarkable progress has been made since the discovery of the MPS a decade ago, the mechanisms regulating this lattice within neurons are so far poorly understood. By using super resolution fluorescence microscopy techniques (STED and MINFLUX) in hippocampal primary neurons, we identified Paralemmin-1 as a novel component of the MPS. Paralemmin-1 is a highly hydrophilic phosphoprotein anchored to the inner face of the plasma membrane and involved in membrane expansion and dendritic spine maturation. In our study, we demonstrate that Paralemmin-1 alone is able to regulate the nanoscale organization of the MPS and affects the neuronal development. Indeed, the overexpression of Paralemmin-1 leads to a strong enhancement of the MPS periodicity and neuronal arborization. Conversely, its depletion negatively impairs the MPS periodicity, delays neuronal development, and affects the electrophysiological properties of the neurons. Finally, using MINFLUX, a microscopy technique which allows localization of a target with single-digit nanometer precision, we determine the precise position of Paralemmin-1 within the MPS, namely along the actin/adducin ring-like structures. Together, our work identifies Paralemmin-1 as a novel component of the MPS and reveals a new mechanism regulating this structure.

Disclosures: V. Macarron Palacios: None. **J. Hubrich:** None. **M. do Rego Barros Fernandes Lima:** None. **C. Acuna:** None. **M. Trapp:** None. **A. Patrizi:** None. **E. D'Este:** None. **M. Kilimann:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.15/B18

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R35 GM142726
NIH Grant P20 GM125528
College of Medicine Alumni Association Grant
OUHSC Graduate College McNair Alumni Award

Title: Soluble amyloid precursor protein limits axon outgrowth in developing hippocampal neurons

Authors: *D. BARBER[1,3], S. HOUMAM[2,3], C. LACY[2,3], K. SHUKLA[2,3], H. RICE[2,3];
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Abstract: Abstract: **(Introduction)** Neuronal morphological development is a complex process critical to proper brain function. The amyloid precursor protein (APP), a protein normally associated with the pathogenesis of Alzheimer's, has also been shown to affect neuronal morphological development, including neurite outgrowth and branching. Recently, we discovered that sAPPα functions as a $GABA_BR1a$-isoform specific ligand to modulate synaptic transmission in neurons. Curiously, the $GABA_B$ Receptor has also been implicated in shaping neuronal morphology throughout development; thus, we investigated whether the effects of sAPP on neurite outgrowth are mediated through $GABA_BR$ signaling. **(Methodology)** Utilizing immunocytochemistry (ICC) of primary mouse neuron culture, we investigated morphological changes to both axons and dendrites in response to treatment with sAPPα purified protein and baclofen ($GABA_B$ agonist) in combination with CGP55845 ($GABA_B$ Inverse agonist). Axons and dendrites were measured using Beta III Tubulin (neurite marker), MAP2 (dendritic marker), and Tau (axon marker). **(Results)** We found that axons specifically showed a reduction in neurite outgrowth (DIV 3) that persisted into later stages of development (DIV 7) when treated with sAPPα or a 17 amino acid peptide corresponding to the specific binding region of sAPPα to the sushi domain of the $GABA_B$ R1a receptor. In addition, we have found that NGN2 doxycycline inducible neurons express $GABA_B$ R1 receptor as early as 6 days post differentiation. (Conclusions) These findings expand on the functional outcomes of sAPPα- $GABA_B$ signaling in the developing brain, suggesting that this interaction may govern more than synaptic activity. The NGN2 iNs also could serve as a model system for future studies to study how well this interaction is conserved in human neurons.

Disclosures: D. Barber: None. **S. Houmam:** None. **C. Lacy:** None. **K. Shukla:** None. **H. Rice:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.16/B19

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

Support: NIH NINDS NS086794

Title: Nmnat2 in cortical glutamatergic neurons exerts both cell and non-cell autonomous influences to shape cortical development and to maintain neuronal health

Authors: *Z.-X. NIOU;
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Abstract: Nicotinamide mononucleotide adenylyl transferase 2 (NMNAT2) is neuroprotective in numerous preclinical models of neurodegeneration. It belongs to NMNAT family as an essential NAD synthesizing enzyme and molecular chaperone. NMNAT2 is the major NMNAT expressed in the brain and is highly expressed in post-mitotic cortical neurons. NMNAT2 has been identified as a key neuronal maintenance factor using nerve injury model. However, it is unclear what is its endogenous role in brains. Here we specifically deleted NMNAT2 in glutamatergic neurons and examined different aspects of brain development. By using several axonal markers, we found loss of NMNAT2 will lead to early on-set axonal degeneration. The abnormal accumulations of Amyloid Precursor Protein (APP) in axons together with activated astrocytes and microglia in NMNAT2 conditional KO brains indicates neurodegenerations. Taken together, we observed critical evidence for NMNAT2 plays an important role to maintain axon stability during the mouse brain development. Using a genetic approach, we found that only complete loss of Sarm1 function in NMNAT2 cKO mice was able to prevent the impact of NMNAT2 loss on axonal integrity and inflammation. Taken together, our studies provide new in vivo supportive evidence for NMNAT2's roles in the formation and maintenance of the neurons in the CNS.

Disclosures: Z. Niou: None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.17/B20

Topic: A.05. Axon and Dendrite Development

Support: BR4910/1-2 (SPP1738)
BR4910/2–2 (SPP1935)
SE697/4-2 (SPP1738)
SE697/5–2 (SPP1935)

Fi573/15-2 (SPP1935)
Fi573/20-1

Title: Cytosolic Ptbp2 modulates axon growth in motoneurons through axonal localization and translation of Hnrnpr

Authors: *S. SALEHI[1], A. ZARE[1], G. PREZZA[1], J. BADER[2], C. SCHNEIDER[3], U. FISCHER[3], F. MEISSNER[2], M. MANN[2], M. BRIESE[1], M. SENDTNER[1];
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Abstract: The neuronal RNA-binding protein Ptbp2 regulates neuronal differentiation by modulating alternative splicing programs in the nucleus. Such programs contribute to axonogenesis by adjusting the levels of protein isoforms involved in axon growth and branching. While its functions in alternative splicing have been described in detail, cytosolic roles of Ptbp2 for axon growth have remained elusive. Here, we show that Ptbp2 is located in the cytosol including axons and growth cones of motoneurons, and that depletion of cytosolic Ptbp2 affects axon growth. We identify Ptbp2 as a major interactor of the 3' UTR of *Hnrnpr* mRNA encoding the RNA-binding protein hnRNP R. Axonal localization of *Hnrnpr* mRNA and local synthesis of hnRNP R protein are strongly reduced when Ptbp2 is depleted, leading to defective axon growth. Ptbp2 regulates hnRNP R translation by mediating the association of *Hnrnpr* with ribosomes in a manner dependent on the translation factor eIF5A2. Our data thus suggest a mechanism whereby cytosolic Ptbp2 modulates axon growth by fine-tuning the mRNA transport and local synthesis of an RNA-binding protein.

Disclosures: S. Salehi: None. **A. zare:** None. **G. Prezza:** None. **J. Bader:** None. **C. Schneider:** None. **U. Fischer:** None. **F. Meissner:** None. **M. Mann:** None. **M. Briese:** None. **M. Sendtner:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.18/B21

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS107342

Title: Axonal targeting of the TrkA neurotrophin receptor by transcytosis

Authors: *G. MOYA, V. AHER, K. REJJI;
Johns Hopkins Univ., Baltimore, MD

Abstract: The complex morphology of neurons imposes unique challenges in controlling cellular functions in distal compartments such as axons and dendrites. A fundamental question in neuronal cell biology is how membrane proteins are targeted to axons after their biosynthesis in cell bodies. Axonal delivery of membrane proteins has been proposed to occur via several modes, including direct trafficking via the secretory pathway and transcytosis. Transcytosis is an atypical endocytosis-based mechanism, where newly synthesized proteins are first inserted on cell body surfaces, internalized, and anterogradely transported to axons. Previously, we found that the TrkA receptor for the neurotrophin, Nerve growth factor (NGF), is actively recruited to axons of sympathetic neurons via transcytosis, in a manner triggered by the ligand acting on distal axons. These results provide the first evidence for a non-canonical ligand-promoted mode of axonal targeting of membrane proteins and suggest a positive feedback mechanism that dynamically scales up receptor availability in axons during times of need. However, little is known about the kinetics of transport, the organelles involved, and the functions of TrkA transcytosis. Using live imaging in microfluidic chambers, we observed that soma surface-labeled FLAG-TrkA receptors exhibit distinct behaviors upon transcytosis to distal axons, with anterograde movements, pausing, recycling to the axonal membrane, and intriguingly, retrograde movements. Electron microscopy revealed transcytosing TrkA receptors in endosomes and multi-vesicular bodies. Further, local injection of non-cell-permeable biotin and FLAG antibodies to cell bodies in sympathetic ganglia of mice resulted in TrkA receptors appearing in axon terminals innervating a target tissue, suggesting that TrkA transcytosis occurs *in vivo*. Finally, we found that transcytosed TrkA receptors are localized to axonal varicosities, thought to be synaptic sites, in sympathetic axons. Using newly generated TrkA knock-in mice to disrupt receptor transcytosis, we found impaired formation of pre-synaptic sites in sympathetic axons. Together, this work defines an under-appreciated pathway for long-distance delivery of membrane proteins to axons and highlights the physiological relevance of TrkA transcytosis.

Disclosures: G. Moya: None. **V. Aher:** None. **K. Rejji:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.19/B22

Topic: A.05. Axon and Dendrite Development

Support: DGAPA-PAPIIT, UNAM IN210716

Title: Analysis of actin polymerization by redox regulation in cerebellar granule neurons

Authors: *G. MEDINA RUIZ[1], J. MORAN[2];
[1]Univ. of Pittsburgh, PITTSBURGH, PA; [2]Natl. Univ. of Mexico, Apdo. Postal 70-253, Mexico

Abstract: Reactive Oxygen Species (ROS) are essential in cellular growth, proliferation, and cell death. NADPH oxidase 2 (NOX2) is one of the principal sources of ROS in the cells.

Interestingly, the only known function of this complex is the production of superoxide, which is an anion radical. NOX2 activity is essential for the respiratory burst in immune system cells, and there is evidence of its participation in signaling processes in neurons. The main interest in the study of NOX2 has focused mainly on its involvement in pathological processes. However, little is known about its role in physiological processes, such as neuronal development. In this work, we were interested in determining the role of NOX2 in actin polymerization regulation during neuronal development. To explore this phenomenon, we analyze F-actin changes in real-time using a fluorescent marker, LifeAct-GFP, to perform Fluorescence After Photobleaching (FRAP). We expressed LifeAct-GFP in primary cultures of cerebellar granular neurons and analyzed changes in actin polymerization in response to different oxidant or antioxidant conditions. We found that the overexpression of the cytosolic p47 subunit of NOX promotes a faster actin polymerization recovery than the control. This effect is reversed through the genetic (Overexpress the negative dominant p22 subunit) or pharmacological inhibition of NOX2. Our results indicate that the dynamic of actin polymerization is promoted by oxidant conditions and is inhibited by antioxidant conditions. This study raises the possibility of NOX as a positive physiological regulator of actin polymerization in the elongation of the neurites in the cerebellar granule neurons during development.

Disclosures: G. Medina Ruiz: None. **J. Moran:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.20/B23

Topic: A.05. Axon and Dendrite Development

Support: KAKENHI #18K06459, #17KK0144, and #21K19305 to M.N.
KAKENHI #18H04013, #18H04670; AMED-CREST #19gm1210007s0101, #20gm1210007s0102, #21gm1210007s0103, and #22gm1210007s0104 to M.I.

Title: F-actin induces the accumulation of an axon guidance receptor in the non-adhesive surfaces of growth cones, inducing the formation of filopodia

Authors: *M. NOZUMI, M. IGARASHI;
Dept Neurochem & Mol Cell Biol, Niigata Univ, Grad Sch. Med. Dent. Sci., Niigata, Japan

Abstract: F-actin in growth cones, regulated by axon guidance molecules, is crucial for their precise advance. We previously reported that local endocytosis simultaneously was performed together with bundling of F-actin near the leading edge of the growth cone (Nozumi et al., Cell Rep, 2017). To further investigate this phenomenon, we analyzed the three-dimensional structure of growth cones using super-resolution microscopy (3D-SIM) and total internal reflection microscopy. We observed that F-actin bundles were arranged in a regular pattern near the

adhesive surface of the growth cone, also near the leading edge. In contrast, F-actin bundles in the central domain of the growth cone, were distributed in the non-adhesive surface. There were irregularly extended F-actin bundles protruding and forming non-adhesive filopodia. Notably, these filopodia exhibited a very frequent turnover, and repeated growth and retraction for one minute, as observed through live imaging using GFP. Further analysis of the actin bundles comprising the non-adhesive filopodia revealed a higher concentration of an actin-depolymerizing factor, cofilin, than in others. This high cofilin content was considered to be responsible for the short lifetime of these filopodia. In addition, we found that an axon guidance receptor neuropilin-1, and one of its ligands, semaphorin 3A, were localized in such non-adhesive filopodia. Neuropilin-1 was localized in lipid rafts, and we also detected the accumulation of endocytosis-related proteins, endophilin, and dynamin, involved in lipid raft internalization, in the non-adhesive filopodia. To further investigate the relationship between cofilin and the formation of non-adhesive filopodia, we employed an optogenetic method to inactivate cofilin, resulted in an increase in neuropilin-1-positive non-adhesive filopodia. These findings suggest that F-actin in growth cones serves not only as a driving force for growth cone advance, but also it actively extends filopodia from the growth cone surface to capture extracellular axon guidance molecules, thereby accumulating neuropilin-1 receptors there.

Disclosures: **M. Nozumi:** None. **M. Igarashi:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.21/B24

Topic: A.05. Axon and Dendrite Development

Support: NRF-2020R1A5A1019023
NRF-2022R1A2C1004913
KHIDI-HU21C0071
BK21 Four Biomedical Science Program

Title: Deneddylating enzyme SENP8 regulates neuronal development

Authors: *S. LEE, J.-M. SONG, M. KANG, Y. SUH;
Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Neddylation is a cellular process in which the neural precursor cell expressed, developmentally down-regulated 8 (NEDD8) is conjugated to the lysine residue of target proteins via serial enzymatic cascades. Recently, it has been demonstrated that neddylation is required for synaptic clustering of metabotropic glutamate receptor 7 (mGlu7) and postsynaptic density protein 95 (PSD-95), and the inhibition of neddylation impairs neurite outgrowth and excitatory synaptic maturation. Similar to the balanced role of deubiquitylating enzymes (DUBs) in the ubiquitination process, we hypothesized that deneddylating enzymes can regulate neuronal

development by counteracting the process of neddylation. We find that the SUMO Peptidase Family Member, NEDD8 Specific (SENP8) acts as a key neuronal deneddylase targeting the global neuronal substrates in primary rat cultured neurons. We demonstrate that SENP8 expression levels are developmentally regulated, peaking around the first postnatal week and gradually diminishing in mature brain and neurons. We find that SENP8 negatively regulates neurite outgrowth through multiple pathways, including actin dynamics, Wnt/β-catenin signaling, and autophagic processes. Alterations in neurite outgrowth by SENP8 subsequently result in the impairment of excitatory synapse maturation. Our data indicate that SENP8 plays an essential role in neuronal development and is a promising therapeutic target for neurodevelopmental disorders.

Disclosures: **S. Lee:** None. **J. Song:** None. **M. Kang:** None. **Y. Suh:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.22/B25

Topic: A.05. Axon and Dendrite Development

Support: RGC
HMRF
CUHK direct grant scheme
United College endowment fund
TUYF Charitable Trust

Title: The roles of insulin in stimulating ARF6-Rac1-mediated neurite outgrowth

Authors: *K.-F. LAU;
The Chinese Univ. of Hong Kong, Shatin, NT, Hong Kong

Abstract: Neurite outgrowth is a crucial process for the formation of new projections for the establishment of neural connectivity during neuronal development. Emerging evidence suggests insulin, the essential hormone in maintaining blood glucose homeostasis, possesses neurotrophic functions in stimulating neurite outgrowth. However, the precise mechanisms of insulin signalling in the central nervous system is not fully understood. Deciphering the precise involvement of insulin in neurite outgrowth may furnish a novel molecular basis for developing new approaches to enhance the regeneration and re-wiring of injured neurons. To this end, we found that insulin upregulates neurite outgrowth mediated by ADP-ribosylation factor 6 (ARF6)-Ras-related C3 botulinum toxin substrate 1 (Rac1) signalling through the neuronal adaptor protein FE65. During the process, insulin stimulates atypical protein kinase Cι/λ (PKCι/λ) to induce phosphorylation of FE65 at serine 459 (S459). The phosphorylation of FE65 S459 further tiggers ARF6-Rac1-mediated neurite outgrowth by potentiate FE65-ARF6 interaction. Our findings reveal a novel mechanism that insulin stimulates neurite outgrowth.

Disclosures: K. Lau: None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.23/B26

Topic: A.05. Axon and Dendrite Development

Support: NCATS Award TL1TR001431
NIDCD R01-DC018040-01

Title: Molecular Mechanisms of Type II Spiral Ganglion Neuron Development

Authors: *D. GEORGE[1], A. CUI[2], S. THIRU[2], M. R. DEANS[3], T. M. COATE[2];
[1]Biol., [2]Georgetown Univ., Washington, DC; [3]Surgery, Univ. of Utah, Salt Lake City, UT

Abstract: The molecular mechanisms dictating spiral ganglion (SGN) neuron growth and cochlear innervation must be determined to understand normal auditory development. SGNs are bipolar neurons that relay auditory input to the cochlear nuclei after receiving glutamatergic input from mechanosensitive receptor hair cells. Type II SGNs represent a fascinating subdivision of neurons in the inner ear with a highly stereotyped projection pattern whereby they project past the IHCs, make a 90 turn toward the cochlear base then synapse with 10-15 outer hair cells (OHC). Planar cell polarity (PCP) proteins have previously been shown to mediate type II SGN turning, but whether additional axon guidance mechanisms are involved remains unknown. In this study, I am investigating axon guidance mechanisms that facilitate type II SGN guidance and OHC innervation.
I generated *Efna3* and *Vangl2* null mice carrying *Neurog1*^{CreERT2} and *R26R*^{tdTomato}, permitting SGN sparse labeling. Conversely, *Efna3; Vangl2* double knockouts (DKOs) are examined using anti-NF200. Immunostaining, confocal imaging, and 3D rendering in Imaris software was used to quantify type II SGN turning, branching and other navigation characteristics.
Immunostaining experiments exhibited EPHRIN-A3 expression on the membranes of inner pillar cells (IPCs) and Deiters' cells of the cochlear epithelium at E16 and P0. Compared to controls, *Efna3* null mice showed a small, but significant increase in type II SGNs incorrectly turning toward the apex. Both *Efna3* null and heterozygous mice showed increased numbers of type II SGNs with abnormal navigation behaviors. In particular, *Efna3* nulls displayed decreased branch numbers, suggesting EPHRIN-A3 may normally act as a positive growth cue. However, E15 *in vitro* outgrowth assays examining temporal aspects of EPHRIN-A3 on type II SGNs suggest it may cause growth cone repulsion. As predicted, *Vangl2* nulls displayed an immense rise in type II SGNs incorrectly turning to the apex. *Vangl2* null and heterozygous cochleae both displayed an increased (but rare) number of type II SGNs possessing abnormal navigation behaviors, similar to *Efna3* mutants. *Vangl2* null cochleae also displayed a lower number of branches per fiber compared to control littermates, suggesting VANGL2 may also act as a positive growth cue. *Efna3; Vangl2* DKO type II SGN turning defects resemble *Vangl2* nulls, suggesting the

Eph/Ephrin and PCP signaling systems operate in a linear pathway.
Taken together our findings suggest that Eph/Ephrin signaling may act downstream of PCP signaling to mediate type II SGN guidance during development.

Disclosures: **D. George:** None. **A. Cui:** None. **S. Thiru:** None. **M.R. Deans:** None. **T.M. Coate:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.24/B27

Topic: A.05. Axon and Dendrite Development

Support: NIH NCI 2R01CA205255-06A1
NIH NCI 1T32CA236754-01

Title: Human induced sensory neurons adopt adult morphology in co-culture with rodent satellite glia

Authors: ***C. J. LEBLANG**, M. P. MURPHY, T. MILLER, O. E. TASDEMIR YILMAZ, R. A. SEGAL;
Cancer Biology/Neurobiology, Dana Farber Cancer Institute/Harvard Med. Sch., Boston, MA

Abstract: Chemotherapy Induced Peripheral Neuropathy (CIPN) is a neurodegenerative disorder impacting the distal axons of peripheral sensory nerves, leading to debilitating symptoms including allodynia, paresthesia, temperature sensitivity, and numbness. More than half of patients receiving chemotherapy develop CIPN, and in severe cases, symptoms lead to discontinuation of treatment and may persist thereafter. Recently, induced sensory neurons (iSNs) generated from pluripotent stem cells have been used to model human CIPN *in vitro* to better understand its pathogenesis, identify molecular targets of intervention, and test potential therapeutics. However, current differentiation protocols produce sensory neurons with an immature phenotype, mimicking the morphology and physiology of embryonic sensory neurons. Since CIPN and other peripheral neuropathies occur postnatally, it is crucial to develop a differentiation protocol that will produce sensory neurons with mature functionality. Current evidence suggests that peripheral glial cells play an important role in maturation of sensory neurons *in vivo*, leading to the development of their hallmark pseudo-unipolar morphology, which is integral to adult DRG cell signaling. Here, we have tested the hypothesis that differentiation of induced human neural crest stem cells in co-culture with rodent E15 dorsal-root peripheral glia (rDRG), will quickly and efficiently produce mature hiSNs. Our results indicate that iSNs differentiated in co-culture with rDRGs transition to pseudounipolar morphology significantly more frequently than iSNs differentiated alone. Our data suggest that this transition requires physical contact between rDRG satellite glial cells and developing iSNs, and the mechanism underlying this transition is not mediated by a glial secreted factor. We have

interrogated potential receptor-ligand pairs that may drive a contact mediated morphologic change. These new methods for generating iSNs with adult morphology will allow for targeted studies of degenerating peripheral axons, as these are the projections that are specifically impacted in CIPN and in other neuropathies.

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Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.25/B28

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant T32 HL007062

Title: Sufficient iron is essential for proper axon growth and mitochondria localization

Authors: *T. MONKO, K. DEVGUN, S. OSTERGREN, E. TRIPP, D. MICKELSON, T. BASTIAN;
Pediatrics, Univ. of Minnesota, Minneapolis, MN

Abstract: Gestational and perinatal iron deficiency is one of the most common insults to brain development, yet there is a paucity of knowledge about iron requirements during the earliest periods of neuron development from axon initiation and elongation to pathfinding and synapse formation. Developing axons have high metabolic requirements to facilitate cytoskeletal rearrangement and transport of cellular cargo, including mitochondria. Neuronal iron homeostasis is intimately linked with mitochondrial activities, having been shown to regulate mitochondrial transport and energetics to support proper dendritic arborization. Based on these connections, we hypothesized that iron-dependent mitochondrial function might also be necessary for proper regulation of axon initiation, elongation, and branching.
Primary neurons cultured from embryonic (E) day 16.5 wildtype mouse hippocampus were incubated from 3 days in vitro (DIV) with either the iron chelator deferoxamine (DFO; iron deficient) or vehicle (iron sufficient) to avoid disrupting axon initiation. Sholl analysis performed at 7 DIV, revealed that iron deficient neurons had shorter, but more branched axon morphology compared to iron sufficient neurons, suggesting an altered growth trajectory. To study the earliest stages of growth, we developed a novel model to investigate axon initiation and elongation using primary neurons cultured from E14.5 wildtype mouse neocortex. Neurons were treated with the intracellular iron chelator deferiprone (DFP; iron depleted) or vehicle (iron sufficient) beginning at 2 hours in culture (HIC). As early as 18 HIC, iron depleted neurons showed reduced specification of a single axon and disrupted mitochondrial trafficking to the nascent axon. From 24 to 72 HIC, axon morphology and mitochondrial localization were observed over time and analyzed using novel machine learning-based image analysis with

Python and napari.
These findings suggest that iron plays an essential role in early axon development by promoting axon elongation, minimizing exuberant axonal branching, and facilitating the localization of mitochondria to sub-axonal compartments. Understanding the roles of iron and mitochondria in axonal growth is crucial for revealing a unifying mechanism of neuronal development.

Disclosures: T. Monko: None. **K. Devgun:** None. **S. Ostergren:** None. **E. Tripp:** None. **D. Mickelson:** None. **T. Bastian:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.26/B29

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: R01NS110590

Title: Zn^{2+}decoration of microtubules arrests axonal transport and displaces tau, doublecortin, and MAP2C

Authors: *Y. QIN;
Univ. of Denver, Denver, CO

Abstract: Intracellular Zn2+ concentrations increase via depolarization-mediated influx or intracellular release, but the immediate effects of Zn2+ signals on neuron function are not fully understood. By simultaneous recording of cytosolic Zn2+ and organelle motility, we find that elevated Zn2+ (IC50 ~ 5-10 nM) reduces both lysosomal and mitochondrial motility in primary rat hippocampal neurons and HeLa cells. Using live cell confocal microscopy and *in vitro* single-molecule TIRF imaging, we reveal that Zn^{2+} inhibits activity of motor proteins (kinesin and dynein) without disrupting their microtubule binding. Instead, Zn^{2+} directly binds to microtubules and selectively promotes detachment of tau, DCX, and MAP2C, but not MAP1B, MAP4, MAP7, MAP9 or p150glued. Bioinformatic predictions and structural modelling show that the Zn^{2+} binding sites on microtubules partially overlap with the microtubule binding sites of tau, DCX, dynein, and kinesin. Our results reveal that intraneuronal Zn^{2+} regulates axonal transport and microtubule-based processes by interacting with microtubules.

Disclosures: Y. Qin: None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.27/B30

Topic: A.05. Axon and Dendrite Development

Support: Charles University Grant Agency, project no. 268723

Title: Trak1 role in mitochondrial dynamics and neuronal development

Authors: *M. RUIZ ESTRADA[1,2], M. PRAKASH[3], M. RUSKOVÁ[1,2], R. WEISSOVÁ[1,2], M. BRAUN[3], Z. LANSKY[3], M. BALASTIK[2];
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Abstract: Tight control of mitochondrial dynamics is essential for neuronal function and survival. Its deregulation has been associated with several neurodevelopmental and neurodegenerative disorders, such as epilepsy and Alzheimer's disease; however, the underlying molecular mechanisms are still not fully understood. Mitochondrial dynamics is regulated on multiple levels by several motor and adaptor proteins. Here we examine TRAK1 - an adaptor protein that links mitochondria to microtubule-based molecular motors kinesins and dynein - and its role in mitochondrial dynamics in vitro and in primary neurons. We use microtubule-based in vitro assays to assess the effect of TRAK1 on kinesin and dynein-based transport, and live-cell imaging confocal microscopy to quantify mitochondrial motility in WT or TRAK1-deficient primary neuron cultures. Our results demonstrate that TRAK1 regulates kinesin- as well as dynein-based transport on microtubules in vitro. Moreover, we show that its downregulation in neurons increases stationary mitochondria and affects particularly anterograde mitochondrial transport, significantly decreasing the number of transported mitochondria, their velocity, traveled distance and time. Taken together, these data demonstrate that TRAK1 is an essential regulator of mitochondrial dynamics both in vitro and in neurons. A more detailed analysis of its deficiency will provide new information on how mitochondrial dynamics contribute to neural development and disease.

Disclosures: M. Ruiz Estrada: None. **M. Prakash:** None. **M. Rusková:** None. **R. Weissová:** None. **M. Braun:** None. **Z. Lansky:** None. **M. Balastik:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.28/Web Only

Topic: A.05. Axon and Dendrite Development

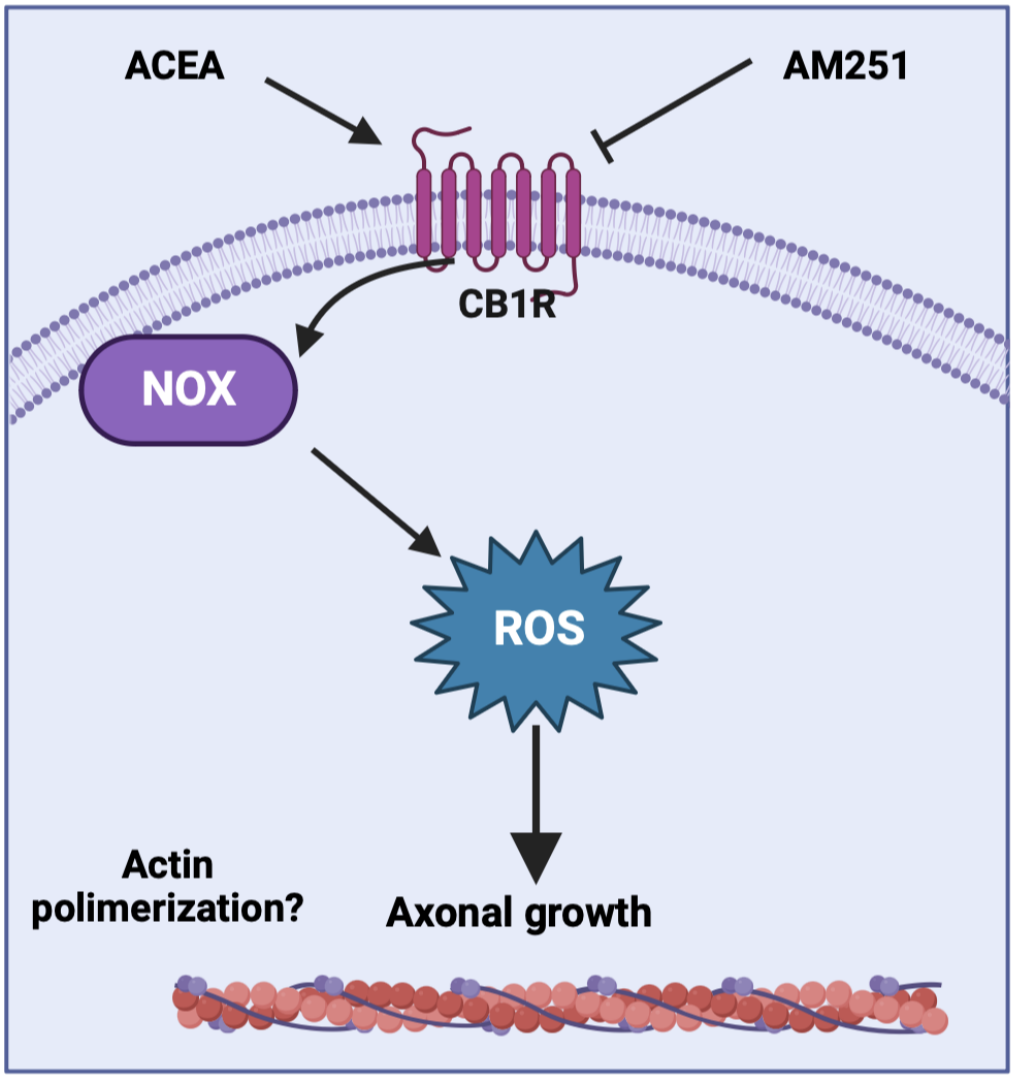
Support: CONACYT Grant No. 285184
PAPIIT Grant No. IN216422
CONACYT Ph.D. Fellowship No. 463895

Title: Cb1 cannabinoid receptor induces axonal growth by modulation of reactive oxygen species production during the development of cerebellar granule neurons

Authors: *B. GARCIA- HERNÁNDEZ[1], T. DURÁN-GONZALES[1], J. MORAN[2]; [1]Natl. Univ. of Mexico, Ciudad de Mexico, Mexico, Mexico City, Mexico; [2]Natl. Univ. of Mexico, Natl. Univ. of Mexico, Ciudad de Mexico, Mexico

Abstract: CB receptors are targets of phytocannabinoid molecules such as THC from the *Cannabis Sativa* plant. They form part of the endocannabinoid system that comprises CB1 (CB1R) and CB2 receptors, endogenous ligands such as anandamide and 2-AG and enzymes involved in the metabolism of the ligands. Cerebellar granule neurons (CGN) present a high expression of CB1R and the enzyme diacylglycerol lipase (DAGL), which produces the endogenous ligands for CB1R. On the other hand, in models of oxidative stress, the activation of the CB1R decreases the oxidant environment. In cerebellar granule neurons, axonal growth is mediated by reactive oxygen species (ROS). In this study, we explored the regulation of axonal growth by CB1 receptor activation and whether this action is mediated by ROS. By using primary cultures of CGN we confirm previous results on an increase in basal ROS content after 2 and 3 days *in vitro* (DIV), a period when axonal growth occurs. At these times, the pharmacological activation of the CB1R by ACEA increased the ROS content. Similarly, the neurite length increases by 50% at 2 and 3 DIV after 24 and 48 h of ACEA treatment. To evaluate the actin polymerization dynamics during axonal growth induced by the activation of the CB1R, we used the technique of fluorescence recovery after photobleaching (FRAP). Our data showed that ACEA treatment modulates the actin polymerization rate. Together these data suggest that the CB1R induces axonal growth of the cerebellar granule neurons by modulating the ROS content and the actin polymerization dynamics.

Graphical abstract



Disclosures: **B. Garcia- Hernández:** None. **T. Durán-Gonzales:** None. **J. Moran:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.29/B31

Topic: A.05. Axon and Dendrite Development

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

Title: Regulation of sympathetic growth by ephrinB1 in the spontaneously hypertensive rat heart

Authors: M. M. BUYUKOZTURK, P. DUJMIC, R. E. KREIPKE, S. J. BIRREN;
Brandeis Univ., Waltham, MA

Abstract: The Spontaneously Hypertensive Rat (SHR) has increased sympathetic drive to the periphery that precedes and contributes to the development of high blood pressure, making it a useful model for the study of neurogenic hypertension. Intrinsic activity and synaptic properties of SHR neurons have been shown to be altered in comparison to the normotensive Wistar Kyoto (WKY), and while hyperinnervation is seen in SHR vasculature, little is known about fiber growth in the heart. In this study we examine the growth properties of sympathetic peripheral neurons into the heart during the early postnatal period. Immunocytochemistry on of SHR left ventricle (LV) slices with TH antibody shows an increase in innervation density compared to the WKY as early as the first week after birth, even though hypertension onset is not until 8 weeks of age. In contrast, while SHR neurons dissociated from the superior cervical ganglion (SCG) and cultured alone *in vitro* do not show a significant difference in growth from WKY neurons, co-culture of these neurons with cardiomyocytes leads to a significant increase in arborization in the SHR on top of their myocyte targets. This correlation with target contact suggests a membrane-bound factor as the driver behind this growth pattern, and one such factor is the membrane-tethered ligand ephrinB1. RT-qPCR points towards ephrinB1 as a potential regulator of this phenomenon in our system, as it is expressed both in the SCG and the LV. Its expression appears higher in SHR SCG versus WKY SCG, and lower in the SHR LV versus WKY LV. This suggests ephrinB1 acts as a repulsive signal for fiber growth in the context of target cardiomyocyte contact, its presence redirecting neurons away from the SCG, and its absence permitting growth onto myocytes. Paradoxically, results from *in vitro* application of a soluble external domain of ephrinB1 in SHR N-only culture show an increase in fiber growth, but no change in WKY conditions or in either strain in the presence of glia. Since the SCG also contains non-neuronal cells such as satellite glia (SG), this suggests a role for SGs in regulating ephrinB1-mediated growth in the SCG. When fiber growth is measured in co-culture of neurons with SGs, there is no significant difference between SHR and WKY, and no effect on fiber growth of ephrinB1 soluble domain application in either strain. All together, these data suggest ephrinB1 signaling in the SCG behaves differently than it does at the point of contact with target cardiomyocytes, that the effect of ephrinB1 on sympathetic fiber growth can differ depending on the presence of non-neuronal cells in our system, and that this regulation is altered in the SHR compared to WKY.

Disclosures: M.M. Buyukozturk: None. **P. Dujmic:** None. **R.E. Kreipke:** None. **S.J. Birren:** None.

Poster

PSTR516. Mechanisms Underlying Axon Growth and Targeting

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR516.30/B32

Topic: A.05. Axon and Dendrite Development

Support: NSF-CRCNS award #2112862
California NanoSystems Institute

Title: Primary neuron culture systems with tunable serotonergic-fiber densities

Authors: *J. H. HAIMAN[1], G. DUNN[2], M. AHUJA[1], N. ELYASI[1], H. WOO[1], S. JANUSONIS[1];
[1]Psychological and Brain Sci., [2]Molecular, Cellular, and Developmental Biol., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: The self-organization of the serotonergic fiber matrix depends on the structural and dynamical properties of single serotonergic axons (fibers). These fibers have a number of interesting features, including pliable morphological characteristics, strongly stochastic trajectories, and the ability to regenerate in the adult brain. In addition, detailed single-cell studies have revealed a remarkable transcriptional diversity of serotonergic neurons, which suggests that any brain region is likely to contain serotonergic fibers representing different (and perhaps partially flexible) transcriptional programs. The analysis of single serotonergic fibers remains a challenge because in many brain regions they do not have a preferred orientation and achieve very high densities. Recently, progress has been made in tracing individual serotonergic fiber trajectories in the brain, including high-resolution microscopy (Maddaloni et al., 2017) and Brainbow AAV-based approaches (Mays et al., 2023). However, these methods do not allow access to the natural dynamics of the fibers, as well as to their real-time responses to experimental manipulations. The current study extends our recent work on serotonergic fibers in primary brainstem cultures (Hingorani et al., 2022). Specifically, it seeks to produce cultures with tunable densities of neurons with the serotonergic phenotype, for applications in standard (2D) and hydrogel-based (3D) environments. The approach is based on fluorescence-activated cell sorting (FACS), followed by verification of cell viability in fixed preparations and direct live imaging with confocal microscopy or holotomography (a refractive index-based method). In particular, this system can mimic relatively low (e.g., cerebellar-like) and high (e.g., amygdala-like) serotonergic-fiber density environments and will facilitate studies of interactions among fibers, as they extend and branch *ex vivo*. Hydrogel systems, enriched with serotonergic neurons and their fibers, may also find applications in the biomedical field, due to their potential in supporting the regeneration and plasticity of neural tissue. The study is a part of our larger program that investigates the structure and self-organization of serotonergic fibers. It includes supercomputing simulations of these fibers as paths of fractional Brownian motion (Janusonis et al., 2020, 2023) and potential applications of their properties in artificial neural networks (Lee et al., 2022).

Disclosures: J.H. Haiman: None. **G. Dunn:** None. **M. Ahuja:** None. **N. Elyasi:** None. **H. Woo:** None. **S. Janusonis:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.01/B33

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

Support: NIH R01 HD092369

Title: Irisin Regulation of Glutamatergic Synaptogenesis in the Developing Hippocampus

Authors: *M. JOSTEN[1], K. PARKER[2], J. RODRIGUEZ[2], C. DILLON[2], G. WAYMAN[2]; [1]Washington State Univ. Grad. IPN, Pullman, WA; [2]Washington State Univ., Pullman, WA

Abstract: Increased diagnostic sensitivity and/or increased incidence have led to increased diagnosis rates of neurodevelopmental disorders (NDDs) including autism spectrum disorders and schizophrenia in recent decades. These disorders are associated with altered hippocampal synaptic phenotype and connectivity in both human and rodent models. Therefore, understanding of hippocampal synaptic and circuit development is critical to advancing therapeutic and preventative treatments for NDDs. The endogenous mechanisms underlying synaptic development, including activation of intracellular signaling pathways that regulate and promote synaptogenesis, are regulated in part by environmental cues derived from the maternal intrauterine milieu, such as hormones, nutrients, and gases. Leptin - the satiety adipokine - and irisin - the exercise myokine - are two such hormones whose expression levels and/or signaling activity are regulated by maternal health status. In adults, leptin and irisin promote hippocampal synaptogenesis and hippocampus-dependent learning and memory. Additionally, their effects on multiple neuronal and physiological systems are phenocopies. However, little is known about how these hormones and their signaling cascades interact, particularly in the context of brain development. Here I demonstrate the interrelated effects of leptin and irisin on synaptogenesis in developing hippocampal pyramidal neurons. *In vitro*, we investigated irisin- and leptin-stimulated signaling pathways and the dependence of these pathways on the expression of the leptin receptor and the irisin-binding integrin receptors. *In vivo*, we characterized the effects of post-natal irisin injections and the effects of moderate maternal exercise throughout gestation on hippocampal synaptogenesis. These data support a codependent signaling interaction between irisin and leptin, as well as a role for irisin in hippocampal synaptic development.

Disclosures: M. Josten: None. **K. Parker:** None. **J. Rodriguez:** None. **C. Dillon:** None. **G. Wayman:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.02/B34

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

Support: NIH Grant R01 NS117695
NIH Grant R21 MH131947

Title: Population Level Analysis of Cortical Interneuron Synapses Through Spatial Statistical Analysis

Authors: *P. D. DUMMER[1], D. I. LEE[2], S. HOSSAIN[1], D. CABRERA[3], A. C. EVARD[4], R. WANG[5], E. KOUTSILIANOS[6], G. NEWMAN[1], C. HO[1], A. CAVANAGH[1], M. CAMPBELL[7], V. MENON[2], E. AU[1];
[1]Pathology & Cell Biol., [2]Neurol., [3]Anesthesiol., [4]Computer Sci., [5]Statistics, [6]Biomed. Engin., Columbia Univ., New York, NY; [7]Neurosciences, UCSD, San Diego, CA

Abstract: Synaptic connections between neurons are a key node in neuropsychiatric disorders. They are the primary targets for psychopharmacology and numerous genetic studies link dysfunction of synaptic genes with psychiatric illness. Traditionally, synapses are studied using electrophysiology, neuroanatomical reconstructions (both are high resolution, low throughput) or biochemistry (low resolution, high throughput). To bridge the divide, we developed a machine learning-based method that measures the spatial statistics of genetically-labeled synapses in an imaged confocal stack. Since these metrics are relatively accessible, our approach is scalable to hundreds of thousands of interneuron synaptic boutons in any given confocal stack. Thus, for the first time, we have the capability to examine interneuron synapses at the population level. Using this approach, we can automatically assign target probability score for dendrite-, soma- and AIS-targeting inhibitory synapses, which reveals that interneuron subclasses form distinct connections throughout cortical layers and across cortical areas. Further analysis reveals that each of the canonical synapse types are composed of synaptic subgroups, which correspond to distinct postsynaptic subcompartments. Some of these subgroups are exquisitely localized within different layers of the cortex, strongly suggesting functional differences. Going forward, we believe that our approach for population-level analyses of interneuron synapses will be a useful complementary tool for existing approaches to understand interneuron function in health and disease.

Disclosures: P.D. Dummer: None. **D.I. Lee:** None. **S. Hossain:** None. **D. Cabrera:** None. **A.C. Evard:** None. **R. Wang:** None. **E. Koutsilianos:** None. **G. Newman:** None. **C. Ho:** None. **A. Cavanagh:** None. **M. Campbell:** None. **V. Menon:** None. **E. Au:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.03/B35

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

Support: NIH Grant 2R01MH113743
NIH Grant R21MH115353
Charles H. Hood Foundation

Title: Astrocytes modulate a critical window of microglia-mediated synapse remodeling

Authors: *Y.-H. LEE, T. E. FAUST, C. O'CONNOR, M. BOYLE, D. P. SCHAFER;
Neurobio., Univ. of Massachusetts Chan Med. Sch., Worcester, MA

Abstract: Neuroplasticity is required for learning and adaptation to the ever-changing environment. During early development, changes in neuronal activity lead to removal of less active synapses. Microglia and astrocytes participate in activity-dependent synapse remodeling by engulfing the synapses from less active neurons. However, the clearance of synapses is less robust after specific "critical windows" of development. How these two glial cells precisely clear up specific synapses and regulate the closure of the critical window remains an open question. Previously, our lab demonstrated that microglia are responsible for the removal of thalamocortical synapses in somatosensory cortex upon removal of whiskers on the snout. We have new data that astrocytes do not engulf the synapses, but instead decrease their contact with the synapses at early postnatal stage. We are now exploring whether the degree of astrocyte-synapse contact dictates a critical developmental time window for activity-dependent synaptic pruning by microglia.

Disclosures: Y. Lee: None. **T.E. Faust:** None. **C. O'Connor:** None. **M. Boyle:** None. **D.P. Schafer:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.04/B36

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

Support: 1R15 AG060461-(01)

Title: Alterations in excitatory synaptic content of PSD95 in the hippocampus of transgenic mice expressing of chimeric NMDA receptor GluN2 subunits indicate a link to GluN2A ionotropic signaling

Authors: *G. A. WILD[1], R. E. KEITH[4], J. I. ESCOBAR[2], L. C. MCAULIFFE[3], T. C. DUMAS[2];
[1]Psychology, George Mason Univ., Sterling, VA; [3]Psychology, [2]George Mason Univ., Fairfax, VA; [4]Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: Postsynaptic density protein 95 kD (PSD95) is a glutamate receptor anchoring protein at excitatory synapses. In the hippocampus, expression levels of PSD95 are developmentally

regulated and increase across the first three postnatal weeks in naïve mice. However, training in a hippocampus dependent task at postnatal day (P) 17 produces a lasting increase in PSD95 expression. This activity dependent increase in PSD95 expression is associated with a premature alteration in the subunit composition of N-methyl-D-aspartate receptors (NMDARs) such that GluN2A subunit incorporation into NMDARs overtakes GluN2B at an earlier age than seen in naïve mice. Vice versa, prevention of the developmental increase in PSD95 prevents the GluN2B to GluN2A subunit shift. Given that NMDARs interact with PSD95 via the GluN2A or GluN2B carboxy terminal domain (CTD) in a nonselective manner, how PSD95 regulates the late postnatal GluN2B to GluN2A shift likely involves GluN2 CTDs but might also involve changes in calcium conductance dynamics. To better understand interactions between GluN2A- or GluN2B-type NMDARs and PSD95, we performed Western blot analyses on PSD95 in hippocampus samples taken from transgenic mice expressing GluN2 chimeric subunits having the CTDs swapped between subunits (GluN2A-B^{ctd} and GluN2B-A^{ctd}). We prepared postsynaptic density fractions from hippocampal homogenates (and neocortical homogenate control samples) collected from GluN2A-B^{ctd}, GluN2B-A^{ctd}, and WT littermates and labeled for PSD95. Results indicate no change in expression levels of PSD95 from P17 to P60 in the hippocampus or neocortex of wildtype mice. An increase in PSD95 occurred in the hippocampus, but not the neocortrex, of the GluN2A-B^{ctd} line at P17-19 compared to age-matched GluN2B-A^{ctd} or wildtype control mice. Since the total NMDAR synaptic pool is not altered in these transgenic lines and the background is predominantly GluN2B at P17-19, the molecular alteration in the GluN2A-B^{ctd} line is a premature increase in the calcium conductance domains of GluN2A (the GluN2B^{ctd} CTD matches the native background). Thus, NMDARs interact with PSD95 at immature hippocampal synapses via GluN2A-type ionotropic NMDAR signaling.

Disclosures: G.A. Wild: None. **R.E. Keith:** None. **J.I. Escobar:** None. **L.C. McAuliffe:** None. **T.C. Dumas:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.05/B37

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

Support: Research Grant Council Hong Kong GRF 17106018
Research Grant Council Hong Kong GRF 17117720
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University Grants Committee of Hong Kong Theme-based Research Scheme (T13-605/18-W)

Health and Medical Research Fund (06172986) of the Food and Health Bureau of the Hong Kong SAR Government

Title: The translation initiating factor eIF4E and arginine methylation underlie G3BP1 function in dendritic spine development of neuron

Authors: X. LI, R. DONG, A. FLORES, *K.-O. LAI;
Dept. of Neurosci., City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Communication between neurons relies on neurotransmission that takes place at synapses. Excitatory synapses are located primarily on dendritic spines which possess diverse morphologies, ranging from the elongated filopodia to the mushroom-shaped spines. Failure in the proper development of dendritic spines has detrimental consequences on neuronal connectivity, but the molecular mechanism that controls the balance of filopodia and mushroom spines is not well understood. G3BP1 is the key RNA-binding protein that assembles the stress granules in non-neuronal cells to adjust protein synthesis upon exogenous stress. Emerging evidence suggests that the biological significance of G3BP1 extends beyond its role in stress response, especially in the nervous system. However, the mechanism underlying the regulation and function of G3BP1 in neuron remains elusive. Here we found that G3BP1 suppresses protein synthesis and binds to the translation initiation factor eIF4E via its NTF2-like domain. Notably, the over-production of filopodia caused by G3BP1 depletion can be alleviated by blocking the formation of translation initiation complex. We further found that the interaction of G3BP1 with eIF4E is regulated by arginine methylation. Knockdown of the protein arginine methyltransferase PRMT8 leads to elevated protein synthesis and filopodia production, which is reversed by the expression of methylation-mimetic G3BP1. Our study therefore reveals arginine methylation as a key regulatory mechanism of G3BP1 during dendritic spine morphogenesis, and identifies eIF4E as a novel downstream target of G3BP1 in neuronal development independent of stress response.

Disclosures: X. Li: None. **R. Dong:** None. **A. Flores:** None. **K. Lai:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.06/B38

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

Support: FRQNT Team grant

Title: Distinct forms of structural plasticity of spines of adult-born interneurons induced by different odor learning paradigms.

Authors: *A. FERREIRA[1,2,3], V. S. CONSTANTINESCU[2], S. MALVAUT[4,3], A. SAGHATELYAN[5,3,2], S. V. HARDY[6,3];

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Abstract: The ability to store new information is a crucial process underlying our everyday life. This capability has been linked to modifications of the efficacy of synaptic transmission that are partly due to activity-dependent structural alterations of dendritic spines. It remains elusive whether different forms of learning and sensory stimulations induce distinct forms of structural plasticity. To address these questions, we developed a computational pipeline to reconstruct dendritic spines from confocal microscopy images into a 3D mesh model and analyzed their number and morphometric properties after distinct learning and sensory stimulation paradigms. We used simple and complex odor learning paradigms, as well as sensory deprivation known to modulate the structuro-functional properties of adult-born interneurons in the olfactory bulb. After dimension reduction and spine clustering into five populations, each population of spines showed distinct morphology. Interestingly, distinct learning and sensory stimulation paradigms involved specific forms of structural plasticity. A simple go/no-go odor learning task induced changes in morphometric properties of existing spines, without any changes in their number. In contrast, the complex go/no-go odor learning task increased the spine density and only slightly affected the spine morphology, while the sensory deprivation decreased the spine density without affecting their morphology. Our results reveal that distinct learning paradigms and sensory stimulation differently affect the number and morphometric properties of dendritic spines.

Disclosures: A. Ferreira: None. **V.S. Constantinescu:** None. **S. Malvaut:** None. **A. Saghatelyan:** None. **S.V. Hardy:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.07/B39

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

Title: Cellular interactions in the nociceptive pathway of Drosophila melanogaster during synapse formation and pruning

Authors: *C. GUALTIERI, F. J. VONHOFF;
Univ. of Maryland Baltimore County, Baltimore, MD

Abstract: Synaptic pruning is a neuroplastic process leading to the withdrawal of ectopic synapses formed during the initial phases of neuronal development. However, the molecular mechanisms underlying synaptic pruning remain incompletely understood. The process of synapse pruning is crucial during development in multiple organisms as it has also been linked to the onset of neurodevelopmental disorders like autism. First, we determined the anatomical effects of candidate autism genes in vivo using the Drosophila model. Starting from the

hypothesis that candidate autism genes would lead to the presence of ectopic synapses that branch off stereotypic connectivity patterns, we assessed the stereotypic synaptic innervations of cIV nociceptive sensory neurons development. The candidate autism genes of the transsynaptic adhesion proteins neurexin-1 and neuroligin-3 were downregulated using RNAi constructs. Anatomical defects were assessed by counting the number of ectopic neurites. Data shows an increased number of ectopic neurites in the stereotypic ladder structure formed in the CNS by the axonal projection of nociceptive neurons when the candidate autism gene neurexin-1 is downregulated. Then we assessed the synaptic connectivity between cIV sensory neurons and the postsynaptic basin interneurons in the CNS of Drosophila during embryonic and larval development using the GFP Reconstitution Across Synaptic Partners (GRASP) technique, revealing the synaptic partnership between nociceptors and basin interneurons -1 and -4 at different stages of Drosophila development. Our findings will provide the groundwork for determining the potential role of synaptic pruning on the synaptic connectivity of nociceptors to basins and will offer the basis for investigating the processes leading to the failure in the elimination of ectopic synapses providing insights into the molecular mechanisms regulating synaptic refinement.

Disclosures: C. Gualtieri: None. **F.J. Vonhoff:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.08/B40

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

Support: NIH R01MH105426

Title: Molecular and synaptic interactions between Kirrel3 and IgSF8

Authors: *A. WEINBROM[1], A. JOHNSON[1], F. YE[1], J. N. SAVAS[2], M. WILLIAMS[1]; [1]Neurobio., Univ. of Utah, Salt Lake City, UT; [2]Northwestern Univ., Northwestern Univ., Chicago, IL

Abstract: A healthy neuronal synapse requires both accurate partner recognition and appropriate structural assembly. Cell type-specific patterns of cell surface proteins are thought to form the basis for target recognition, adhesion, and specific synapse formation. However, individual surface proteins are rarely sufficient to induce synaptogenesis on their own, suggesting that surface protein-protein interactions have an essential role in linking cell adhesion to synapse assembly. Despite this, little is known about how synaptic surface proteins work together to mediate synapse assembly. Previously, the Williams lab discovered that the homophilic cell adhesion protein Kirrel3 is necessary to form a specific type of hippocampal synapse, but neither a protein complex or a functional mechanism for this process have been identified. Importantly, Kirrel3 is sufficient to induce synapses between neurons but is not sufficient when presented to

neurons on the surface of non-neuronal cells, suggesting that Kirrel3 likely has an essential binding partner that is present in neurons. Thus, we conducted a proteomic screen for Kirrel3 interactors and discovered that Kirrel3 binds another surface protein; IgSF8. I validated this interaction in the mouse brain and, here, I will present my latest findings investigating the role of IgSF8 in Kirrel3-mediated synapse formation. This work will shed light on how synaptic surface proteins work together to assemble synapses and may be clinically relevant since mutations in Kirrel3 are repeatedly found in patients with autism spectrum disorders and intellectual disability.

Disclosures: **A. Weinbrom:** None. **A. Johnson:** None. **F. Ye:** None. **J.N. Savas:** None. **M. Williams:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.09/B41

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

Support: NIH R01MH105426

Title: Analysis of Kirrel3-Mediated Synapse Formation from a Genetic Angle

Authors: *O. SHENNIB, A. JOHNSON, A. MOHNKE, O. RAINES, M. WILLIAMS; Neurobio., Univ. of Utah, Salt Lake City, UT

Abstract: Synapse specificity describes the developmental process during which neurons form a synapse with a particular type(s) of target cell. When synaptic specificity goes awry some synapses and neuronal circuits fail to develop, which can affect brain function and may lead to neurodevelopmental disorders. Previously, our lab showed that Kirrel3 is a homophilic, cell adhesion protein that mediates synapse specificity. Analysis of Kirrel3 knockout mice revealed a specific loss of mossy fiber filopodia synapses, which connect excitatory DG neurons to inhibitory GABA neurons in the hippocampus. In addition to the loss of filopodia synapses, we observed an increase in the activity of CA3 neurons that is likely due to a loss of feed-forward inhibition in the DG-GABA-CA3 circuit. Because this work was done using germline knockout mice, it remains unknown precisely when, where, and how much KIRREL3 protein is needed for normal hippocampal synapse formation and function. To address these open questions, we obtained Kirrel3 conditional mice. Here, I will present my recent work analyzing mossy fiber synapse formation and CA3 activity in mice in which Kirrel3 is specifically deleted from DG or GABA neurons, heterozygotes, and from adult mice after synapses initially form. Together, my work expands our understanding of the mechanism of Kirrel3-dependent synapse formation. Importantly, this work adds new insight to mechanisms of synapse specificity and will contribute to our understanding of the neurobiology of brain disorders because Kirrel3 variants have been repeatedly identified as risk factors for autism spectrum disorders and intellectual disabilities.

Disclosures: **O. Shennib:** None. **A. Johnson:** None. **A. Mohnke:** None. **O. Raines:** None. **M. Williams:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.10/B42

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

Support: University of Utah Department of Biochemistry

Title: Structural and biophysical studies of synaptic adhesion proteins Kirrel3 and IgSF8

Authors: *Y. GUO[1], A. WEINBROM[2], M. WILLIAMS[2], J. BRASCH[1];
[1]Biochem., [2]Neurobio., Univ. of Utah, Salt Lake City, UT

Abstract: Synaptic adhesion proteins are essential for neuronal communication and cell adhesion in all vertebrate species. In the hippocampus, the CA3 region receives signals from excitatory and inhibitory pathways in circuits whose specificity and integrity depend on synaptic adhesion proteins, maintaining intellectual cognitive function. Kirrel3, a member of the immunoglobulin superfamily, has been identified as a key component in synaptic formation where it mediates feed-forward inhibition of hippocampal CA3. Dysregulations in Kirrel3 gene copy number and pathogenic mutations underlie neurological deficits such as Autism, Jacobsen's Syndrome, and intellectual disorders. While Kirrel3 is critical to proper intellectual functioning, much about its molecular mechanism remains unknown. Kirrel3 was previously shown to be insufficient to promote synaptogenesis when presented on a non-neuronal cell, suggesting the need for neuron-specific cofactors for synaptogenesis. Our collaborator, the Williams' lab, identified IgSF8 as a novel Kirrel3 binding partner using a proteomics approach. The molecular properties, structure, binding mechanism, and functional relevance of hippocampal IgSF8 are also unknown. Therefore, we investigated the homophilic and heterophilic binding interactions of Kirrel3 and IgSF8 ectodomains using structural and biophysical approaches to understand the structure and function of Kirrel3/IgSF8 at the synapse. We characterized the molecular interactions of Kirrel3/IgSF8 independently and in combination, and our results suggest that they likely form parallel dimers with high affinity, likely indicating cis interactions (formed between proteins on the same membrane). We used a unique liposome reconstitution assay developed by us to mimic a near-native environment for proteins to form assemblies that can be directly visualized by cryo-electron tomography (cryo-ET). We found that Kirrel3:IgSF8 assemblies at membrane contact sites appear to be tightly packed, while Kirrel3 assemblies alone are disordered at contact sites, suggesting a direct function of the heterophilic interaction in synapse formation. Furthermore, initial experiments of Kirrel3 pathogenic mutations and domain deletions suggest that Kirrel3 trans interactions are likely impaired through slowed kinetics and, notably, interactions to IgSF8 are impaired, likely targeting the binding interface. These comprehensive studies help us to visualize the effects of Kirrel3 mutants in disease on the

molecular level, which will inform strategies to develop treatments for human neurological disorders.

Disclosures: Y. Guo: None. **A. Weinbrom:** None. **M. Williams:** None. **J. Brasch:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.11/B43

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

Support: NIH Grant 1R15MH126345-01
NIH Grant P20GM103434
NIH Grant 1P20GM121299
NIH Grant 2U54GM104942
NASA Agreement 80NSSC20M0055

Title: Sex differences in alpha-2-delta-1-mediated synaptic development: Regulation by estrogen

Authors: J. C. WILLIAMSON, A. MAZUR, *W. C. RISHER;
JC Edwards-Marshall Univ. Sch. of Med., Huntington, WV

Abstract: Synaptogenesis is the process by which neurons form synapses allowing for communication throughout the body. In recent years, astrocytes, the most abundant glial cells in the brain, have been revealed to strongly regulate the process of central nervous system (CNS) synaptogenesis, notably by way of secreted proteins. One such protein family, the thrombospondins (TSPs), has been shown to function through neuronal calcium channel subunit α2δ-1. Previous studies from our lab revealed that astrocytic TSP/neuronal α2δ-1 signaling is significantly sex-biased, being a prominent synaptogenic mechanism in males but having a strongly diminished effect in females. A promising target for a molecular mechanism to explain this sex difference is estrogen. Previous results from our lab have shown that modulating estrogen levels promotes significant shifts, both positive and negative, in TSP2-induced synaptogenesis between cultured neurons. Here, we investigated the extent to which estrogen regulates α2δ-1-mediated cortical synaptic development in vivo. Wild-type (WT) and forebrain-specific α2δ-1 knockout (KO) male and female mice were injected with either E2 (a biologically active form of estrogen), letrozole (aromatase inhibitor that prevents E2 production), or saline from postnatal day 7 (P7) to P40±2 (accounting for the 4-day mouse estrous cycle in females). Immunohistochemical staining of postsynaptic protein PSD95 and presynaptic protein vesicular glutamate transporter-1 (VGluT1) in primary visual cortex (V1) was visualized by confocal microscopy. WT females showed a significant decrease in excitatory synapse density with E2 treatment, but only when in proestrus. Furthermore, α2δ-1 KO females were no different from WT when accounting for estrus phase and treatment. Finally, male α2δ-1 KO mice had the lowest synapse counts of any group analyzed thus far. Though letrozole experiments are still

ongoing, our findings so far have strengthened our hypothesis that regulation of intracortical synaptic connectivity by α2δ-1 is strongly influenced by sex and estrogen signaling. Taken together, our work strongly indicates that this mechanism is critical to the establishment of sex differences in brain circuitry.

Disclosures: J.C. Williamson: None. **A. Mazur:** None. **W.C. Risher:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.12/B44

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

Support: NIH Grant EY13584
NIH Grant NS060125

Title: Glutamatergic signaling and neuroligin/neurexin adhesion play opposing roles that are mediated by major histocompatibility complex I molecules in cortical synapse formation

Authors: G. L. SELL[1], S. L. BARROW[2], ***A. MCALLISTER**[1];
[1]Ctr. for Neurosci., UC Davis, Davis, CA; [2]Vertex Phamaceuticals, San Diego, CA

Abstract: Although neurons release neurotransmitter before contact, the role for neurotransmitter release in synapse formation remains unclear. Cortical synapses do not require synaptic vesicle release for formation (Sudhof 2021), yet glutamate clearly induces new spines in hippocampal slices (Kwon et al., 2011). Using cultures to dissect molecular mechanisms, we found that glutamate not only does not directly induce synapse formation, but rather it has the opposite effect in young cortical neurons. Acute (10min) pharmacological activation of NMDARs reduces synapse density, defined by colocalization of pre- and postsynaptic proteins, by about 30% in both mouse and rat dissociated cultures, while NMDAR blockade has the reverse effect. This glutamate-induced reduction in synapses was confirmed using whole-cell patch-clamp electrophysiology, with a significant reduction in mEPSC frequency and no change in amplitude. Using live imaging, we found that acute glutamate treatment decreases the mobility and surface expression of NMDARs and the proportion of mobile NMDARs that are transported with neuroligin-1 (NL1). This effect was spatially limited, as determined by focal application of glutamate, and dependent on NMDAR-mediated Ca^{2+} influx. Importantly, these effects of glutamate are prevented specifically at sites of association of the NMDAR/NL1 complex with neurexin (Nrxn) in a mixed co-culture assay and overexpression of NL1-mCherry rescues the glutamate-induced synapse loss, indicating that NL1 adhesion is an opposing signal to glutamate for regulating synapses. We also determined that major histocompatibility complex I (MHCI) molecules, which negatively regulate synapses (Glynn et al., 2011), also bidirectionally and negatively regulate NL1 protein levels. Finally, MHCI molecules are necessary for the glutamate-induced synapse loss, through their direct negative regulation of NL1 protein levels.

Together these data show that young cortical neurons undergo a novel type of homeostatic plasticity that involves surprisingly rapid changes in glutamatergic synapse density, as opposed to the activity-induced changes in synaptic strength that occur in older neurons. This new form of plasticity is mediated by MHCI molecules on neurons. Moreover, glutamate release appears to regulate when and where glutamatergic synapses are formed by destabilizing postsynaptic components (NMDAR/NL1) that fail to make contact with presynaptic partners, similar to the role for glutamate in synapse loss at the NMJ (Personius et al. 2016) as well as the opposing roles for acetylcholine and agrin in synapse formation at the neuromuscular junction (Misgeld et al. 2005).

Disclosures: G.L. Sell: None. **S.L. Barrow:** None. **A. McAllister:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.13/B45

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

Support: NIH Grant R01NS065856
NIH Grant F31 NS118799

Title: Characterizing the role of Plexin-B receptor domains in synapse formation

Authors: S. ADEL[1], ***R. D. RAY**[2], C. CARMONA[3], S. PARADIS[4];
[1]Brandeis Univ., Brandeis Univ. Grad. Neurosci. Program, Waltham, MA; [2]Biol., Brandeis Univ. Undergraduate Neurosci. Program, Waltham, MA; [4]Biol., [3]Brandeis Univ., Waltham, MA

Abstract: Proper neural circuit function relies on the dynamic regulation of excitatory and inhibitory synapse formation in the mammalian brain. The misregulation of synapse development and identity specification has been associated with neurological disorders such as epilepsy and autism spectrum disorder. Despite the known importance of synapse formation in neural processes, the molecular interactions that direct synapse assembly and specify synapse properties remain largely elusive. We previously demonstrated that Class IV Semaphorin (Sema4) ligands signal with Plexin-B receptors to promote synapse formation in the rodent hippocampus. Sema4D signals with Plexin-B1 to promote GABAergic synapse formation, and Sema4A signals with Plexin-B2 to promote glutamatergic synapse formation. However, the molecular mechanisms underpinning Plexin-B1 and Plexin-B2 synaptogenic functions remain unclear. In addition to the role of Plexin-B2 in glutamatergic synapse formation, Plexin-B2 is also necessary for GABAergic synapse formation. To understand the functional divergence and overlap in Plexin-B1/B2 signaling, we constructed two chimeric Plexin-B proteins containing domain swaps between the intracellular, transmembrane, and extracellular domains of Plexin-B1 and Plexin-B2. The expression of each chimera, along with Cre-recombinase, is induced through viral-mediated gene transduction in dissociated hippocampal neurons isolated from transgenic

mice harboring a Cre-specific conditional Plexin-B2 allele. After 16 days in culture, the neuronal cultures are fixed and stained for markers of glutamatergic and GABAergic synapse proteins. By examining the clustering and colocalization of presynaptic and postsynaptic molecules, we seek to determine whether the unique transmembrane domains of Plexin-B1 and Plexin-B2 may direct distinct steps of GABAergic and glutamatergic synapse development. Uncovering the functions of specific Plexin-B domains in synapse development will enhance our understanding of the signaling mechanisms that govern different steps in this process, as well as provide insight on how those processes differ between GABAergic and glutamatergic synapse development.

Disclosures: **S. Adel:** None. **R.D. Ray:** None. **C. Carmona:** None. **S. Paradis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent US-10626163-B2, Severin Therapeutics, Inc..

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.14/B46

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

Support: NIMH R01_MH125528

Title: Investigating morphological and synaptic deficits in neuropsychiatric disorders using human neural models

Authors: *L. CHERUVU[1], X. SU[2], Z. P. PANG[2];
[1]Rutgers Univ., Hillsborough, NJ; [2]Neurosci. and Cell Biol., Child Hlth. Inst. of New Jersey, Rutgers Robert Wood Johnson Med. School, New Brunswick, NJ, New Brunswick, NJ

Abstract: Dendrite morphology, such as the complexity of dendritic branching and the density of dendritic spines, directly impacts synapse formation and synaptic transmission. Spines are the main sites receiving synaptic inputs, and the dendritic trees on which they reside can develop and change with brain maturity. Disrupting synapse formation and dendritic branching during development can lead to dysregulation of synaptic transmission; this impairment has been associated with many neurodevelopmental and neuropsychiatric disorders. For example, rare de novo variants and heterozygous mutations in SETD1A (encodes a component of the histone methyltransferase complex) are strongly associated with autism spectrum disorders (ASD), schizophrenia, and other neurodevelopmental disorders. The studies of $Setd1a^{+/-}$ mice showed disrupted dendrite development and spine formation and impaired synaptic transmission. Here, we hypothesize that deficits in dendritic morphology are associated with the alteration of synaptic transmission and underlie the pathophysiology of mental disorders. We have generated human-induced neurons (iNeurons) from induced pluripotent stem cells (iPSCs) that carry SETD1A heterozygous mutations and the isogenic controls. We are focused on illustrating the dendritic morphological changes in these iNeurons and investigating whether SETD1A

heterozygous mutations cause deficits. To investigate the dendritic morphology, we sparse transfected iNeurons with vectors expressing GFP and reconstructed their morphology using confocal images; we also performed immunohistochemistry using antibodies specific to presynaptic proteins such as synapsin I and VGLUT1, and postsynaptic protein PSD95. Ongoing experiments focus on evaluating the changes in these morphometric parameters and investigating if SETD1A or other gene mutations associated with mental disorders affect dendritic development and functionality. Results from this study using a human neuronal model will shed light on the pathophysiology of mental disorders.

Disclosures: L. Cheruvu: None. **X. Su:** None. **Z.P. Pang:** None.

Poster

PSTR517. Molecular Mechanisms of Synapse Formation, Maturation and Remodeling

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR517.15/B47

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

Support: R21MH1079660
R56RAG058593
Competitive Medical Research Fund (UPMC)
Whitehall Foundation Grant
NRSAD Young Investigators Award

Title: Rhoa signaling is a convergence point for nogo receptor and brain-derived neurotrophic factor modulation of synapse stabilization.

Authors: T. KALPATTHI, Y. ZHAO, L. EISENMAN, D. STANISLAUS, *Z. WILLS; Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: While the respective synapse growth and inhibition signaling of brain-derived neurotrophic factor (BDNF) and the Nogo receptor (NgR) are well described, how these pathways function together to regulate the assembly of excitatory synapses is unclear. Employing live dual-sensor imaging of dissociated hippocampal neurons undergoing synapse development, we map in individual neurons BDNF calcium events in concert with the GTPase activity of RhoA, an intracellular mediator of NgR's inhibition of synapse formation. We find endogenous RhoA and calcium signals negatively correlate with one another in pyramidal neuron dendrites but not spines or axons, suggesting these pathways may oppose one another specifically in this domain. Consistent with this hypothesis, pharmacological, genetic, and optogenetic activation of RhoA or calcium signaling inhibits the opposing pathway in dendrites. Our imaging and electrophysiological studies reveal BDNF promotes synaptogenesis by Protein Kinase A (PKA) activation of T-type calcium channel currents, a function mediated in part by inhibition of RhoA signaling. Rho Kinase (ROCK), a key mediator of RhoA signaling, phosphorylates the T-channel CaV3.1, inhibiting its ability to initiate calcium-dependent synapse

development. Expression of a CaV3.1 ROCK phosphomutant ($CaV3.1^{M1,M2}$) increases synapse number and reverses Nogo-dependent spine loss, revealing how NgR signaling inhibits T channels to block calcium-dependent synapse development. Further, time-lapse kymographic imaging of early steps in synaptogenesis pinpoints axon-filopodial contact stabilization as a key step that may be inhibited by NgR-RhoA signaling. In total, this work identifies the GTPase RhoA as a point of convergence among competing synaptic signaling pathways, one which may insure proper orchestration of the early stages of synapse assembly.

Disclosures: T. Kalpatthi: None. **Y. Zhao:** None. **L. Eisenman:** None. **D. Stanislaus:** None. **Z. Wills:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.01/B48

Topic: A.07. Developmental Disorders

Support: Pooler Charitable Foundation

Title: A TrkB partial agonist rescues autistic-like behavior in juvenile mice prenatally exposed to valproic acid

Authors: M. ABDOLLAHI[1], F. M. LONGO[3], ***M. FAHNESTOCK**[2];
[1]Med. Sci. Grad. Program, [2]Psychiatry & Behavioural Neurosciences, McMaster Univ., Hamilton, ON, Canada; [3]Dept. of Neurol. and Neurolog. Sci., Stanford Univ. Med. Ctr., Stanford, CA

Abstract: Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interactions and repetitive behavior. In humans and rodents, exposure to valproic acid (VPA) during pregnancy leads to idiopathic ASD in both male and female offspring. Human idiopathic ASD cortical tissue exhibits decreased signaling through the brain-derived neurotrophic factor (BDNF) receptor, TrkB. Notably, exposure to VPA during pregnancy in rodents is associated with decreased TrkB signaling and ASD-like behavior in offspring. This study asked whether decreased TrkB signaling contributes to ASD-like behavior in idiopathic autism. To do so, we pharmacologically increased TrkB signaling in the VPA mouse model. **Methods:** We investigated the potential of the partial TrkB agonist, LM22A-4, to reduce autistic-like behavior in the VPA mouse model. Pregnant C57Bl/6N mice were intraperitoneally injected with either 600 mg/kg VPA or saline (vehicle) on embryonic day 12.5. Male and female offspring received daily intraperitoneal injections of either 50 mg/kg LM22A-4 or saline from postnatal days 21 (weaning) to 35 (euthanasia). Behavioral assays included tests of anxiety using the step-down test and elevated plus maze on postnatal days 29 and 30, respectively. Repetitive behavior was assessed using the marble-burying test on postnatal day 31, while sociability and locomotor activity were examined using the 3-chamber test on postnatal

days 32-33. Olfactory function was evaluated using the buried food seeking test on postnatal day 34. Following euthanasia, brain tissue was dissected. **Results:** Both male and female VPA-exposed mice exhibited impaired sociability and increased repetitive behavior, consistent with ASD-like symptoms. No significant differences were observed in anxiety, locomotion, or olfactory function between VPA-exposed mice and control groups. Importantly, treatment with LM22A-4 effectively rescued the core symptoms associated with ASD in both sexes in the VPA mouse model. **Conclusions:** These findings provide evidence that decreased TrkB signaling plays a role in the etiology of idiopathic ASD. The potential therapeutic benefits of targeting BDNF/TrkB signaling using LM22A-4 highlight a promising avenue for the development of novel treatments for ASD.

Disclosures: M. Abdollahi: None. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PharmatrophiX. **M. Fahnestock:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.02/B49

Topic: A.07. Developmental Disorders

Title: Cell specific transcriptomic analysis identifies EPAC2 as a novel target to rescue atypical sensory processing in Fragile X knockout mouse.

Authors: *A. SURESH[1], C. PORTERA-CAILLIAU[2];
[1]Neurol., Univ. of California Los Angeles, Los Angeles, CA; [2]UCLA, UCLA, Los Angeles, CA

Abstract: Fragile X Syndrome (FXS) is a prototypical neurodevelopmental disorder (NDD) characterized by intellectual disability, autistic traits, and atypical sensory processing. FXS arises from transcriptional silencing of the *FMR1* gene, which leads to the near complete loss of the RNA binding protein fragile X messenger ribonucleoprotein 1 (FMRP). Functionally, FMRP is a repressor of protein translation and regulates the expression of several hundred genes. Exactly how loss of FMRP and the resulting dysregulation of molecular signaling pathways affects brain circuit function have not yet been understood. Recent studies have implicated changes in excitatory and inhibitory circuits in the etiology of FXS, including reduced firing and density of parvalbumin (PV) neurons, the major subtype of inhibitory interneurons in the cerebral cortex. To investigate whether loss of FMRP similarly affects the transcriptome of excitatory and inhibitory neurons, we used a Ribo-Tag approach to isolate mRNA from Ca^{2+}/calmodulin-dependent protein kinase II (CAMK2) and PV neurons in primary somatosensory (S1) and visual (V1) cortices of adult *Fmr1 KO* mice and wild-type (WT) controls. Intersectional analysis identified 194 differentially expressed genes shared between both CAMK2 and PV neurons. These included several autism risk genes and genes regulated by FMRP. Gene enrichment analysis of the shared genes, identified pathways enriched for GTPase signal transduction and

Golgi organization. Among these shared genes, we identified upregulation of *Epac2* (also called *Rapgef4*), a cAMP dependent guanine-exchange factor, which is both an autism risk gene and whose expression is regulated by FMRP. EPAC2, the protein product of *Epac2*, is an important regulator of synapse turnover, and stability. Chronic treatment with a selective EPAC2 antagonist, rescued tactile defensiveness in *Fmr1* KO mice. These studies identify EPAC2 as a novel target for rescuing atypical sensory processing in FXS.

Disclosures: A. Suresh: None. **C. Portera-Cailliau:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.03/B50

Topic: A.07. Developmental Disorders

Support: NNSFC Grant no.81871079

Title: Gigyf2 disruption leads to autism and interferes with synaptic development and akt-mtor signaling

Authors: *Y. BIN[1], S. ZHU[2], S. TAN[2], K. XIA[2], H. GUO[2];
[1]Ctr. South Univ., changsha, China; [2]Central South Univ., Changsha, China

Abstract: Autism spectrum disorder (ASD) represents a group of neurodevelopmental phenotypes with a strong genetic component. *De novo* variants in *GIGYF2* have been identified in individuals with ASD; however, its clinical significance remains unclear. In addition, the neurobiological and molecular mechanisms of *GIGYF2* in ASD are unknown. Combining human genetics, conditional knockout mouse models and a variety of molecular biology methods, we explored the genotype-phenotype relationships of *GIGYF2* and the underlying neurobiological and molecular mechanisms. We investigated 6 likely gene-disruptive (LGD) and 13 *de novo* missense variants within *GIGYF2* identified in individuals with neurodevelopmental concerns. We found that *GIGYF2* mutations are more related to autism, intellectual disability and language problems in human. Using conditional (Nestin-cre) knockout mouse models, we found that *Gigyf2* haploinsufficiency or knockout mice showed autistic-like behaviors, such as social impairments and over-grooming behaviors. In vivo and in vitro analyses revealed decreased dendrited spine density, accompanied by decreased expression of PSD95 and synaptophysin. Mechanismly, we found *Gigyf2* haploinsufficiency or knockout increased phosphorylation level of AKT, mTOR and S6 in the mouse cortex at postnatal day 30. Our findings indicate that *GIGYF2* deficiency causes autism-like behaviors and leads to abnormal synaptic development likely through interference with AKT-mTOR signaling pathway.

Disclosures: Y. Bin: None. **S. Zhu:** None. **S. Tan:** None. **K. Xia:** None. **H. Guo:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.04/B51

Topic: A.07. Developmental Disorders

Support: Simon Foundation #206683
NIMH Grant MH112808
NIMH Grant MH128765
NARSAD Young Investigator Grant

Title: Dysregulated GluA2-Y876 Phosphorylation Contributes to Loss of Synaptic Upscaling In GRIP1 Mutant Mice with Impaired Social Interaction and Repetitive Behavior

Authors: *M. HAN[1,2], H. L. TAN[3], J. KIM[4,5], R. MEJIAS[2,6], S.-L. CHIU[3,7], R. L. HUGANIR[3], T. WANG[2];
[1]NIMH, NIH, Bethesda, MD; [2]The McKusick-Nathans Dept. of Genet. Med., [3]The Solomon H Snyder Dept. of Neurosci., [4]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD; [5]Korea Brain Res. Inst., Daegu, Korea, Republic of; [6]Dept. of Physiol., Univ. of Seville, Seville, Spain; [7]Inst. of Cell. and Organismic Biol. and Neurosci. Program of Academia Sinica, Academia Sinica, Taipei, Taiwan

Abstract: Synaptic upscaling is a post-synaptic homeostatic plasticity characterized by increasing surface levels of AMPA receptors (AMPARs) in response to reduced neural activity and is regulated in part by phosphorylation of AMPA receptor 2 (GluA2) at tyrosine 876 (GluA2-pY876). Loss of synaptic upscaling has been found in several autism mouse models. However, the underlying mechanisms remain poorly understood. Glutamate receptor interacting protein 1 (GRIP1) binds the c-terminal domain of GluA2 via its PDZ domains 4-6 where several rare functional variants were identified in autism patients. We studied mice carrying one variant, *GRIP1*-I586L (murine I507L) in PDZ5, resulting in an increase in binding with GluA2 and accelerated GluA2 recycling in transfected neurons. *Grip1*-I507L mice show impaired social interaction and increased repetitive behavior as described in established autism mouse models. Electrophysiology studies identified increased neuronal excitability and excitatory-to-inhibiory ratio in layer 2/3 of the medial prefrontal cortex, a brain region known to involve in autism pathogenesis. Furthermore, *Grip1*-I507L cortical neurons show loss of synaptic upscaling in response to tetrodotoxin (TTX) induced inactivity. Importantly, while basal GluA2-pY876 and its protein tyrosine kinase, Fyn, were increased, TTX treatment failed to induce additional GluA2-pY876 in *Grip1*-I507L neurons compared to wide type controls. These data are consistent with that enhanced Fyn expression results in increased GluA2-pY876 and a stronger binding of GRIP1-GluA2 at basal condition while lack of additional induction of GluA2-pY876 by TTX contributes to loss of synaptic upscaling in *Grip1*-I507L neurons. These results support a novel mechanism that dysregulated GluA2-pY876 contributes to loss of synaptic upscaling in *Grip1*-I507L mice with autism-associated social and repetitive behaviors.

Disclosures: **M. Han:** None. **H.L. Tan:** None. **J. Kim:** None. **R. Mejias:** None. **S. Chiu:** None. **R.L. Huganir:** None. **T. Wang:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.05/B52

Topic: A.07. Developmental Disorders

Support: NIH NIMH K00 MH133250

Title: Investigating the mechanism underlying cholinergic function in neurodevelopmental mouse model of TRIO

Authors: *N. CERTAIN[1], A. JENG[1], S. MYERS[2], A. KOLESKE[1];
[1]Yale Univ., New Haven, CT; [2]La Jolla Inst., La Jolla, CA

Abstract: The widely distributed cholinergic system actively neuromodulates several brain functions including attention, learning, and memory. Cholinergic dysfunction is consistently reported in neurodevelopmental disorders (NDDs). Here, we address cholinergic alterations following excitatory neuron-specific deletion of *TRIO*, a high-risk gene for autism, schizophrenia, and related developmental disorders. The integrity of the cholinergic system depends critically on proper synthesis, release, and hydrolysis of acetylcholine. Our comparative proteomic analyses revealed that several key regulators of cholinergic signaling (e.g. acetylcholinesterase, choline acetyltransferase) are significantly altered in *TRIO*-deficient mice. We are investigating if changes to cholinergic tone differentially impact attention, learning, and memory in *TRIO*-deficient mice compared to wild type littermates. We are also focusing on whether these cholinergic deficits contribute to synaptic pathophysiology using electrophysiology and if these deficits can be rescued with cholinergic-specific pharmacological intervention. Our overall goals are to understand how reduced TRIO function impacts cholinergic tone and how this contributes to circuit dysfunction and behavioral deficits in *TRIO*-deficient mouse model of NDDs.

Disclosures: **N. Certain:** None. **A. Jeng:** None. **S. Myers:** None. **A. Koleske:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.06/B53

Topic: A.07. Developmental Disorders

Title: The Role of Endosomal Trafficking in Dendritic Spine Morphology and Synapse Formation in SHANK3B +/1 Mouse Model of Autism

Authors: D. BIELAMOWICZ, R. HAGEL, K. ROACH, S. B. B. DUTTON, ***J. LARIMORE**; Agnes Scott Col., Atlanta, GA

Abstract: Dendritic morphology and dendritic spine receptor insertion is necessary for typical neuronal development and synaptic formation. Previous studies have proposed that aberrant connectivity among neurons underlies autism phenotypes, and that altered connectivity is a result, in part, of altered dendritic spine volume and density of patients with autism. Postsynaptic densities (PSD) composition requires proper endosomal trafficking at the level of the recycling endosome as well as other scaffolding proteins to be present. The SHANK family of proteins are responsible for synapse formation and synaptic plasticity at glutamatergic synapses. SHANK3B codes for key PSD proteins that are part of the glutamate receptor protein complex that physically links ionotropic NMDA receptors to metabotropic mGlu5 receptors, a linkage necessary for induction of plasticity. Using immunoblotting, immunohistochemistry, and qt-PCR we describe how AGAP1-dependent endosomal trafficking kinetics and endosomal protein levels are altered in SHANK3B +/- mice. We also describe how those alterations regulate receptor trafficking, receptor localization in the PSD, and spine morphology. Understanding how AGAP1-dependent endosomal pathways may contribute to neurodevelopment will further elucidate how proper neuronal connections are formed.

Disclosures: D. Bielamowicz: None. **R. Hagel:** None. **K. Roach:** None. **S.B.B. Dutton:** None. **J. Larimore:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.07/B54

Topic: A.07. Developmental Disorders

Support: DOD, 13196175
R01HD054453 (NIH-NICHD),
R01NS117597 (NIH-NINDS),

Title: The NKCC1 inhibitor bumetanide restores E/I balance and rescues sensory hypersensitivity in developing Fragile X mice

Authors: *N. KOURDOUGLI[1], T. NOMURA[2], A. HEUVELMANS[4], A. CONTRACTOR[3], C. PORTERA-CAILLIAU[5];
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[3]Northwestern Univ., Chicago, IL; [4]Univ. of Amsterdam, UVA, Amsterdam, Netherlands; [5]Neurol., Carlos Portera-Cailliau, Los Angeles, CA

Abstract: Exaggerated responses to sensory stimuli leading to tactile defensiveness is a hallmark of Fragile X syndrome (FXS) that is also present in *Fmr1* KO mice, the mouse model of FXS. Such sensory hypersensitivity is thought to contribute to or exacerbate other characteristic phenotypes of FXS, such as inattention, anxiety, and learning disability. Various studies in *Fmr1 KO* mice have implicated GABAergic inhibition in the origin of these deficits, because of elevated balance of excitation to inhibition (E/I ratio), immaturity and hypoactivity of fast-spiking interneurons, or delayed switch in GABA polarity. For example, our labs recently showed that administering bumetanide, a NKCC1 co-transporter blocker, to rectify chloride imbalance in *Fmr1* KO mice, restored GABA polarity and ameliorated circuit plasticity in primary somatosensory cortex (S1) during the critical period. Here, we sought to investigate the effect of bumetanide on circuit dysfunction in S1 barrel cortex of early postnatal *Fmr1* KO mice and on sensory hypersensitivity. Using in vivo 2-photon calcium imaging, we demonstrate that layer (L) 2/3 pyramidal cells in S1 of *Fmr1* KO mice show a higher frequency of early synchronous events at postnatal day (P) 6 compared to wild-type (WT) controls. This could be reversed by acute bumetanide administration) at P6 in *Fmr1* KO mice, but not by a control diuretic, chlorothiazide (CTZ), that does not cross the blood-brain-barrier. Furthermore, chronic administration of bumetanide from P5 to P14 rectifies E/I ratio by increasing feedforward GABAergic inhibitory currents, as assessed with slice electrophysiology. Finally, 2P calcium imaging revealed that chronic bumetanide treatment restored S1 circuit differences in *Fmr1* KO mice (including reduced L2/3 neuronal adaptation to whisker stimulation) and, importantly, ameliorated tactile avoidance. Thus, by restoring E-I balance, the FDA-approved drug bumetanide, can reduce sensory symptoms in FXS.

Disclosures: **N. Kourdougli:** None. **T. Nomura:** None. **A. Heuvelmans:** None. **A. Contractor:** None. **C. Portera-Cailliau:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: NIH Grant MH079407
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NIH Grant MH133014

Title: Sexually dimorphic phenotypes and the molecular mechanisms in UBE3A-dependent autism spectrum disorder

Authors: *Y. TIAN, H. QIAO, H. MAN;
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Abstract: Autism spectrum disorders (ASDs) are characterized by social, communication, and behavioral challenges. UBE3A is one of the most common ASD genes, and transgenic mice with UBE3A overexpression exhibit typical autistic behaviors. ASDs display a remarkable sex difference with a 4:1 male to female prevalence ratio; however, the underlying mechanism remains largely unknown. Using the UBE3A-overexpressing mouse model, we studied sex differences at behavioral, genetic, and molecular levels. We found that male mice with extra copies of Ube3A exhibited greater impairments in social interaction, repetitive self-grooming behavior, memory, and pain sensitivity, whereas female mice with UBE3A overexpression displayed greater olfactory defects. Social communication was impaired in both sexes, with males making more calls and females preferring complex syllables. At the molecular level, androgen receptor (AR) levels were reduced in both sexes due to enhanced degradation mediated by UBE3A. However, AR reduction significantly dysregulated AR target genes only in male, not female, transgenic mice. Importantly, restoring AR expression effectively rescued male-biased alterations in the expression of AR target genes, social preference, grooming behavior, and memory in male mice with extra copies of Ube3A, without affecting females. These findings suggest that AR plays an essential role in mediating the sexually dimorphic changes in UBE3A-dependent ASD.

Disclosures: Y. Tian: None. **H. Qiao:** None. **H. Man:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

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Program #/Poster #: PSTR518.09/B55

Topic: A.07. Developmental Disorders

Support: SFARI

Title: Developmental progression of prefrontal-mediodorsal thalamus circuit dysfunction in a SHANK3$^{-/-}$ mouse model of Autism Spectrum Disorder

Authors: *G. DEVIENNE[1], G. VANTOMME[2], J. R. HUGUENARD[1];
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Abstract: Autism Spectrum Disorders (ASDs) are heterogeneous behavioral disorders of neural development. Despite numerous studies focusing on ASD-related pathophysiological circuit mechanisms in mature mice, little is known regarding mechanisms underlying ASD onset and its evolution through development. Using the Shank3 complete knockout (Shank3$^{-/-}$) ASD mouse model our team aims to fill this knowledge gap. The medial prefrontal cortex (mPFC) circuit plays an important role in social and cognitive behavior. We studied mPFC circuitry in mice at two developmental stages: adults and 14 days old, a developmental period in which Shank3

protein expression is transiently reduced. Our local field potential (LFP) recordings in mPFC slices revealed a global hyperfunction in Shank3$^{-/-}$ adult mice, especially in deeper layers. In sharp contrast, we did not detect any changes in P14 Shank3$^{-/-}$ mouse compared to WT. This novel observation is the first evidence of progressive establishment of ASD circuit dysfunction in mPFC. Next, we show subtle changes in excitability specific to mPFC layer 5 (L5) pyramidal cells in adults. Whole-cell recordings revealed an increased capacitance of Shank3$^{-/-}$ cells. We also observed an increased total dendritic length and complexity from post hoc analysis of cells filled with biocytin. In addition, the resting membrane potential is depolarized, suggesting hyperexcitability of these cells. We also noticed that the composite excitatory/inhibitory (E/I) ratio, is increased. Interestingly, P14 mice also display differences in some of these features, indicating that the subtle intracellular changes at P14 are not yet affecting overall circuit function, as measured with LFP**.** L5 mPFC neurons provide strong driver inputs to the MD thalamus. We obtained a data set of MD cell responses to optogenetically stimulating mPFC axons expressing channelrhodopsin. In contrast to mPFC cortical neurons, MD cells from Shank3$^{-/-}$ mice did not display changes in intrinsic properties. However, we observed a large difference in the nature of the evoked excitatory synaptic response of the direct output from mPFC neurons to MD in adult Shank3$^{-/-}$ mice. Individual responses show a slower decay (τ_D = 40 ± 6.7 ms vs. 21.9 ± 2.98 in WT). In addition, 10 Hz trains of stimulation evoked slow residual depolarizing current outlasting the stimulus, and persisting for 100s of ms. This delayed synaptic response evident in Shank3$^{-/-}$ would further enhance the efficacy of mPFC outputs in terms of their ability to recruit MD firing. Preliminary evidence supports the involvement of NMDA and mGluR receptors in these responses, providing a potential target for modifying abnormal circuits in ASD.

Disclosures: G. Devienne: None. **G. Vantomme:** None. **J.R. Huguenard:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

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Topic: A.07. Developmental Disorders

Support: NIH Grant 5R01MH129749-02

Title: Relating cerebellar glutamatergic dysfunction and age effects on behavioral phenotype in Shank3$^{\Delta e4\text{-}22}$ mutant mice

Authors: *R. KSHETRI, J. BEAVERS, A. HUEBSCHEN, S. PORCAYO, R. EWA, B. D. RICHARDSON;
Pharmacol., Southern Illinois Univ. Sch. of Med., Springfield, IL

Abstract: Post-synaptic glutamate receptors (AMPAR, NMDAR, and mGluR) are supported and regulated by the SHANK3 scaffold protein (*Shank3* gene), deletions/mutations in which are a

prominent monogenic cause of autism spectrum disorder (ASD). Despite high SHANK3 expression in cerebellar granule cells (CGCs), the primary integrator of cerebellar input, the role of SHANK3 in cerebellar glutamatergic transmission and signal integration is poorly understood. We hypothesize that SHANK3 shapes the nature of glutamatergic transmission and circuit development in CGCs, the loss or dysfunction of which may result in ASD-like motor and non-motor behavioral phenotypes. The purpose of this study is to understand how the loss of SHANK3 affects the function and structure of glutamatergic CGC synapses that may affect cerebellar modulation of motor and non-motor behaviors. In order to identify gene-, sex-, and age-related interactions and main effects on behavioral phenotype, adolescent (5-7 weeks old) and adult (3-5 months old) mice of both sexes carrying the wildtype *Shank3* gene (*Shank3*$^{+/+}$) or that were heterozygous (*Shank3*$^{+/-}$) or homozygous (*Shank3*$^{-/-}$) for a version of the *Shank3* gene lacking exons 4-22 (all isoforms) were used in a behavioral battery to assess motor function, anxiety, repetitive behavior, memory, and social interaction. We found more prominent *Shank3* genotypic differences in motor function, increased anxiety, and repetitive behavior in adult mice compared to adolescent mice. Spontaneous and pharmacologically-evoked glutamate receptor-mediated responses in CGCs were evaluated by whole-cell patch clamp electrophysiology and glutamate photo-uncaging. Our preliminary electrophysiological findings suggest a parallel relationship between the age-related increase in behavioral deficits and the enhancement of spontaneous excitatory postsynaptic currents (sEPSCs) amplitude in CGCs of adult *Shank3*$^{-/-}$ mice. Faster decay kinetics were observed in the evoked excitatory postsynaptic currents (eEPSCs) of *Shank3*$^{-/-}$ mice, in comparison to *Shank3*$^{+/+}$ mice. Ongoing glutamate photo-uncaging experiments in mature adult mice showed an increased AMPA/NMDA ratio and enhanced responsivity of glutamate receptors (AMPA and NMDA combined) in CGCs of *Shank3*$^{-/-}$ mice, relative to *Shank3*$^{+/+}$ mice. These findings suggest the possible role of SHANK3 in maintaining glutamatergic receptors and synapses in CGCs, as well as the potential involvement of the cerebellum in ASD. These insights may underlie and present a novel treatment target for motor and certain cerebellum-modulated non-motor behavioral deficits observed in the absence of *Shank3* activity or function.

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Poster

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Program #/Poster #: PSTR518.11/B57

Topic: A.07. Developmental Disorders

Support: P30ES0302283

Title: Proteomic analysis identified alterations in WNT-Semaphorin-GABA signaling in BTBR cerebellum

Authors: *E. GOZAL[1], M. J. RANE[2], M. T. BARATTI[2], R. JAGADAPILLAI[3], G. N. BARNES[4];
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Abstract: Introduction: Common biological processes are involved in brain vascular and neurological development with crosstalk between blood cells, vascular and neuronal cells. Genetic evidence has implicated gene variations associated with the GABA, WNT, and semaphorins systems in ASD pathogenesis in humans and in mouse genetic ASD models. As a neurotransmitter, GABA signaling has been shown, both in human and in non-human models, to play a role the immune system and in endothelial cells. The GABABR agonist baclofen, currently in autism clinical trials, is a known neutrophil chemoattractant. However, in a neutrophil driven acute lung injury model, baclofen upregulated GABABR2 signaling and inhibited neutrophil-mediated lung damage. Although neutrophil activation is known to be modulated in ASD patients, their exact contribution to ASD pathogenesis is unknown. Therefore, targeting selective GABAergic signaling components and their interactions with other developmental pathways in neuronal, endothelial, or immune cells, could open new therapeutic perspectives in ASD patients. Recent studies show that the cerebellum (CE) affects cognition and social behavior. Evidence from human and preclinical models of ASD suggests that the CE may play a pivotal role in autistic behavior. Proteomic study of C57/bl6 and BTBR mice CE, identified several pathways including neuroinflammation, neutrophil degranulation, GABA receptor, Wnt, and Semaphorin signaling, that were differentially regulated in BTBR as compared to C57/bl6.
Methods: C57/bl6 and BTBR CE homogenates were subjected to proteomic analysis. The data was validated by immunofluorescence and/or immunoblotting.
Results: Our proteomic analysis identified over 150 proteins related to neuroinflammation, neutrophil degranulation, over 25 related to GABA, synthesis, release, or metabolism and over 20 proteins related to WNT signaling, regulated in BTBR mice compared to C57/bl6 control. Data validation showed an increase in inflammatory complement C3, along with presence of neutrophils in cortical tissue. We also demonstrated increase in expression of SNARE proteins, critical for cellular degranulation. Furthermore, we demonstrated changes in expression of GABA receptors, Semaphorin 4D and Wnt signaling proteins.
Conclusions: We demonstrated WNT-Sema-GABA signaling was dysregulated in BTBR CE compared to C57bl/6, with increase in inflammatory markers, and appearance of neutrophils. These data support the hypothesis that altered cerebellar neurovascular and immune signaling may impact ASD relevant neural circuits and promote the appearance of autistic behavior.

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Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

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Program #/Poster #: PSTR518.12/B58

Topic: A.07. Developmental Disorders

Title: The role of CC2D1 genes in neuronal proteostasis and integrated stress response

Authors: *L. TURKALJ, M. KHALID, A. BHATTACHARYA, C. MANZINI;
Rutgers, New Brunswick, NJ

Abstract: One of the emerging pathogenetic mechanisms in neurodevelopmental disorders (NDD), including autism spectrum disorder (ASD) and intellectual disability (ID), involves perturbations in neuronal proteostasis and the integrated stress response (ISR). Proteostasis, or protein homeostasis, is essential for proper neuronal function and is maintained by a complex regulatory network involved in protein synthesis, maintenance, trafficking, and degradation. Under conditions of proteostasis disruption, the ISR is engaged as an adaptive mechanism fine-tuning the cell proteome to restore homeostasis. However, recent studies identified maladaptive ISR as a potential driver of neuronal dysfunction in different models of NDD. Here we focus on understanding how disruption in CC2D1A leads to ASD and ID. Recessive mutations in *CC2D1A* lead to ID and ASD in humans, however, the exact pathogenetic mechanisms of CC2D1A loss-of-function (LoF) are not fully understood. CC2D1A is a protein scaffold that is involved in protein trafficking through the endolysosomal pathway. It is characterized by four unique DM-14 protein-binding domains and a C-terminal C2-domain. CC2D1A has been shown to regulate endosome biogenesis by binding CHMP4B, a component of the Endosomal Sorting Complexes Required for Transport (ESCRT)-III, while the C2 domain serves to tether the protein to the endosomal membrane. Notably, previous studies suggested that a paralogue gene, CC2D1B, likely plays a compensatory role to CC2D1A. Our initial findings in *Cc2d1a*/*Cc2d1b*-deficient murine cortical neurons demonstrate a large accumulation of ubiquitinated proteins within enlarged early endosomes, demonstrating impairment in endosomal protein trafficking. In parallel, our preliminary data indicate that CC2D1A LoF disrupts the integrated stress response (ISR) in embryonic *Cc2d1a* knockout (KO) cortices. Protein accumulation can drive synaptic impairment through different mechanisms. One possibility is that protein accumulation triggers a broad cellular response to proteostasis stress, such as the ISR. Based on these results, we propose a central hypothesis that the failure of protein trafficking through the endolysosomal pathway triggers widespread changes in neuronal function by dysregulating the ISR signaling in CC2D1A/CC2D1B or CC2D1A LoF scenarios. This investigation has the potential to deepen our understanding of the intricate relationship between neuronal proteostasis, ISR signaling, and NDD.

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Poster

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Title: Tracking Postnatal Brain Development in Neurodevelopmental Disorder Models with DevATLAS

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Abstract: Early postnatal brain development is the critical stage when the symptoms of many neurodevelopment disorders (NDDs) start manifesting. Often these functional deficits are caused by abnormal neural circuit maturation without accompanying gross alteration to brain architecture, making it challenging to pinpoint disruptions in these NDD models. There is an urgent need for genetic tools that would allow for tracking the sequence of neural circuit maturation on the whole brain level during the early postnatal period. Since one of the key driving factors of neural circuit maturation is neuronal activity. To overcome this challenge, our lab has developed *DevATLAS*, the Developmental Activation Timing-based Longitudinal Acquisition System, based on the expression of the immediate early gene *Npas4*. *Npas4* is selectively induced by neuronal activity and its activation during development triggers activity-dependent synapse development, a critical step during functional maturation of neural circuits. DevATLAS permanently labels neurons with *tdTomato* (tdT) as they are activated by neuronal activity to express *Npas4*. We demonstrate that DevATLAS captures the sequence of functional neural circuit maturation across the whole brain during the early postnatal period. Using DevATLAS in conjunction with both confocal and whole-brain imaging, we have examined several genetic and environmental NDD models such as Fragile-X syndrome and prenatal valproic acid exposure. Significant developmental perturbations are observed within these NDD models that are correlated with behavioral deficits associated with these conditions. Our analyses have also uncovered potential shared points of disruption in postnatal brain development among several NDD models, suggesting the possibility of "hub" regions in the brain that are critical for overall brain development. We further show that *DevATLAS*, combined with single-cell RNA-seq is a powerful tool in uncovering molecular mechanisms underlying activity-dependent neuronal maturation in vivo. Finally, using an early beneficial intervention of environmental enrichment, we observe normalization of learning and memory development in the Fragile-X mutants, which is captured by DevATLAS. Our results demonstrate that DevATLAS can be applied to study other NDD models. When combined with single-cell sequencing methods, DevATLAS will facilitate the discovery of molecular and genetic pathways perturbed in NDD-

affected brain regions, paving way to understand the underlying disease etiology and to derive new therapeutic interventions to ameliorate NDD deficits.

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Poster

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Topic: A.07. Developmental Disorders

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Title: Captopril, an Angiotensin-Converting Enzyme (ACE) Inhibitor, Attenuates Microglial Activation and Improves Social Behavior in a Mouse Model of Autism Spectrum Disorder

Authors: *B. SPIELMAN, C. BAGNALL-MOREAU, C. CRUZ, L. BRIMBERG;
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Abstract: In utero exposure to maternal anti-brain antibodies (IgG) is linked to an increased risk of autism spectrum disorder (ASD) in offspring. Caspr2 protein (Contactin-associated protein-like 2, encoded by the ASD risk gene, CNTNAP2) is a common target of these maternal antibodies. We have developed a mouse model in which dams are immunized with human Caspr2 protein, and hence develop endogenous anti-Caspr2 IgG. Male offspring, but not female offspring, born to dams harboring anti-Caspr2 IgG throughout gestation exhibit an ASD-like behavioral phenotype including impaired social behavior. We examine the role of microglia in mediating the effects of in utero exposure to Anti-Caspr2 or to Control IgG. We observe microglial activation with altered synaptic pruning in the hippocampus of adult Anti-Caspr2 male mice compared to Controls. Anti-Caspr2 male microglia display increased engulfment of VGAT-labeled inhibitory presynapses and decreased engulfment of VGLUT2-labeled excitatory presynapses, as compared to Control males. Micro-positron emission tomography (MicroPET) scan confirms increased neuroinflammation in the hippocampus of Anti-Caspr2 males, as measured using a [11C]PBR28 radiotracer. We next study whether we can pharmacologically ameliorate the ASD-like phenotype in Anti-Caspr2 males by dampening microglial activation using the Angiotensin Converting Enzyme (ACE) inhibitor captopril. Control and Anti-Caspr2 male mice were given daily intraperitoneal injections of ACE inhibitors captopril (BBB-permeable) or enalapril (BBB-impermeable), or the appropriate vehicle, beginning at 3 weeks of age and continuing for 2 weeks. Anti-Caspr2 males treated with captopril show suppressed microglial activation and preserved dendritic arborization in CA1 pyramidal cells, compared to Anti-Caspr2 males treated with enalapril or vehicle. Additionally, in a dyadic play assay, Anti-Caspr2 males treated with captopril demonstrate longer reciprocal social interaction than do

Anti-Caspr2 males treated with vehicle, and similar reciprocal social interaction time to Control males. Single cell RNA-sequencing of hippocampal microglia confirms that in utero exposure to anti-Caspr2 IgG affects microglia at the transcriptional level, and that captopril ameliorates transcriptional changes by downregulating inflammatory genes and upregulating homeostatic genes. Overall, our model of in utero exposure to anti-Caspr2 IgG can be used to examine the role of microglia in ASD and the potential benefits of using microglia-modulating therapeutics.

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Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

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Title: Investigating the Molecular Effects of Valproic Acid on IPSC-Derived Neurons

Authors: C. LEUNG[1], S. ROSENZWEIG[2], ***B. YOON**[2], N. A. MARINELLI[3], E. HOLLINGSWORTH[4], A. M. MAGUIRE[2], M. COWEN[3], M. SCHMIDT[2], J. IMITOLA[4], E. D. GAMSIZ UZUN[2], S. B. LIZARRAGA[5];
[1]70 Ship St, [2]Brown Univ., Providence, RI; [3]Univ. of South Carolina, Columbia, SC; [4]Univ. of Connecticut, Storrs, CT; [5]Brown, Providence, RI

Abstract: Autism spectrum disorder (ASD) is a highly heritable, neurodevelopmental disorder that is characterized by social, behavioral, and physical deficits. Evidence suggests that several environmental risk factors could contribute to prevalence and etiology of ASD. One such environmental risk factor is valproic acid (VPA), an anticonvulsant drug that helps treat seizures. Several lines of evidence suggest that VPA taken prenatally significantly increases the risk of ASD. VPA is a known histone deacetylase (HDAC) inhibitor which can hyperacetylate histones H3 and H4 and lead to an open configuration of chromatin. Furthermore, dysregulation of the chromatin environment has been shown to also modulate RNA splicing, an essential mechanism of gene regulation for eukaryotic organisms. The molecular mechanisms associated with VPA exposure and chromatin dysregulation and alternative splicing are unclear. Here, we show that VPA exposure results in differential transcript usage (DTU) of high risk ASD genes. Furthermore, we found that ASD risk genes that are differentially expressed or show DTU are

enriched in different molecular pathways. Our results demonstrate that changes in alternative splicing are an additional mechanism of disease in ASD along with chromatin dysregulation.

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Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

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Topic: A.07. Developmental Disorders

Support: NRF Korea 2020R1C1C1014779
2021 Yeungnam University Research Grant

Title: A DLG2/PSD-93 deficiency decreases a number of excitatory synapses and spines in the dorsolateral striatum

Authors: T. YOO[1], S. JOSHI[2], S. PRAJAPATI[2], Y. CHO[3], Y. BAE[4], E. KIM[1], ***S. KIM**[2]; [1]IBS, Daejeon, Korea, Republic of; [2]Col. of Pharm., Yeungnam Univ., Geongsan, Korea, Republic of; [3]KNU, Daegu, Korea, Republic of; [4]Kyunpook Natl. Univ., Daegu, Korea, Republic of

Abstract: The loss of Discs large MAGUK scaffold protein 2 (DLG2), also known as postsynaptic density protein-93 (PSD-93) or chapsyn-110, a postsynaptic scaffolding protein, has been previously associated with a higher susceptibility to psychiatric disorders like schizophrenia and autism in humans. In this study, we utilized a mouse model with a deficiency in DLG2/PSD-93 (Dlg2-/- mice) to investigate the functional consequences on synaptic transmission and the structural characteristics of excitatory synapses in the dorsolateral striatum and cortex.Our findings revealed a significant impairment in corticostriatal synaptic transmission in Dlg2-/- mice, without observable changes in presynaptic releases. Specifically, we observed a notable reduction in the frequency of miniature excitatory postsynaptic currents (mEPSCs) in spiny projection neurons (SPNs) within the dorsolateral striatum, although the amplitude remained unaffected. Additionally, both the density of postsynaptic densities and the fraction of perforated synapses were significantly decreased in the Dlg2-/- dorsolateral striatum. Furthermore, there was a significant decrease in dendritic spine density in striatal SPNs, whereas no significant changes were observed in cortical pyramidal neurons of Dlg2-/- mice. As a compensatory response, the deletion of Dlg2 led to an increase in DLG4/PSD-95 expression and a decrease in the expression of TrkA, a protein known to be influenced by the level of DLG2/PSD-93 expression in the striatum, while not significantly affecting the cortex.Taken together, these results suggest that a deficiency in DLG2 leads to synaptic deficits primarily in the striatum, resulting in aberrant synaptic transmission within striatal circuits.

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Poster

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Title: Characterizing the effects of SSRI exposure on histone serotonylation in autistic and typical iPSC-derived neurons

Authors: *C. M. AAMODT[1], A. SHARMA[4], K. PIERCE[2], E. COURCHESNE[5], N. E. LEWIS[1], M. C. MARCHETTO[3];
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Abstract: Prenatal selective serotonin reuptake inhibitor (SSRI) exposure has been linked to increased risk for autism spectrum disorder (ASD), but the effects of SSRI exposure on histone serotonylation have not yet been characterized in ASD. We generated induced pluripotent stem cells (iPSCs) from the fibroblasts of donors with idiopathic ASD and controls. iPSCs were then differentiated to neural progenitor cells (NPCs) and neurons. NPCs and neurons from all subjects were exposed to an acute 48hr dose of fluoxetine (50 ng/ml) or vehicle. Using an antibody for the dual modification histone 3 lysine 4 trimethylation adjacent to glutamine 5 serotonylation (H3K4me3Q5ser) we first probed for signal differences using western blots. NPCs showed a minimal H3K4me3Q5ser signal. Conversely, ASD and control neurons showed similar H3K4me3Q5ser signal after vehicle exposure, but H3K4me3Q5ser was lower in ASD after SSRI exposure. To better characterize this difference we performed chromatin immunoprecipitation for H3K4me3Q5ser from ASD and control neurons with or without SSRI exposure (N=4 per group). ChIP-seq reads were aligned with hisat2, converted to bam files using samtools, and peaks were called using macs2. Differential enrichment was assessed using diffbind. Ongoing research will further characterize the relationship between genetic background, SSRI exposure, histone serotonylation, and gene expression. The results of this project will be valuable for assessing histone serotonylation as a potential target for ASD prevention, diagnostics, and therapeutics.

Disclosures: **C.M. Aamodt:** None. **A. Sharma:** None. **K. Pierce:** None. **E. Courchesne:** None. **N.E. Lewis:** None. **M.C. Marchetto:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.18/C1

Topic: A.07. Developmental Disorders

Support: Max Planck Society

Title: Loss of PTEN from somatostatin (SOM+) interneurons results in decreased lateral inhibition in the central amygdala and increased expression of fear and anxiety

Authors: *T. W. HOLFORD, C. VON-WALTER, K. LETOURNEAU, D. MONCALEANO, M. BOLTON;
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Abstract: Autism spectrum disorder (ASD) is a complex disorder with large individual variability, where every case has differences in the type and severity of symptoms. Despite the recent increase in diagnoses, scientists have advanced considerably less in their understanding of the mechanisms of ASD because few individual genes that are implicated in ASD are mutated in much more than 1% of patients. One proposed mechanism is that the dysfunction of GABAergic interneurons may play a role in the development and progression of the disorder by interrupting the excitatory and inhibitory balance of neural networks. In our research, we elucidate the role of one class of interneurons in ASD by knocking out a high-risk gene (phosphatase and tensin homologue on chromosome ten, or PTEN) selectively in somatostatin-expressing (SOM+) interneurons. Since many symptoms of autism spectrum disorder present themselves as social anxieties, we test our mouse model in a variety of settings to observe social interaction and social preference, fear and anxiety-like behavior, and repetitive stereotyped behavior. We found that in the SOM+ conditional knockout of PTEN, mice had elevated levels of anxiety and fear recall, suggesting a potential disruption of amygdala function. We then investigated potential dysfunction at the cellular and circuit levels using electrophysiology and 2P local circuit mapping. We found that SOM+ cells in the central amygdala (CeA) lacking PTEN had elevated levels of excitatory drive from the basolateral amygdala (BLA) as well as a drastic disruption of lateral inhibition within the CeA, seen by decreased connection probability and reduced inhibitory post synaptic currents. Given what is known about central amygdala circuitry, these deficits in CeA SOM+ neuron activity conceivably underlie the fear and anxiety-related phenotype observed in mice with a conditional SOM+ PTEN knockout.

Disclosures: T.W. Holford: None. **C. Von-Walter:** None. **K. Letourneau:** None. **D. Moncaleano:** None. **M. Bolton:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR518.19/C2

Topic: A.07. Developmental Disorders

Support: SFARI Pilot Award #724187

Title: Arid1b haploinsufficiency disrupts the development of synapses onto inhibitory interneurons

Authors: *A. H. MARSHALL, D. BOYLE, M. A. HANSON, N. BIBI, A. SAFA, D. NAGARAJAN, A. JOHANTGES, J. C. WESTER;
Dept. of Neurosci., Ohio State Univ., Columbus, OH

Abstract: An imbalance in the ratio of excitation to inhibition (E/I) is a potential pathophysiological circuit mechanism of autism spectrum disorder (ASD). Recent work suggests pathology in cortical inhibitory interneurons may play a key role, but interneurons are diverse, and it is unclear how different subtypes contribute to ASD or the developmental stage during which circuit deficits emerge. Here, we used a mouse model of Arid1b haploinsufficiency (Arid1b (+/-)) to study synaptic connectivity and physiology of PV- and SST- expressing interneurons in neonatal and mature mice. We used the Nkx2.1-Cre line to conditionally knockout one copy of Arid1b from these cells starting during embryonic development (referred to as IN Arid1b(+/-) mice). We then then performed whole-cell patch clamp recordings from interneurons and pyramidal cells in slices of hippocampus and neocortex. During the first postnatal week, mouse cortical circuits spontaneously generate waves of synchronous activity called giant depolarizing potentials (GDPs). Thus, we used GDPs as a high-throughput assay of changes in synaptic physiology in neonatal IN Arid1b(+/-) mice. We recorded an interneuron and neighboring pyramidal cell simultaneously in voltage-clamp to measure total synaptic currents during GDPs at postnatal day 5 - 7. In IN Arid1b(+/-) mice, we found decreased GDP frequency in both interneurons and pyramidal cells. However, synaptic charge transfer during GDPs was only reduced in interneurons, suggesting deficits in the development of synapses selectively onto these cells. Next, we investigated circuits in mature mice using paired whole-cell recordings. Furthermore, we parsed interneurons based on their firing properties and morphologies as fast-spiking (FS) PV cells and non-FS SST cells. Strikingly, we observed a loss of facilitation at synapses from pyramidal cells to non-FS SST cells. Thus, synaptic input to SST interneurons remains weak into adulthood. In contrast, we found enhanced strength at pyramidal cell to FS PV cell synapses. This change may be homeostatic, because the density of FS PV cells was significantly reduced in IN Arid1b(+/-) mice. Finally, connectivity rate and physiology of synapses from FS PV cells to pyramidal neurons was not affected. Our data suggest that Arid1b(+/-) in interneurons impairs synaptic function during early development, which persists into adulthood. However, pathology occurs selectively at synapses onto interneurons to cause E/I imbalance.

Disclosures: **A.H. Marshall:** None. **D. Boyle:** None. **M.A. Hanson:** None. **N. Bibi:** None. **A. Safa:** None. **D. Nagarajan:** None. **A. Johantges:** None. **J.C. Wester:** None.

Poster

PSTR518. Synaptic and Cellular Mechanisms of Autism III

Location: WCC Halls A-C

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Program #/Poster #: PSTR518.20/C3

Topic: A.07. Developmental Disorders

Support: NIH/NIMH R01 MH127081

Title: Examining the Role of Methyltransferase ASH1L in Neuronal Arborization during Human Neural Development

Authors: *J. A. WARD[1], F. D. RITCHIE[2], C. S. LEUNG[1], H. XU[3], S. B. LIZARRAGA[1]; [1]Mol. Biology, Cell Biology, and Biochem., Brown Univ., Providence, RI; [2]Univ. of South Carolina, [3]Biol. Sci., Univ. of South Carolina, Columbia, SC

Abstract: Autism spectrum disorders (ASD) usually present with repetitive behaviors, impaired social interactions, and language deficits. Multiple pathways have been implicated in ASD pathogenesis, in particular chromatin and transcriptional regulatory mechanisms are among the most commonly dysregulated pathways associated with ASD. Our work focuses on the study of the histone methyltransferase ASH1L, which is a major ASD genetic risk factor. Humans with mutations in ASH1L present with ASD, intellectual disability, and seizures. Molecularly, ASH1L dimethylates histone H3 at lysine residue 36 (H3K36me2), which renders chromatin in a more open state and leads to transcriptional activation. H3K36me2 histone marks provide steric hindrance to the activity of the polycomb repressor complex 2 (PRC2), a group of methyltransferase proteins that repress transcription by trimethylating histone H3 at lysine residue 27 (H3K27me3). Our previous work on human excitatory cortical neurons with knockdown for ASH1L suggests that PRC2 works antagonistically with ASH1L to regulate neuronal arborization. However. the extent to which pathogenic mutations in ASH1L leads to defects in neuronal arborization are largely unknown. Using CRISPR/CAS9 genome editing, we introduced heterozygous pathogenic mutations in the catalytic and chromatin interacting domains of ASH1L in human induced pluripotent stem cells (iPSCs) from a neurotypical male. We find that iPSC-derived human cortical neurons with pathogenic mutations in ASH1L leads to underdeveloped and less complex neuronal arborization patterns and decreased neurite outgrowth length. Additionally, we also show a novel increase in size and area of end-of-neurite growth cone structures in mutant neurons, suggesting possible changes in neuronal outgrowth and axon guidance. We interrogated the potential molecular pathways regulated by ASH1L using a candidate approach to analyze changes in gene expression of genes previously implicated in neuronal arborization and ASD pathogenesis, finding a novel significant decrease in the levels of FOXP1 in human cortical neurons with pathogenic mutations in ASH1L. We propose that loss of ASH1L leads to a reduction in methylation of H3K36me2 at specific genes such as FOXP1. In the absence of H3K36me2 PRC2 catalytic activity is unopposed, leading to increased levels of H3K27me3, which may in turn lead to transcriptional repression of ASH1L target genes important for regulating neuronal morphogenesis and growth.

Disclosures: **J.A. Ward:** None. **F.D. Ritchie:** None. **C.S. Leung:** None. **H. Xu:** None. **S.B. Lizarraga:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.01/C4

Topic: A.07. Developmental Disorders

Support: IRSF
Henry Engel Fund

Title: Genome-widecrispr screensidentify druggable mecp2 modulators

Authors: *A. ANDERSON, R. MEYER SCHUMAN, H. ZOGHBI;
Baylor Col. of Med., Houston, TX

Abstract: Methyl-CpG Binding Protein 2 (MeCP2) is a critical regulator of neuronal function. Loss- or gain-of-function mutations in MECP2 cause severe neurological disorders, Rett Syndrome (RTT) or MECP2 duplication syndrome(MDS), respectively. Several studies in mice show that normalizing MeCP2 levels in the brain, either by increasing MeCP2 levels in RTT models or decreasing MeCP2 levels in MDS models, can rescue key symptoms of both disorders. In the context of RTT, even boosting mutant MeCP2 levels in mice with hypomorphic mutationscan extend survivability and improve neurological symptoms. Therefore, we set out to investigate genes and pathways that modulate MeCP2 levels to identify candidate targets that can provide therapeutic opportunities.To this end, we developed a transgenic HEK293T cell line that expresses, bi-cistronic RFP-MeCP2-eGFP, and CAS9. This clonal cell line enables the identification ofcells with low or high MeCP2 levels via a fluorescently activated cell sorting (FACS) strategy. Next, we performedtwo independent CRISPR screens using a whole genome library and a kinome sublibrary to identify druggable modulators of MeCP2 protein levels. For these experiments, we then used the CRISPRCloud2 platform to find gRNAs enriched in either low MeCP2 or high MeCP2 groups relative to bulk samples.We found ~2000 potential MeCP2 regulators that either increased or decreased MeCP2 levels. We subsequently validated top hits by evaluating their effect on endogenous MeCP2 protein levels using either independent gRNAs or drug inhibitors. We are now testing their effect on MeCP2 levels in the mouse brain and patient-derived cell lines, while also working on their mechanism of action. These results provide promising avenues of future research into both the basic biology of MeCP2 regulation and potential therapeutic treatments for MeCP2-related disorders.

Disclosures: **A. Anderson:** None. **R. Meyer Schuman:** None. **H. Zoghbi:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.02/C5

Topic: A.07. Developmental Disorders

Support: NIH Grant HD111864

Title: Targeting MeCP2-Associated Disorders with miRNA Site-Blocking Oligonucleotides

Authors: *A. M. VANDERPLOW, G. E. DODIS, Y. RHEE, J. J. CIKOWSKI, R. G. GOGLIOTTI;
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Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder caused by loss-of-function mutations in the methyl CpG binding protein 2 (MeCP2), a crucial methyl-reader protein. Despite extensive research spanning nearly two decades, progress in developing effective therapies for RTT has been limited. However, seminal studies have demonstrated that RTT can be reversed in mouse models by restoring MeCP2 levels. While gene therapy appears to be a promising approach for RTT at first glance; MeCP2 is a dose-sensitive gene, and even a 1-fold increase in expression could result in a related neurodevelopmental disorder known as MeCP2 duplication syndrome (MDS). This poses a significant practical challenge, as achieving efficient delivery of MeCP2 to the entire human brain is necessary while ensuring that each cell receives a precise and relatively small amount of the protein.

The 3'untranslated region (3'UTR) plays a critical role in the post-transcriptional regulation of mRNA, where numerous microRNAs (miRNAs) and RNA binding proteins collectively contribute to fine-tuning gene expression. The impact of each miRNA on protein levels is generally modest, making strategies that disrupt miRNA regulation of the 3'UTR potentially valuable tools for disorders with a narrow therapeutic window for the target gene. The repressive role of miRNAs on MeCP2 expression has been shown at multiple sites and when overexpressed and this strategy can reverse MDS symptoms in mice. However, its therapeutic potential in RTT remains understudied.

We have developed an innovative approach that utilizes a series of locked nucleic acid site-blocking antisense oligonucleotides (sbASOs). These sbASOs are specifically designed to “out-compete” with endogenous miRNAs for binding to MeCP2’s 3'UTR. This strategy effectively enhances or releases the repression of MeCP2 expression in a controlled manner, capping it at sub-toxic levels. We believe that this strategy could be a promising intervention for patients with MeCP2 mutations characterized by missense or late-truncating variants, as the increase of MeCP2 can compensate for the partial loss of function. Our initial data demonstrates that targeting microRNA binding sites using sbASOs can effectively elevate MeCP2 levels in RTT patient-derived fibroblast cell lines in a dose-dependent manner. Importantly, the increases are capped at non-toxic levels due to the modest impact of each miRNA on gene regulation. Ongoing studies aim to assess the efficacy of sbASOs in RTT-derived iPSCs neurons and *in vivo*, to explore whether the elevated expression of mutant MeCP2 variants is associated with improved function and mitigation of pathological features.

Disclosures: **A.M. Vanderplow:** None. **G.E. Dodis:** None. **Y. Rhee:** None. **J.J. Cikowski:** None. **R.G. Gogliotti:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.03/C6

Topic: A.07. Developmental Disorders

Title: In vitro and in vivo characterization of pathogenic gain-of-function mutations in the GB2 subunit of $GABA_B$ receptors

Authors: *M. STAWARSKI[1], D. FERNÁNDEZ-FERNÁNDEZ[1], B. FRYCZ[1], D. ULRICH[1], L.-Y. CHEN[2], M. CHOI[3], M. TAFTI[2], M. GASSMANN[4], B. BETTLER[1];
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Abstract: Healthy brains maintain a constant balance between excitatory and inhibitory neuronal activity (E/I balance) despite transient imbalances exhibited by groups of neurons. A disturbed E/I balance in the brain may cause epilepsy, autism spectrum disorder and cognitive impairments. $GABA_B$ receptors (GBRs) are G protein-coupled receptors for the neurotransmitter γ-aminobutyric acid (GABA). GBRs are crucially involved in the control of the E/I balance by providing pre- and postsynaptic inhibition in response to ambient and synaptically released GABA. GBRs are obligatory heterodimers composed of a GB1 and GB2 subunit. Genetic approaches identified several pathogenic monoallelic *de novo* variants in *GABBR2*, the gene encoding the GB2 subunit. Mutations in the transmembrane domains of GB2 were shown to underlie Rett-like phenotypes (RTT: GB2 A567T) and epileptic encephalopathy (EE1: GB2 S695I, EE2: GB2 I705N) (Vuillaume et al., *Ann Neurol, 83*, 2018; Yoo et al., *Ann Neurol, 823*, 2017). Patients carrying these mutations exhibit phenotypes consistent with the E/I balance shifted towards increased excitation. We functionally analyzed these pathogenic GB2 variants in heterologous cells. All three GB2 variants represent gain-of-function mutations that increase agonist potency and/or constitutive receptor activity. The inverse agonist CGP54626 only partially blocks the activity of mutant receptors. The A567T and I705N variants reduce the efficacy of agonists, while the S695I variant is unresponsive to agonist. We generated heterozygous (reflecting the patient situation) and homozygous GB2-I704N mice to study the neuronal effects of the human I705N mutation. Biochemical analysis of GB2-I704N knock-in mice reveals adaptive changes that counteract functional alterations induced by the mutation. In addition, an ongoing electrophysiological characterization of GB2-I704N mice indicates synaptic and network alterations. We will discuss how increased agonist potency and constitutive activity of mutant receptors can be reconciled with a shift of the E/I balance in patients towards increased excitation.

Disclosures: **M. Stawarski:** None. **D. Fernández-Fernández:** None. **B. Frycz:** None. **D. Ulrich:** None. **L. Chen:** None. **M. Choi:** None. **M. Tafti:** None. **M. Gassmann:** None. **B. Bettler:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.04/C7

Topic: A.07. Developmental Disorders

Support: NINDS R01NS100738

Title: Forniceal deep brain stimulation rescues MeCP2-deficiency induced neuronal maturation deficits in the dentate gyrus of Rett syndrome mice

Authors: *Q. WANG[1,2], B. TANG[1,2], Z. WU[1,2], J. TANG[1,2];
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Abstract: Adult hippocampal neurogenesis (AHN) encompasses the proliferation, differentiation, and maturation of new dentate granule cells (DGCs) that are continually added to the hippocampus in adult animals. Impaired AHN results in cognitive deficits in diverse models of neurodegenerative and neurodevelopmental diseases, while interventions increasing AHN (e.g. enriched environmental experiences and deep brain stimulation, DBS) enhance hippocampal circuit function and behavioral performance. Rett syndrome (RTT), a postnatal neurodevelopmental disorder mainly caused by mutation in the X-link gene *MECP2*, is characterized by motor deficits and intellectual disability. We previously reported that forniceal DBS promoted the generation of DGCs and rescued the hippocampal memory in a mouse model of RTT. Here we further demonstrated that ablating AHN abolished the benefits of forniceal DBS on contextual fear memory, suggesting a causal relationship between the DBS-induced AHN and hippocampal memory enhancement. Importantly, besides generation, the following maturation and functional integration of newborns into the hippocampal network indispensably contribute to DBS-induced memory improvement. To understand how DBS regulates neuronal maturation, we used retrovirus expressing green fluorescent protein to selectively label newborn DGCs and analyze their maturation and integration processes. Newborn DGCs in RTT mice that do not express MeCP2 due to random inactivation of X chromosome displayed impaired morphological development and synaptic dysfunction. Forniceal DBS, however, sufficiently ameliorated these deficits. This is likely due to that DBS restored the delayed conversion of GABA-induced depolarization into hyperpolarization caused by MeCP2 loss and reinstated the level of KCC2, a K^+-Cl^- cotransporter that regulates GABAergic development. Together, this study suggests that MeCP2 loss impairs the maturation of adult-born DGCs. Restoration of AHN deficits may serve as a potential mechanism underlying forniceal DBS-induced memory benefits in RTT mice. This work was supported by NINDS R01NS100738 and 2R01NS100738.

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Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.05/C8

Topic: A.07. Developmental Disorders

Support: MIT Research Scholars Program
Wenner-Gren Fellowship
Picower Institute Innovation Fund
NIH grant MH085802

Title: Long Distance Cortical Projections from Anterior Cingulate Cortex to Visual and Retrosplenial Cortical Areas in Rett Syndrome Model Mice

Authors: *J. M. HARPE[1], S. K. ÄHRLUND-RICHTER[1], M. SUR[2];
[1]MIT, Cambridge, MA; [2]Dept. of Brain and Cognitive Sci., MIT Grad. Brain and Cognitive Sci., Cambridge, MA

Abstract: Neurodevelopmental disorders, such as autism spectrum disorders, are commonly characterized as "connection disorders", with emerging evidence suggesting disruptions in local and long-range connections as features underlying their phenotypes. Rett Syndrome is a severe neurodevelopmental disorder primarily affecting girls, caused by mutations in the MECP2 gene. Rett model mice with MeCP2 mutations are known to have disrupted synaptic function and plasticity as well as reduced neuronal maturation, with a variety of motor and cognitive deficits likely related to altered brain connections. Whether or not MECP2 mutations disrupt long-range connections in the brain is unknown. The anterior cingulate cortex (ACA) is a crucial brain region involved in cognitive processes. It plays a significant role in top-down control of attention, decision-making and emotional regulation, via its long-range influences on diverse brain structures. Here, we examined whether top-down long-range projections from the ACA to visual and adjacent areas of neocortex are altered in MeCP2 mutant mice. AAV1-CAG-TdTomato was injected in ACA of 3-month-old MECP2 heterozygous mice to trace anterograde projections in target regions, and compared with projections in age-matched wild-type control mice. Confocal microscopy was used to capture serial sections, and brain regions were aligned and registered with the Allen Brain Atlas for quantifying anterograde axonal densities based on anatomical location. In particular, we aimed to identify whether cortical regions such as primary visual cortex, medial and lateral higher visual cortical areas, and retrosplenial cortex, displayed differences in areal and laminar termination sites and densities. Preliminary analysis of the data reveals that similar to wild-type mice, Rett model mice show dense ACA projections to retrosplenial cortex with sparser projections to lateral visual areas, especially targeting superficial cortical layers including layer 1. Axonal termination densities in the Rett model mice may exhibit a tendency towards a broader distribution across the layers and borders of target

cortical areas when compared to wild-type mice. Thus, MECP2 mutations may thus be associated with a more generalized pattern of top-down axonal projections originating from the ACA, potentially leading to altered laminar and areal specificity in target regions. While further analyses are required to confirm these observations, they are consistent with the hypothesized reduced neuronal maturation as a key feature of Rett Syndrome, suggesting that changes in long-range cortical projection systems may be an important feature of the disorder.

Disclosures: **J.M. Harpe:** None. **S.K. Ährlund-Richter:** None. **M. Sur:** None.

Poster

PSTR519. Rett Syndrome

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.06/C9

Topic: A.07. Developmental Disorders

Support: HeART Neurohabilitation Award GL-1400315

Title: Virtual color sensor computerized game improves voluntary control of upper motor extremities in girls with rett syndrome

Authors: *S. KYLE[1], J. PAULY[1], V. BEAUDOIN[1], E. CHEN[1], H. HINES[1], I. SNEE[1], G. YIP[1], W. EMIGH[2,3], N. MISHLER[2,3], I. POTTMEYER[2], P. S. DIENER[1];
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Abstract: Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder that causes individuals to lose voluntary control over their upper extremities and develop unique involuntary stereotypic arm movements. To restore voluntary upper extremity function, we developed a color sensor computer game (CSCG) with the objective of encouraging purposeful upper extremity movement through a visual and auditory reward-based system personalized to each participant. Individuals with RTT (females, n=7) participated in the study in a completely virtual environment over the span of approximately 4-5 months. The study period consisted of a total of 5 function reach tests (fRT), and 108 intervention sessions with the CSCG, each lasting 15 minutes. The participants began with a baseline fRT followed by a 4 week period with no intervention outside of the participants' normal routine. A second fRT was then conducted followed by a brief training period using electronic applications to reinforce the concept of cause and effect. After the training period, a third fRT was conducted, immediately followed by the beginning of the intervention period. At the end of approximately 12 weeks of 9 interventions per week, the fourth fRT was conducted. Intervention was then completely withdrawn for 4 weeks, ending with a fifth fRT. Other outcome measures, which included a baseline of each participant's unique stereotypic upper extremity movements and clinically-relevant goal attainment scores (GAS), were determined in partnership with the participants' guardians prior to any intervention. GAS measured arm use during daily activities such as separating arms to dress,

eat, and reach for objects. fRTs and intervention sessions were recorded and independently analyzed by two trained reviewers for evaluation of quantity and duration of independent hand use and attention measured through glances and sustained attention. GAS were re-evaluated with the participants' guardians at the end of the study. While preliminary results (n=3) show inconsistency in improvements in visual attention, there was a reduction in quantity and duration of stereotypic upper extremity movements and an increase in quantity and duration of independent upper extremity use. Improvement in voluntary control of upper extremity movement during activities of daily living was demonstrated with positive changes in GAS. GAS effectively measured qualitative clinical changes that may impact quality of life. Our results suggest that CSCG may be effectively conducted in a virtual environment and should be customized based on the individual 's unique stereotypic movements and motor skills.

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Poster

PSTR519. Rett Syndrome

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.07/C10

Topic: A.07. Developmental Disorders

Support: Brain/MINDS project of AMED JP15dm0207001
Rett Syndrome Supporting Organization

Title: MECP2 mutant marmosets exhibit primate-specific phenotypes of Rett syndrome

Authors: *N. KISHI[1,3], J. OKAHARA[1,4], K. SATO[4], D. YOSHIMARU[1,5], K. ONISHI[2], T. ITOU[1], M. OKUNO[1], H. J. OKANO[1,5], J. HATA[1], E. SASAKI[1,4], T. SHIMOGORI[2], H. OKANO[1,3];
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Abstract: Rett syndrome (RTT) is a severe and progressive neurodevelopmental disorder primarily affecting girls with a prevalence rate of one in 10,000-15,000. Children with RTT develop relatively normally for 6-18 months, after which they undergo a period of rapid regression, with loss of purposeful hand use, deceleration of head growth, and autistic behaviors. Mutations of the *MECP2* gene on the X chromosome are found in over 95% of cases of classic Rett syndrome. While the functions of MECP2 in the central nervous system have been studied using *Mecp2* mutant mice, it is important to note that these mouse models do not always accurately replicate the symptoms observed in RTT patients due to the differences in brain structure and function between primates and rodents. The establishment of nonhuman primate

(NHP) models that are similar to humans in many aspects is very important to understand the pathogenesis of RTT. The common marmoset (*Callithrix jacchus*) is a small New World primate that is native to the Atlantic coastal forests in northeastern Brazil, offering several advantages as a model organism. In this study, using genome editing technology, *MECP2* mutant marmosets were generated as a primate model of RTT. The marmosets lacking MECP2 were born without complications, but during the weaning period, they exhibited impaired brain growth and reduced activity. By conducting single-nucleus and spatial transcriptome analyses, abnormal gene expression related to neuronal maturation was identified in *MECP2*-deficient neurons, varying across different types of neurons and cortical layers. These findings demonstrate that the marmoset model faithfully mirrors the pathophysiology observed in RTT patients and have potential for advancing our understanding of the underlying mechanisms of RTT and for conducting preclinical research.

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Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.08/Web Only

Topic: A.07. Developmental Disorders

Support: IRSF-3515

Title: Cellular phenotypes of cortical neurons from R255X MECP2 knock-in mice in vitro are improved by expression of wildtype MeCP2 or read-through with G418

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Abstract: Approximately 60% of individuals with Rett Syndrome (RTT) carry a nonsense mutation in the *MECP2* gene; thus, there is an unmet need to identify novel nonsense suppression compound(s) that can restore full length MeCP2 protein levels and function. A recent high-throughput screen using dual luciferase reporters in cell lines expressing R168X, R255X, R270X, and R294X *MECP2* nonsense mutations, identified 11 novel compounds that increased full-length MeCP2 protein levels. Before testing their efficiency in preclinical RTT models, we first characterized neuronal phenotypes in cultured cortical neurons from newborn knock-in mice harboring the *MECP2* R255X mutation. After 2 weeks *in vitro*, R255X mutant neurons showed smaller cell bodies, shorter dendrites, fewer dendritic branches, and a lower density of excitatory synapses when compared to wildtype (WT) neurons. Ca^{2+} imaging revealed that R255X mutant neurons have heightened spiking activity, reflected in significantly shorter

inter-event intervals between spontaneous Cal-520 transient events than in WT neurons. We are analyzing the correlation of Ca^{2+} signaling networks. Consistent with the "genetic" rescue of *Mecp2* knockout mice by the proper expression of *Mecp2* from its endogenous promoter (Guy et al. 2007), transduction of AAV9-MeCP2-GFP in R255X mutant neurons made these cellular phenotypes similar to those in WT neurons, including soma size, dendritic length and branching, and excitatory synapse density. As proof of principle for the potential clinical use of 'readthrough' compounds, cultured R255X mutant neurons treated with the aminoglycoside G418 (50ug/mL) for 72h *in vitro* showed cell body size and excitatory synapse density similar to WT neurons. Planned experiments include testing how newly identified 'readthrough' compounds compare to G418 as a positive control. We expect these combined approaches will identify effective compounds to suppress nonsense mutations, which can be moved to preclinical functional and behavioral studies in R255X *MECP2* knock-in mice.

Disclosures: X. Xu: None. **J. Merritt:** None. **S.J. Gray:** None. **J. Neul:** None. **L. Pozzo-Miller:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.09/C11

Topic: A.07. Developmental Disorders

Title: Novel unsupervised modeling of sleep EEG brain state dynamics shows that Rett syndrome brains do not conform to conventional sleep stage classification

Authors: *C. HUANG[1,2], A. MAHAT[3], D. GLAZE[1,4], M. MALETIC-SAVATIC[1,2], A. BUCKLEY[5], M. MCGINLEY[1,2,6];
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Abstract: Conventional interpretation of the dynamics of brain state during wakefulness and sleep relies on manual scoring of the sleep polysomnography (PSG) into 5 stages (Wake, rapid eye movement (REM), and Non-REM 1-3). However, sleep brain state dynamics may be so significantly altered in some developmental brain disorders (DBD), such as Rett syndrome, that conventional PSG may provide an inaccurate and incomplete model. Here we developed a Hidden Markov Model (HMM) approach to describe sleep brain activity as continuous transitions between quasi-stable states. We compared differences between the inferred states from healthy subjects at different age groups and the states from Rett syndrome patients. 150 neurotypical subjects (age: 7.9 ± 4.6 years, 68 females) and 30 Rett syndrome girls (age: 11.6±5.4 years) participated in the study. The neurotypical subjects were equally divided into 5 age groups (in years): 2-3, 4-5, 6-8, 9-12,13-18. As the model input, we extracted the power spectrum from seven frequency bands (in Hz): 0.5-2, 2-4, 4-6, 6-8, 8-12,12-14, and 14-16 of

electroencephalogram (EEG) from Fp1, Fp2, C3, C4, O1, O2, T3, T4, Cz, Oz leads. Additional features were signal root mean squared (RMS) and per-channel sample entropy and the EMG RMS. We then employed HMM to infer the hidden states, including initial states probability, the mean activity and covariance matrix, and states transition probabilities. For each neurotypical age group, 82 inferred states well-described the sleep EEG statistics, based on the leave-one-out likelihood approach. Some states were highly specific to a PSG stage, while some states appeared to reflect within-stage microstructure that was not captured by conventional PSG staging and varied across age groups. The state transition matrix modularity first increased, and then decreased, with age, indicating novel age-dependent brain states dynamic. For the Rett group, the optimal state number was only 24, suggesting simpler overall dynamics, and the states that were highly specific to the REM stage varied between individuals. Furthermore, the states highly related with sleep spindles found in the control group were not found in the Rett group. We conclude that altered sleep-state generation and rhythms, as uncovered here in the proposed HMM framework, can help define brain state changes, without imposing (perhaps false) assumptions about sleep stage structure. This approach offers insight into underlying neuropathology in DBDs. Furthermore, it holds promise for diagnosis, as well as evaluation of treatment efficacy, particularly when treatments aim to reverse early developmental neuromodulatory brain state aberrancies in DBDs.

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Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.10/C12

Topic: A.07. Developmental Disorders

Support: Simons Collaboration on the Global Brain
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National Institute of Neurological Disorders & Stroke (R01NS057819)
Howard Hughes medical institute

Title: Home-cage assisted measurements of decision-making reveal deficits in Mecp2$^{+/-}$ mice

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Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder characterized by a wide range of symptoms, with severe apraxia being a notable feature. Apraxia is the inability to perform motor planning and is often associated with basal ganglia dysfunction. However, our knowledge of the circuit alterations in the basal ganglia and how they relate to the behavioral symptoms in RTT is limited. Here we used a novel approach to analyze circuit malfunction underlying behavior in a mouse model of RTT that carries a *methyl-CpG-binding protein 2* (*Mecp2*)-null allele (RTT mice).
In an automated home-cage system (Hao et al, eLife, 2021), self-motivated mice engaged in tactile decision-making tasks for over several months without human supervision. In the decision-making task, mice discriminated object location using whiskers and reported object location using directional licking. Parallel testing allowed us to assay two dozen cages at the same time. Instead of cross-sectional analysis, this approach longitudinally tracked the onset and progression of behavior deficits in the RTT mice over time relative to littermate wild-type (WT) mice. We discovered that RTT mice were able to learn the decision-making task similarly to WT mice at 12 to 16 weeks of age. However, RTT mice exhibited abnormal licking patterns and slower reaction time, which deteriorated with age. Once the mice achieved proficiency in the decision-making task, we conducted an additional assessment of their flexible motor planning by reversing the sensorimotor contingency. The new sensorimotor contingency allowed us to examine the mice's ability to adapt to new task rules. RTT mice exhibited slower reversal learning compared to WT mice at 16 to 20 weeks old. Over repeated sensorimotor reversals, both RTT and WT mice exhibited accelerated reversal learning. We are currently combining this approach with multi-Neuropixels probe recordings from a frontal cortico-basal-ganglia loop required for tactile decision-making. This loop includes anterior lateral motor cortex, lateral striatum, and ventromedial thalamus. Preliminary analyses suggest that there is reduced choice-related neural activity during decision-making in RTT mice.
Our study outlines a platform to assay motor planning deficits in the Rett mouse model and allows largescale analysis of the underlying neural dynamics.

Disclosures: Y. Ki: None. **H. Zoghbi:** None. **N. Li:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.11/C13

Topic: A.07. Developmental Disorders

Support: 1R01NS106285

Title: Altered expression and biochemical properties of perineuronal nets in a mouse model of Rett syndrome

Authors: *A. SINHA[1], A. KOWALCHUK[1], R. T. MATTHEWS[2], J. L. MACDONALD[1]; [1]Syracuse Univ., Syracuse, NY; [2]Neurosci., SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Rett Syndrome (RTT) is a severe debilitating neurodevelopmental disorder caused by mutations in *MECP2*. Currently there is no effective treatment for this devastating condition. Previous work in the field has demonstrated that the experience-dependent development of neural circuits in the central nervous system (CNS) is widely disrupted in mouse models of RTT (*Mecp2* $^{-/y}$ mice). This experience-dependent refinement of CNS neural pathways in higher mammals, including humans, occurs during an early hyperplastic developmental period known as critical period (CP). The closure of CP and, therefore, the final maturation of the CNS, is marked by the appearance of perineuronal nets (PNNs), lattice-like substructures within the extracellular matrix (ECM) that enwrap specific subpopulations of neurons. PNNs are essential in the modulation of neuronal plasticity and their disruption can even reopen juvenile forms of plasticity in adult animals. In *Mecp2* $^{-/y}$ mice, PNNs form early in development and therefore, lead to an earlier closure of CP. However, little is known of the cellular, molecular, or biochemical underpinnings of the precocious PNN formation in RTT or *Mecp2* mutant mice. Therefore, an understanding of the molecular composition and structure of the aberrant PNNs formed in RTT is essential to develop strategies to specifically rescue their formation and re-establish the proper developmental trajectory for PNN formation and CP. We present evidence that PNNs in *Mecp2* $^{-/y}$ mice cortices are biochemically distinct from their wild type counterparts and that they structurally mature at an earlier developmental stage. Further, our data suggest that astrocytes play a unique and underappreciated role in regulating PNN formation, that, in turn, we believe contributes to the pathology in RTT. Previous work from our lab has demonstrated that genetic attenuation of the NFκB pathway can lead to rescue of many hallmark phenotypes of RTT. In this study, we additionally investigate whether NFκB pathway attenuation can recover the formation of PNNs in *Mecp2* $^{-/y}$ cortices, demonstrating that this restores the biochemical properties of PNNs in *Mecp2* $^{-/y}$ mice. Together, our work provides deeper insight into a well-known alteration observed in RTT pathology and provide new pathways as targets for therapeutic intervention.

Disclosures: **A. Sinha:** None. **A. Kowalchuk:** None. **R.T. Matthews:** None. **J.L. MacDonald:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.12/C14

Topic: A.07. Developmental Disorders

Support: Rett Syndrome Research Trust Grant 20190460 (to M.E., M.F.)
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The Donna and Benjamin M. Rosen Bioengineering Center
Howard Hughes Medical Institute (to M.E.)

Title: Neurophysiological Abnormalities Rescue in Rett Syndrome Mice using miRNA regulated AAV-MeCP2 Gene Replacement

Authors: *K. MAHE, A. M. H. MAYFIELD, M. FLYNN, M. B. ELOWITZ, V. GRADINARU;
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Abstract: Rett syndrome (RTT) is a monogenic neurodevelopmental disorder predominantly afflicting females, characterized by the loss of motor skills, language regression, and intellectual disability. Mutations in methyl-CpG binding protein 2 (MeCP2) have been causally linked to the clinical presentation of RTT, whereas overexpression of MeCP2 leads to MeCP2 Duplication Syndrome. The extreme dosage sensitivity of MeCP2 requires a narrow window of protein expression in gene replacement approaches. Despite extensive research on the underlying genetic mutations associated with RTT, effective methods to achieve dosage sensitive therapeutic interventions remain elusive. We have developed a miRNA-based synthetic system to achieve control of MeCP2 expression at the single cell level for systemic delivery via AAV-CAP.B22 (Goertsen et al. 2022), an engineered adeno-associated virus (AAV) capsid that crosses the blood-brain barrier and efficiently transduces neurons and astrocytes in the brain. We demonstrate the ability to limit ectopic MeCP2 expression to values comparable to those of endogenous MeCP2, irrespective of AAV dosage per cell. Further, we explore the therapeutic potential of regulated MeCP2 replacement on both behavior and brain activity patterns. Behavioral phenotype was characterized using the validated RTT phenotype score (Guy et al. 2007) which describes motor ability (gait, activity), neurological features (tremor, hindlimb clasp), and general health status (weight, appearance). Preliminary results from female RTT mice injected systemically at 4 weeks of age with regulated AAV-CAP.B22-MeCP2 at a dose of 5E12 viral genomes per mouse demonstrate prevention of progression of the RTT phenotype. In conjunction with this characterization, we are investigating the effects of the regulated construct on activity in the motor cortex (M1) and the hippocampus—areas of the brain important for coordinating movement and cognition. To examine whether the mechanism of this behavioral rescue relies on changes to the baseline neurophysiological hallmarks of RTT, we are assessing synapsin-GCaMP8s neurons using implanted cranial windows over the M1 motor cortex. We are measuring synchronicity, frequency, and firing patterns during rest and movement using head-fixed 2-photon imaging in 2-month-old RTT female mice. We are also assessing bulk firing amplitudes and decay times for neurotransmission in the hippocampus during a ladder descent task using fiber photometry. Our findings will inform the RTT gene therapy field on the potential of regulated replacement of MeCP2 as a strategy for restoring normal cellular and behavioral function.

Disclosures: K. Mahe: None. **A.M.H. Mayfield:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with M.F. as inventor. **M. Flynn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with M.F. as inventor. **M.B. Elowitz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with M.E. as inventor. **V. Gradinaru:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); C.I.T. filed IP for methods with V.G. as inventor;. Other; Capsida Biotherapeutics Cofounder and BoD member.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.13/C15

Topic: A.07. Developmental Disorders

Support: Broad Institute's Stanley Center for Psychiatric Research

Title: X-inactivation-driven functional mosaicism in the female human brain and application to Rett syndrome

Authors: *S. BURGER[1,2,5], M. GOLDMAN[2,5], J. NEMESH[2,5], E. LING[2,5], S. BERRETTA[3,6,4], S. MCCARROLL[2,5];
[1]Harvard Univ., Boston, MA; [2]Dept. of Genet., [3]Program in Neurosci., [4]Dept. of Psychiatry, Harvard Med. Sch., Boston, MA; [5]Stanley Ctr. for Psychiatric Res., Broad Inst. of MIT and Harvard, Cambridge, MA; [6]McLean Hosp., Belmont, MA

Abstract: Every human female is a mosaic of cells that have inactivated one or the other X-chromosome copy (X1 or X2). How X-inactivation patterns vary across cell types — and the nature of functional mosaicism due to X-inactivation — are key questions, especially for persons with X-linked mutations. We developed a computational method for identifying, in single-cell RNA-seq data from any female, which copy of the X chromosome is active in each individual cell. Because X1- and X2-expressing cells share the same environment, genetic background, and postmortem conditions, comparison of these two cellular populations makes it possible to precisely delineate the cell-autonomous effect of X-chromosome differences. Analyzing cortical tissue from 118 female donors, we found this functional mosaicism to be quite modest in most people. However, in each of five females with various Rett-syndrome-causing *MECP2* mutations, thousands of differentially expressed genes distinguished functional- and mutant-*MECP2*-expressing cells. We found that different mutations in *MECP2* elicit highly correlated gene expression changes across individuals, but with magnitudes that vary by mutation and correspond to reported clinical severity of these mutations. We observed a 1.2 - 1.3 fold greater transcriptional impact associated with P101T and R168X mutations relative to R255X, P302L, and R306C mutations. Beyond these persons with clinically diagnosed Rett syndrome, many individuals may unknowingly carry X-linked mutations with less clinically-defined phenotypes; we hypothesized that our analytical approach could be used to identify females with unusual levels of X-linked functional mosaicism. We discovered one such individual with a functional-mosaicism signature identical to that of Rett syndrome patients but with expression fold-changes 3 - 4 times lower than observed for P101T and R168X mutations. Sequencing confirmed the presence of a (previously unrecognized) MECP2 R167W mutation, which has been associated with X-linked intellectual disability and likely contributed to this individual's clinical history and

struggles with learning disabilities. We found that the impact of this mutation had been further attenuated in this patient by favorable X-inactivation. Our approach provides a way to measure the transcriptional impact of any X-linked mutation — comparisons that have historically been complicated by variability in donor clinical history and X-inactivation skew. Our computational approach can be used to identify and characterize X-linked mutations in any tissue or cell type from any female of any genetically diverse species.

Disclosures: **S. Burger:** None. **M. Goldman:** None. **J. Nemesh:** None. **E. Ling:** None. **S. Berretta:** None. **S. McCarroll:** None.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.14/C16

Topic: A.07. Developmental Disorders

Title: Characterizing the maturation of electrophysiological properties in stem cell-derived human neurons lacking methyl CpG binding protein 2 (MECP2)

Authors: *P. GANDHI[1], D. LIU[1], A. SNYDER[1], C. TIAN[1], C. M. PETROSKI[1], N. ANASTASI[2], A. GUNDLFINGER[2], E. HARDE[2], R. JAGASIA[2], R. E. PETROSKI[1], E. C. O'CONNOR[2];
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Abstract: Rett syndrome (RTT) is a rare, genetic neurodevelopmental disorder that remains without cure. RTT is caused by loss-of-function mutations in the gene encoding methyl CpG binding protein 2 (MECP2), which plays an important role in neuronal maintenance and plasticity. The development of novel therapies for RTT critically depends upon the availability of model systems with robust and disease-relevant phenotypes. In this regard, we generated MECP2 KO human embryonic stem cell-derived neural stem cells (hESCs-NSCs) and differentiated them into neurons, as a promising tool for understanding MECP2 loss-of-function on electrophysiological phenotypes in a human neuronal context. Whole-cell patch-clamp recordings were performed on MECP2 KO neurons and isogenic wild-type controls (WT) at different times of differentiation, with an objective to characterize the developmental trajectory of electrophysiological phenotypes. Additionally, gramicidin perforated patch-clamp recordings were conducted to characterize maturation of the GABA reversal potential (E_{GABA}). Over (181) 90 WT and 91 MECP2 KO cells were recorded from 1-3-month-old neuronal cultures. Both WT and MECP2 KO neurons developed a more mature neuronal phenotype over time: 1) Cell capacitance (cell size) increased, 2) Resting membrane potential became more hyperpolarized, 3) Voltage-gated sodium current density increased and 4) $GABA_A$ receptor current amplitudes increased. The majority of both WT and MECP2 KO neurons did not fire spontaneously but

could elicit action potentials with depolarizing current injection. Further characterization of neuronal phenotypes will be presented and discussed in light of the utility of this tool for developing novel therapies for Rett Syndrome.

Disclosures: **P. Gandhi:** A. Employment/Salary (full or part-time):; Neuroservices-Alliance. **D. Liu:** A. Employment/Salary (full or part-time):; Neuroservices-Alliance. **A. Snyder:** A. Employment/Salary (full or part-time):; Neuroservices-Alliance. **C. Tian:** A. Employment/Salary (full or part-time):; Neuroservices-Alliance. **C.M. Petroski:** A. Employment/Salary (full or part-time):; Neuroservices-Alliance. **N. Anastasi:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche. **A. Gundlfinger:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche. **E. Harde:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche. **R. Jagasia:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche. **R.E. Petroski:** A. Employment/Salary (full or part-time):; Neuroservices-Alliance. **E.C. O'Connor:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche.

Poster

PSTR519. Rett Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR519.15/C17

Topic: A.07. Developmental Disorders

Support: NIH Grant 2R01NS057819-16
Henry Engel Philantrophic Fund

Title: Modulating the alternative splicing of MECP2:A potential therapeutic strategy for Rett Syndrome

Authors: *H. PALLIKARANA TIRUMALA[1], L. WANG[1], A. TROSTLE[1], W. WANG[1], S. BAJIKAR[1], Y. LI[1], J. KIM[1], H. CHEN[1], Z. LIU[1], H. ZOGHBI[1,2,3];
[1]Human and Mol. Genet., Baylor Col. of Med., Houston, TX; [2]Texas Children's Hosp., Houston, TX; [3]Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder caused by loss-of-function mutations in *MECP2* (Methyl CpG binding protein 2), a transcriptional regulator essential for maintenance of normal neuronal function. There is no effective treatment for RTT, and studies in mouse models have shown that increasing even mutant MeCP2 protein improves neurological symptoms. We devised a therapeutic strategy to increase mutant MeCP2 protein level by modulating *MECP2* alternative splicing. *MECP2* has two alternatively spliced isoforms: *e1* (exons 1,3,4) and *e2* (exons 1,2,3,4). Despite similar levels of transcription, *e2* is translated at a lower efficiency than *e1*. Studies on mouse models and human RTT patients suggest that E2 is dispensable for the neuronal function of MeCP2. Therefore, we hypothesize that switching *e2* to *e1* will increase MeCP2 protein level and improve symptoms in RTT models. For this, I used

iPSCs from a RTT patient carrying a MeCP2 missense mutation, G118E, which show ~30% lower MeCP2 and when differentiated into neurons, display electrophysiological abnormalities and dysregulated gene expression. I generated a CRISPR/Cas9-mediated deletion of *MECP2* exon 2 (E2KO) in RTT iPSCs and upon differentiation into neurons, found E2KO rescues MeCP2 protein levels and ameliorates the excitability and spontaneous activity deficits of the RTT neurons. RNA-sequencing revealed that E2KO normalizes expression of ~20% of dysregulated genes in RTT neurons. Preliminary studies using exon-skipping morpholino upregulated *e1* and reduced *e2* in RTT neurons. These data set the stage for a promising therapeutic strategy using antisense oligonucleotides to promote isoform switching in RTT patients with partially functioning alleles.

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Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.01/C18

Topic: A.07. Developmental Disorders

Support: British Columbia Children's Hospital Research Institute's Brain, Behaviour and Development Research Theme Boost Award

Title: Progranulin is an FMRP target that influences macroorchidism but not behaviour in a mouse model of Fragile X Syndrome

Authors: *B. LIFE[1], L. BETTIO[2], I. GANTOIS[3], B. R. CHRISTIE[4], B. R. LEAVITT[1]; [1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Xenon Pharmaceuticals, Vancouver, BC, Canada; [3]McGill Univ., Montreal, QC, Canada; [4]Univ. of Victoria, Victoria, BC, Canada

Abstract: Fragile X Syndrome (FXS) is the most prevalent monogenic cause of autism and intellectual disability worldwide. FXS is the product of loss of expression of the gene Fragile X Messenger Ribonucleoprotein 1 (*FMR1*). *FMR1* encodes FMRP, a multifunctional RNA-binding protein responsible for the post-transcriptional regulation of many genes. A better understanding of the role of each target gene in the pathophysiology of FXS will help to inform rational therapeutic development. To this end, we sought to probe the role of the gene progranulin in FXS. Progranulin is important for brain health maintenance and has been implicated in neurodevelopment, lysosomal function, and inflammation. Prior research suggests that increased progranulin expression in the prefrontal cortex is pathologically relevant in male *Fmr1* knockout (*Fmr1* KO) mice, a mouse model of FXS. However, several key knowledge gaps remain. The mechanism of increased progranulin expression in *Fmr1* KO mice is poorly understood and the extent of progranulin's involvement in FXS-like phenotypes in *Fmr1* KO mice has been

incompletely explored. Here, we report a thorough characterization of progranulin expression in *Fmr1* KO mice. We find that the phenomenon of increased progranulin expression is post-translational and tissue specific. We also demonstrate for the first time an association between progranulin mRNA and FMRP, suggesting that progranulin mRNA is an FMRP target. Subsequently, we show that progranulin over-expression in *Fmr1* wild-type mice causes reduced repetitive behaviour engagement in females and mild hyperactivity in males but is largely insufficient to recapitulate FXS-associated behavioural, morphological, and electrophysiological abnormalities. Lastly, we determine that genetic reduction of progranulin expression on an *Fmr1* KO background reduces macroorchidism but does not alter other FXS-associated behaviours or biochemical phenotypes. We conclude that progranulin is an FMRP target with modest effects on FXS pathophysiology.

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Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.02/C19

Topic: A.07. Developmental Disorders

Title: Ciliopathy and altered extracellular vesicles secretion in meningeal fibroblast of$Fmr1^{-/y}$ mice

Authors: *J.-Y. HEO[1], B. HA[2], Y.-J. JANG[2], S.-J. JEONG[3];
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Abstract: The meninges are three layer-tissue membrane consisting of the pia matter, arachnoid matter, and dura matter. There are two major functions such as a supportive framework for the cerebral cortex and a protecting brain against mechanical damage. The meningeal cells produce basement membrane (BM) proteins and extracellular matrix components to form the glial limitans and they control the migration of neurons during early brain development. The meninges are often involved cerebral pathology, but their function has not been explored during cortical development yet. We found the primary cilia on the meninges of the mouse brain as well as in primary meningeal fibroblasts (MFs). The presence of ciliopathy was noted in the meninges and meningeal fibroblasts (MFs) of *Fmr1*$^{-/y}$ mice, previously reported to exhibit ciliopathy in hippocampal neurons. The aberrant formation of cilia in these cells is associated with a disruption in cell integrity and the secretion of extracellular vesicles (EVs), which are known to share a cellular origin with cilia. These results suggest that primary cilia on MFs are essential to secrete EVs and that the phenotypic ciliary deficit is related to the meningeal cell integrity causing abnormal meningeal formation during the cortical formation of *Fmr1*$^{-/y}$ mice.

Disclosures: **J. Heo:** None. **B. Ha:** None. **Y. Jang:** None. **S. Jeong:** None.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

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Program #/Poster #: PSTR520.03/C20

Topic: A.07. Developmental Disorders

Support: NIH Grant - T32MH073124
NIH Grant - R01MH094681-10

Title: Parvalbumin+ GABAergic interneurons are reduced in number in specific subregions of the human Fragile X amygdala and hippocampus

Authors: *B. D. DUFOUR[1], G. CRARY[2], T. BARTLEY[2], E. MCBRIDE[2], A. DEL CID[2], V. MARTINEZ-CERDENO[3];
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Abstract: Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by a CGG repeat expansion (200+ repeats) in the FMR1 gene. It is the most prevalent monogenic cause of both intellectual disability and Autism. Neuroanatomical alterations of the human Fragile X Syndrome (FXS) brain are poorly characterized at the cell and tissue level. Our group has recently identified a reduction in Chandelier cells (ChCs), a type of parvalbumin+ (PV+) GABAergic interneuron, in the human Autistic prefrontal cortex. ChCs provide critical inhibitory control of excitatory glutamatergic neurons in cortical and subcortical structures. Here, we used postmortem brain tissue from 5 human FXS cases and 5 neurotypical controls to test the hypothesis that PV+ interneurons are reduced in the human FXS amygdala and hippocampus, two temporal lobe structures involved in learning/memory, emotional regulation, and social behavior - all behavioral/cognitive processes that are impaired in FXS. 50μm thick coronal serial sections from amygdala and hippocampus were stained for PV using immunohistochemistry; regional boundaries were identified and all PV+ interneurons within each subregion were counted in a series of matched sections. We found subregion specific alterations in PV+ interneurons populations in both the amygdala and the hippocampus, as well as abnormalities in regional size and shape. In the amygdala, PV+ interneurons are exclusively localized to lateral and basolateral subregions. In the lateral amygdala, FXS cases showed a 59.6% reduction in PV+ interneuron number ($p=.012$) and a 23.9% reduction in regional area ($p=.010$). PV+ cell counts and regional area were not altered for the basolateral amygdala ($p>.05$). Within the hippocampus, we found a large 80.5% reduction in PV+ interneuron number ($p=.004$) and a 64.5% reduction in fiber density ($p=.030$) in the granule cell layer of the dentate gyrus. We did not detect significant alterations in parvalbumin neuron number or regional area in other subregions of the dentate gyrus (molecular layer and hilus, $p>.05$) or in the cornu ammonis (CA1 and CA3, $p>.05$). This is the first study to demonstrate a reduction in the number of PV+ interneurons in the human FXS

brain. A loss of PV+ interneurons will invariably lead to disinhibition of excitatory glutamatergic neurons, and thus is a likely contributor to excitation/inhibition imbalance. These findings highlight how neuropathological alterations in the organization of the FXS brain, including deficits in specific brain circuits, could contribute to the clinical profile of FXS.

Disclosures: B.D. Dufour: None. **G. Crary:** None. **T. Bartley:** None. **E. McBride:** None. **A. Del Cid:** None. **V. Martinez-Cerdeno:** None.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.04/C21

Topic: A.07. Developmental Disorders

Title: Alterations in spontaneous and auditory evoked electrophysiological activities in a rat model of Fragile X syndrome

Authors: *C. ROUCARD, V. DUVEAU, A. EVRARD, B. MANDÉ-NIEDERGANG, Y. ROCHE;
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Abstract: Fragile X syndrome is a rare disease caused by a mutation of the Fragile X messenger Ribonucleoprotein 1 (FMR1) gene on the X chromosome. The incidence of this disorder is around 1 in every 3600 males and 1 in 4000-6000 females. This disorder is characterized by 1) a physical phenotype such as large and protruding ears, long face, and hypotonia, 2) autism and social anxiety, 3) intellectual disability with alteration in working memory and 4) psychiatric symptoms. The therapeutical strategy includes both medication (antidepressants and antipsychotics) and behavioral management (speech, behavioral and occupational therapies). Despite a relatively good efficacy, these therapeutical strategies need to be improved and new therapeutical approaches are nowadays developed such as gene therapy, to bring a greater efficacy for these patients. One way to improve the discovery and development of new therapeutical strategies is to use better humanized animal models of the disease and combine them with more translatable tools allowing a better translation between preclinical and clinical research. Among the different translatable tools, electroencephalography (EEG) appears to be the methodology of choice. In this work, we took advantage of quantitative electroencephalography (qEEG) and related methodologies (auditory evoked potentials) to characterize a humanized transgenic rat model of Fragile X. FMR1-KO rats (adults) and wild type (WT) were obtained from Envigo. Cortical EEG electrodes were implanted over the frontal and parietal cortex. Resting EEG was monitored over two consecutive EEG recordings (2 hours) one week apart and analysed. On separated recording sessions, 40Hz Auditory steady states responses were recorded. Evoked power and inter-trial coherence (ITC) were computed using proprietary Matlab algorithms. Quantitative EEG recording and analysis have shown that FMR1-KO rats displayed a decreased power in theta and beta frequency range as compared to WT rats. A significant

increase of power in epsilon frequency range has also been observed in FMR1-KO rats as compared to WT rats. The evoked power and ITC index were significantly reduced in FMR1-KO rats in comparison to their WT littermates. This deficit was consistent between the two independent recording performed. The sensitivity of EEG allows a dynamic characterization and differentiation of translational humanized models of brain disorders. The combination of translational preclinical models with EEG represents the next step for loop translating preclinical trials into clinical practice, opening the era of precision medicine for patients.

Disclosures: C. Roucard: None. **V. Duveau:** None. **A. Evrard:** None. **B. Mandé-Niedergang:** None. **Y. Roche:** None.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.05/C22

Topic: A.07. Developmental Disorders

Support: NIH NICHD

Title: Characterization of gut-brain-bone phenotypes in Fragile X Syndrome (FXS) mice

Authors: *S. ALAM[1], C. PRATER[2], B. FATHEPURE[3], E. LUCAS[4], P. JEYASINGH[2], E. A. MCCULLAGH[2];
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Abstract: Fragile X Syndrome(FXS) is the primary monogenetic cause of autism spectrum disorder (ASD). Ithas no established treatment, but therapeutic approaches can help mitigateFXS-related health complexities. Recent literature elucidated FXS patients alsosuffer from different gastrointestinal disturbances. Therefore, a bidirectionalgut-brain approach is needed to understand the complexities of interactions ofthe gut-brain axis in FXS. In this case, measuring ion abundance across thebrain and gut tissues will facilitate better understanding the underlyingionome traits for etiology, prognosis, diagnosis and ultimately establishingtherapies of FXS. We tested hypothesis that the ionomes in gut and braintissues will differ between sexes and genotypes in Fmr1 Knockout (KO) mousemodel. We measured elemental abundance status by using ICP-OES (InductivelyCoupled Plasma Optical Emission spectroscopy) technique. Our preliminary dataof the whole brain ionomes showed no significant differences between the micegenotypes whereas cecum and subdivided brain regions demonstrated significantdifferences in ion abundance between mice genotypes and sexes. Our initialstudy also showed significant increase in bone-mineral density and decreasedpercentage of fat in FXS female mice compared to wildtype mice. Future work isplanned to measure phylogenetic diversity of gut microbiota and gut barrierintegrity in FXS mice, along with exploring the link between microbialdiversity and bone composition in FXS.

Disclosures: **S. Alam:** None. **C. Prater:** None. **B. Fathepure:** None. **E. Lucas:** None. **P. Jeyasingh:** None. **E.A. McCullagh:** None.

Poster

PSTR520. Fragile X Syndrome

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.06/C23

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 EY025627

Title: Cellular distribution and dendritic organization of defined subtypes in the superior colliculus of fragile X mice

Authors: *A. FETISOVA, J. TRIPLETT, A. BROWN, P. THOTA;
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Abstract: A hallmark feature of fragile X syndrome (FXS) is altered sensory processing, including in the visual domain. Despite this, our understanding of the neural underpinnings of sensory deficits in FXS remains poor, precluding the development of effective therapies. To address this gap, we have focused on the superior colliculus (SC), a critical midbrain structure that receives visual, somatosensory, and auditory inputs to regulate head and eye movements. Previously, we uncovered novel disruptions in the organization of visual inputs to the SC, as well as altered visual function of neurons in the superficial SC of fragile X mice ($Fmr1^{-/y}$). However, the impacts of Fmr1 loss on neuronal organization within the SC remains unclear. To address this, we examined the cytoarchitecture of the SC using immunohistochemistry for molecular markers of distinct neuronal subtypes in the SC, including parvalbumin, calbindin, neurogranin, and reelin. We examined protein expression in male and female control mice ($Fmr1^{+/y}$ and $Fmr1^{+/+}$), as well as heterozygous females ($Fmr1^{+/-}$) and knockout males and females ($Fmr1^{-/y}$ and $Fmr1^{-/-}$) at postnatal day 21. Intriguingly, we observed differential distributions of both calbindin and neurogranin between control and $Fmr1^{-/y}$ mice, indicating disrupted lamination in the superficial SC. To further parse the organization of SC neurons, we examined the distribution and dendritic arborizations of genetically-defined populations. Previously, we found that $Tal1^{CreERT2}$ mice label a subset of neurons in the SC, comprising ~30% of the total population. Here, we characterize the morphological properties of $Tal1^{+}$ neurons, as well as the distributions of excitatory and inhibitory synapses onto them, in control and $Fmr1^{-/y}$ mice. Furthermore, we characterize these characteristics in a novel mouse line, $Hhip^{CreERT2}$, which our data suggests mark a subpopulation of excitatory neurons of stellate morphology. Taken together, these data suggest that Fmr1 is required for the establishment of neuronal subtype-specific distributions in the SC, which may partially underlie the functional deficits we observed previously.

Disclosures: **A. Fetisova:** None. **J. Triplett:** None. **A. Brown:** None. **P. Thota:** None.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.07/C24

Topic: A.07. Developmental Disorders

Support: NSF GRFP
UT Austin Continuing Graduate Student Fellowship
NIH Grant MH100510

Title: Higher hyperpolarization activated current (Ih) in a subpopulation of interneurons in stratum oriens of area ca1 in the hippocampus of fragile x mice.

Authors: *L. T. HEWITT[1], D. H. BRAGER[2];
[1]Inst. for Neurosci., The Univ. of Texas at Austin, Austin, TX; [2]Univ. Texas at Austin, Univ. Texas at Austin, Austin, TX

Abstract: Fragile X syndrome is the most common inherited form of intellectual disability and the leading monogenetic cause of autism. Studies in mouse models of autism spectrum disorders, including the Fmr1 knockout (FX) mouse, suggest that abnormal inhibition in hippocampal circuits contributes to behavioral phenotypes. We and others previously identified changes in multiple voltage-gated ion channels in hippocampal excitatory pyramidal neurons in FX mice. Whether the intrinsic properties of hippocampal inhibitory interneurons are altered in FX remains largely unknown. We made whole-cell current clamp recordings from three types of interneurons in stratum oriens of the hippocampus: fast-spiking (FS) cells and two classes of low threshold spiking cells, oriens-lacunosum moleculare (OLM) and low-threshold high Ih (LTH) neurons. We found that in FX mice, input resistance and action potential firing frequency were lower in LTH, but not FS or OLM, interneurons compared to wild type. Bath application of the h-channel blocker ZD7288 had a larger effect on input resistance in FX LTH cells and normalized input resistance between wild type and FX LTH cells, suggesting a greater contribution of Ih in FX LTH cells. In agreement, voltage clamp recordings showed that Ih was higher in FX LTH cells compared to wild type. Our results suggest that the intrinsic excitability of LTH inhibitory interneurons may contribute to altered inhibition in the hippocampus of FX mice.

Disclosures: L.T. Hewitt: None. **D.H. Brager:** None.

Poster

PSTR520. Fragile X Syndrome

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.08/C25

Topic: A.07. Developmental Disorders

Support: NIH Grant R15S088776

Title: Temporal Assessment of Early Communication in a Mouse Model of Fragile X Syndrome Reveals a Critical Period for Development of syllable complexity

Authors: *K. BLANDIN[1], S. V. CHILUKURI[5], J. PILCHER[2], J. N. LUGO, III[3], J. N. LUGO, Jr[4];
[1]Baylor Univ., WACO, TX; [3]Biol., [4]Psychology and Neurosci., [2]Baylor Univ., Waco, TX; [5]Baylor Col. of Med., Houston, TX

Abstract: Fragile X syndrome (FXS) is the most common monogenetic cause of autism spectrum disorder leading to alterations in social behavior and communication deficits. Alterations in social development are prevalent in those with FXS, with delayed speech and impaired language development being an early indicator of such social deficits. These patterns in FXS individuals in the early phases of life have led to experimental animal paradigms to understand the degree of social delay and variation within FXS. Early alterations in communication have been quantified in mice using ultrasonic vocalizations (USV), following acute isolation from the dam to induce a social communication response. To investigate the alterations in communication seen early in development, male C57BL/6J strain FMR1 knockout (KO) and wildtype mice were generated and identified by genotype for the recording of ultrasonic vocalizations at postnatal days 8, 9, 10 & 11. Each pup was tested at one time point and isolated once. At most, four pups were used from each litter for each time point, to minimize litter effects, testing days were 24 hours apart. Mice underwent recording for 2 minutes in a sound-attenuating chamber during maternal isolation to elicit communication. Repertoire analysis was done using MATLAB DeepSqueak using previously established call classification guidelines. No genotype effects were observed when assessing quantitative measures of communication. Male KO and WT mice did not differ in fundamental frequency, average peak frequency, average amplitude, total duration, average duration, or total calls emitted across all days measured. Qualitatively, we revealed that KO mice on PD 10 emit fewer multi-syllable calls ($p<.05$) and more singular syllable calls ($p<.05$) compared to WT mice. Repertoire analysis shows that also on PD10, KO mice produce fewer frequency step calls compared to WT mice ($p<.05$). Male Fmr1 KO mice do not differ significantly quantitatively when measuring duration, amplitude, frequency, and number of calls emitted, suggesting that within PD 8-11 there is no difference in measurable communication. Qualitative measures highlight that although KO mice are calling at a similar rate, their calls have fewer syllables, potentially identifying PD 10 as a critical day in development within the KO model and heightened sensitivity to insults. Though communication rates are consistent the quality of the communication is impacted at this age, potentially relating to impaired language development.

Disclosures: K. Blandin: None. **S.V. Chilukuri:** None. **J. Pilcher:** None. **J.N. Lugo:** None. **J.N. Lugo:** None.

Poster

PSTR520. Fragile X Syndrome

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Program #/Poster #: PSTR520.09/C26

Topic: A.07. Developmental Disorders

Support: BC Children's Hospital Research Institute Investigator Grant Award (IGAP)
Michael Smith Health Research BC Scholar Award
BC Children's Hospital Foundation

Title: Impact of loss of FMRP on oligodendrocyte development and function

Authors: *S. HOURANI[1,2], C. FERRARI BARDILE[1,2], O. K. OZGOREN[1,2], S. WITT[1,2], J. BEGIN[1,2], S. C. GJERVAN[1,2], J. FENG[1,2], K. H. UTAMI[3], M. A. POULADI[1,2];
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Abstract: Fragile X syndrome (FXS) is a neurodevelopmental disorder encompassing a wide range of clinical phenotypes including cognitive problems, impulsivity, epilepsy, attention deficit, and hyperactivity. It is the leading monogenic cause of intellectual disability and autism spectrum disorder. In most cases, FXS is caused by trinucleotide repeat expansion of the CGG motif in the 5' UTR region of the FMR1 gene, leading to hypermethylation and epigenetic silencing of its promoter and consequent loss of its product, FMRP. FMRP, an RNA-binding protein, plays a central role in gene expression by controlling the translation of potentially hundreds of mRNAs within the brain. While efforts to date have largely focused on the role of FMRP in neuronal development and synaptic plasticity, evidence from human and animal studies indicates that white matter (WM) structures are profoundly affected in FXS, thereby implicating oligodendrocytes in the pathophysiology of the disease. However, the role of FMRP in oligodendrocyte development and function, and therefore etiology of WM abnormalities in FXS remains poorly defined. The current study aims to delineate abnormalities in FXS oligodendrocytes and investigate the underlying mechanisms. To assess the impact of FMRP deficiency on oligodendrocyte development and maturation, we established oligodendrocyte monolayer cultures using an isogenic human pluripotent stem cell model of FXS that shows complete loss of FMRP expression. We have used stage-specific markers to evaluate the impact of FMRP loss on the efficiency and timing of neural and glial induction, OPC specification and oligodendrocyte differentiation and maturation throughout the differentiation process. In addition, changes in the morphology of mature oligodendrocytes in culture are assessed using measures of area, branching and root number of oligodendrocyte processes. Alongside the human oligodendrocyte monolayer studies, structural analyses are also being conducted on Fmr1-deficient mice modelling FXS. Measures of gross and microstructural changes in major WM tracts such as myelin sheath thickness and compaction are being quantified using electron microscopy and related imaging techniques. Finally, transcriptomic analyses will be conducted on both oligodendrocyte monolayer and WM structures in Fmr1-deficient mice to elucidate the molecular processes and pathways dysregulated in the absence of FMRP from oligodendrocytes. Overall, we anticipate that our analyses will shed light on the role of FMRP in oligodendroglia and the contribution of its loss in this cell lineage to the etiology of FXS.

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Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

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Topic: A.07. Developmental Disorders

Support: FAPESP Grant 2022/07948-9
FAPESP Grant 2019/10868-4
FAPESP Grant 2022/09710-0

Title: Distribution of Fmr1 mRNA with intron 14 between the nucleus and cytoplasm

Authors: N. DE ARAUJO, I. C. M. LIMA, L. L. SAMPAIO, *T. G. ALEGRIA, D. K. TAKEMOTO, A. M. LINARDI, ***L. A. HADDAD**;
Genet. and Evolutionary Biol., Univ. of São Paulo, São Paulo, Brazil

Abstract: Fragile X messenger ribonucleoprotein 1 (*FMR1*) gene is functionally important in neurogenesis and synapsis plasticity, with molecular roles in mRNA translation and transport regulation. *FMR1* loss of function may cause fragile X syndrome, the commonest form of inherited intellectual disability among men. In the rat nervous system, *FMR1* primary transcript can undergo alternative splicing of exons 12, 14 and 15, leading to distinct isoforms of the encoded protein, FMRP, with altered post-translation modification motifs and/or RNA-binding domains. We previously demonstrated in the rat forebrain that *Fmr1* exon 14 skipping significantly increases in the third embryonic week. Prior to birth, the total amount of *Fmr1* mRNA reduces and, upon birth, there is a rise of full-length *Fmr1* mRNA, notably expressing all exons. While searching for *FMR1* transcript variability, we computationally identified the retention of intron 14 in rodent, crab-eating monkey and human *FMR1* mRNA retrieved from poly-adenylated transcriptome sequencing (RNA-Seq) data. Here we aimed to understand the subcellular distribution of *FMR1* mRNA retaining intron 14 and its relationship to FMRP levels. Production of *FMR1* mRNAs with intron 14 or lacking exon 14 relies on mutually exclusive usage of introns 13 or 14 3’ splice sites, respectively. When exon 14 expression significantly reduces in E17-E20 rat forebrain, the resulting messages appear prone to decay. Meanwhile, *Fmr1* mRNA with intron 14 is relatively stable when compared to 15 other *FMR1* introns. Intron 14 retention in *FMR1* mRNA is conserved in cell lines. We show in HEK293T cells that intron 14-containing *FMR1* mRNA is enriched in the nucleus. Our *in silico* studies reveal the potential of *FMR1* intron 14 3’ end to assemble a G-quartet structure, which is the major RNA-binding motif previously characterized for FMRP. It has been shown that nuclear intron-retained mRNA can further undergo post-transcriptional splicing, and the resulting mature mRNA then be exported to the cytoplasm. We have thus started testing if FMRP regulates the steady-state of

nuclear-retained non-spliced *FMR1* mRNA, working on a model of FMRP auto-regulation of *FMR1* mRNA intron 14 retention. Additional studies should test the functional effect of G-quartets on intron 14 retention and if *Fmr1* late splicing should regulate neurodevelopment.
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Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.11/C28

Topic: A.07. Developmental Disorders

Title: Altered microtubule dynamics in Fragile X Syndrome: GSK-3 inhibition as a potential therapeutic target.

Authors: *J. KEALY[1], A. FREEBURN[1], F. TRAINI[1], A. THORNTON[2], M. NEUMANN[2], R. MCKENNA[2], B. GARRONE[3], C. MILANESE[3], C. GREENE[1], C. CALLAGHAN[1], M. BIANCHI[1];
[1]Ulysses Neurosci. Ltd., Dublin, Ireland; [2]Trinity Col. Dublin, Dublin, Ireland; [3]Angelini Pharmaceuticals SPa, Rome, Italy

Abstract: Fragile X Syndrome (FXS) is an X-linked neurodevelopmental disorder resulting from a mutation in the *FMR1* gene which codes for Fragile X Messenger Ribonucleoprotein 1, which acts as a shuttle for mRNA during development and in synaptic plasticity. FMRP regulates numerous downstream targets, including glycogen synthase kinase 3 (GSK-3) which is elevated in *Fmr1* knock-out (KO) mice and the *Fmr1* KO phenotype has been shown to respond to GSK-3 inhibition. As GSK-3beta is a regulator of microtubule dynamics, we investigated whether changes in alpha-tubulin post-translational modifications (PTMs) were altered in patients with FXS and in *Fmr1* KO mice. We also determined whether GSK-3beta inhibition had beneficial effects on behaviour and alpha-tubulin PTM in *Fmr1* KO mice. In patients with FXS (n=24), there was a significant decrease in plasma levels of tyrosinated/detyrosinated alpha-tubulin (Tyr/Glu-Tub) when compared to matched controls - specifically in adults (18-46 years old; n=11) but not children (4-12 years old; n=13). There were no changes in plasma levels of acetylated/total alpha-tubulin (Acet/Tot-Tub). In *Fmr1* KO mice, hippocampal levels of Acet/Tot-Tub were significantly increased and Tyr/Glu-Tub were significantly decreased compared to wild type controls. Acet/Tot-Tub also showed a sex difference with females having lower levels of Acet/Tot-Tub compared to males regardless of genotype. Conversely, female mice overall had significantly higher Tyr/Glu-Tub ratios, with *Fmr1* KO mice of both sexes showing significantly lower Tyr/Glu-Tub ratios compared to matched controls. Both male and female *Fmr1* KO mice had significantly lower levels of spinophilin compared to matched

controls, whereas heterozygous female mice were indistinguishable from control females. The GSK-3beta inhibitor SB216763 significantly reduced excessive marble burying and significantly reversed social recognition in *Fmr1* KO mice compared to wild type controls. GSK-3 inhibition had no effect on Acet/Tot-Tub or Tyr/Glu-Tub levels in Fmr1 KO mice. Based on this data, microtubule dynamics appear to be dysregulated in FXS with a tendency towards a less dynamic state (high Acet/Tot-Tub and low Tyr/Glu-Tub). As these changes in alpha-tubulin PTMs are conserved in humans with FXS and in *Fmr1* KO mice, they represent a potential translational biomarker of the syndrome. Though GSK-3beta inhibition showed significant improvements in behaviour in *Fmr1* KO mice, further work on microtubule dynamics in FXS is required to understand their role in the disorder.

Disclosures: **J. Kealy:** A. Employment/Salary (full or part-time):; Ulysses Neuroscience Ltd. **A. Freeburn:** A. Employment/Salary (full or part-time):; Ulysses Neuroscience Ltd. **F. Traini:** A. Employment/Salary (full or part-time):; Ulysses Neuroscience Ltd.. **A. Thornton:** None. **M. Neumann:** None. **R. McKenna:** None. **B. Garrone:** A. Employment/Salary (full or part-time):; Angelini Pharmaceuticals SPa. **C. Milanese:** A. Employment/Salary (full or part-time):; Angelini Pharmaceuticals SPa. **C. Greene:** A. Employment/Salary (full or part-time):; Ulysses Neuroscience Ltd. **C. Callaghan:** A. Employment/Salary (full or part-time):; Ulysses Neuroscience Ltd. **M. Bianchi:** A. Employment/Salary (full or part-time):; Ulysses Neuroscience Ltd..

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.12/C29

Topic: A.07. Developmental Disorders

Title: Social preference learning and cFos expression in young & adult Fragile X mice

Authors: *A. HUSSAIN[1], K. A. RAZAK[2];
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Abstract: BackgroundFragile X Syndrome (FXS) is a leading known genetic cause of autism spectrum disorder, caused by a gene mutation that disrupts the production of fragile X messenger ribonucleoprotein (FMRP). Disruption of FMRP can lead to debilitating symptoms in the cognitive, social, and communication domains. The *Fmr1* knockout (KO) mouse model is a well-studied animal model of FXS to characterize pathophysiological mechanisms. Young mice (~P30) show learned preference for social environment (vs isolation), but this social conditioned place preference (sCPP) learning decreases into adulthood (>P60). In this study, the hypothesis is that the developmental plasticity of sCPP is abnormal in KO mice. MethodsWe utilized the sCPP task that uses a 3-chambered apparatus to measure social and isolation-avoidant behaviors. Wildtype (WT) and KO mice (FVB strain) were tested at two ages, p30 – p40 (young) and p60 – p70 (old). This task uses a 4-day protocol with a 30 min pretest on the first day and a posttest on

the last day where the test mouse has access to the entire chamber with two different bedding types in two of the chambers, and with a neutral middle chamber. Test mouse is conditioned to a social setting in one chamber, with two littermates, and one of the bedding types for 24 hr, followed by a 24 hr isolation conditioning in the opposite chamber with the other bedding. After the posttest, the mouse was left in the apparatus for another 60 mins to induce cFos activation. Test mouse brains were collected to measure cFos density in several brain areas connected to social and avoidant behaviors.
ResultsSplit 15 min analysis show sex differences were seen with old WT females showing almost no social preference throughout the whole task, while the old KO females showed social preference mainly in the last half of the test. Lastly, old KO males showed almost no avoidance of the isolation chamber in the last half of the test.
A negative correlation was observed between cFos density and social preference index as well as avoidance index in old WT and KO in the NAcc and VMH. This suggests that adult KO mice may have different underlying mechanisms, allowing them to reach the same behavioral outcome as WT.
ConclusionsThe results partially support our hypothesis with only older female KO's exhibiting an abnormal social preference. Different relationships were seen between cFos density and social preference in older WT and KO mice. This is a novel finding in several aspects, FMRP deficit does not initially affect social preference learning. However, there is an altered developmental experience that results in differences from WT mice in adulthood.

Disclosures: A. Hussain: None. **K.A. Razak:** None.

Poster

PSTR520. Fragile X Syndrome

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Program #/Poster #: PSTR520.13/C30

Topic: A.07. Developmental Disorders

Support: NIH Grant R01EY023037
NIH Grant R21NS123499

Title: Identifying and correcting deficits in primary visual cortex activity in Fragile X syndrome

Authors: *S. K. SIMPSON[1], M. J. HEINRICH[1], F. A. CHALONER[2], M. Y. LEE[1], P. S. B. FINNIE[3], S. F. COOKE[2], M. F. BEAR[1];
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Abstract: Fragile X syndrome (FXS) is the most common known inherited cause of intellectual disability and autism spectrum disorder (ASD). Therapeutics for the many devastating symptoms of FXS, including deficits in sensory processing, remain elusive. A mouse model of FXS has

been well used to characterize circuit-level electrophysiological phenotypes of the disorder in auditory and somatosensory cortex, but the visual cortex has been less studied. Using the mouse model of FXS, we sought to discover and assess the feasibility of correcting deficits in primary visual cortex (V1) activity by recording local field potentials (LFPs). Our aims were two-fold: (1) to compare phenotypes across sensory modalities and (2) identify a potential translatable biomarker of pathophysiology and treatment response. Awake, head-fixed *Fmr1*$^{-/y}$ mice and control littermates were presented with a variety of visual stimulation conditions, ranging from total darkness to a static, illuminated gray screen to phase reversing gratings, all while recording the LFP in V1. Across all conditions, *Fmr1*$^{-/y}$ mice showed an elevation in power in the gamma frequency range (30-85 Hz) relative to control littermates, consistent with findings from other sensory modalities. The *Fmr1*$^{-/y}$ mice also showed a reduction in power in the theta frequency band (4-10 Hz) in darkness that was enhanced during gray screen and grating presentation, which elicited much stronger narrow-band theta oscillations in control than *Fmr1*$^{-/y}$ mice. Changes in the ratio of theta and gamma power were present in almost every *Fmr1*$^{-/y}$ mouse, suggesting it could be a useful biomarker. To further assess differences in visual responsiveness, visually-evoked potentials (VEPs) were extracted from the LFP following phase reversals of oriented grating presentations, which ramped through different temporal frequencies ranging from 2-15 Hz. Across all temporal frequencies, there was a deficit in response to the onset of visual stimulation in *Fmr1*$^{-/y}$ mice. Following stimulus onset, for frequencies ranging from 4-10 Hz, there was a substantial reduction in the evoked response of *Fmr1*$^{-/y}$ mice. To explore the usefulness of these measures as a potential biomarker, we examined the effects of R-Baclofen, an agonist of $GABA_B$ receptors which is under investigation as a potential therapeutic for FXS. We found that low doses of R-Baclofen shifted sustained and evoked visual activity observed in V1 of *Fmr1*$^{-/y}$ mice closer to wild-type values.

Disclosures: S.K. Simpson: None. **M.J. Heinrich:** None. **F.A. Chaloner:** None. **M.Y. Lee:** None. **P.S.B. Finnie:** None. **S.F. Cooke:** None. **M.F. Bear:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Allos Pharma.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.14/Web Only

Topic: A.07. Developmental Disorders

Title: Astrocytic specific deletion of FMR1 effects on Pyramidal neurons in CA1 Hippocampus, a study of Fragile X Syndrome.

Authors: *S. MAPLES[1], V. WAGNER[2], A. KULINICH[3], I. M. ETHELL[4], P. W. HICKMOTT[5];
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Abstract: Utilizing aspecific FMR1 deletion within CA1 Hippocampus astrocytes has allowed us toexamine the interactions between astrocytes and Pyramidal neuron inhibitory toexcitatory balance. Specifically, tonic GABA currents were investigated usingwhole-cell recordings and various pharmacological agents to explore changes ininhibition onto Pyramidal neurons. Mice aged p21 to p28 were used to mitigateinhibition attributable to interneurons and to reflect developmental specificdeficits. Both male and female mice were used, resulting in a full knockoutcondition (males) and a partial knockout condition (females), compared to maleand female wildtypes. The data was collected in a double-blind study, withidentification made post-hoc through genotyping. The data presented willshowcase a comparison of Hippocampus astrocytic-specific FMR1 deletion, partialdeletion, and gender effects. Based on prior research, it is predicted that thecomplete knock out condition will have the greatest reduction in tonic GABAcurrents resulting in higher baseline excitation of the Pyramidal neurons. Thepartial knock out will have reduced tonic GABA currents but not as severe asthe knockout condition, yet significantly more so than the wild type. However,the astrocyte-specific condition has shown to have two populations with groupshaving greater reduced tonic inhibition than the global knockout condition.There were no significant effects based on age or gender of mice.

Disclosures: S. Maples: None. **V. Wagner:** None. **A. Kulinich:** None. **I.M. Ethell:** None. **P.W. Hickmott:** None.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.15/C31

Topic: A.07. Developmental Disorders

Support: NIH U01NS096767

Title: Developmental Trajectories of Resting State EEG Brain Activity in Children with Fragile X Syndrome

Authors: *E. AUGER[1], E. BERRY-KRAVIS[2], L. ETHRIDGE[3];
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Abstract: Fragile X Syndrome (FXS) is an X-linked monogenic disorder associated with anxiety and executive function deficits. FXS is the most common single gene cause of autism spectrum disorder and intellectual disability. FXS is characterized by altered brain activity, including decreased resting-state alpha power and increased gamma power compared to typically developing controls, particularly in adolescents and adults. However, the developmental trajectories of these neural dynamics in FXS remain understudied. This study examined the effects of a metabotropic glutamate receptor antagonist and language learning intervention in 22 children (ages 36 months to 7 years) with FXS participating in a phase 3 clinical trial. The trial

encompassed up to 20 months with a potential for 9 electroencephalography (EEG) data collection visits, each with up to 3 minutes of resting EEG. No significant drug effects were observed in resting power at the end of the placebo-controlled period or the end of open-label treatment. Consequently, the focus of the study was shifted toward examining the developmental trajectories of participants. The relationship between whole-head resting-state relative power spectral density, visit, and age was analyzed for each of five frequency bands (delta, theta, alpha, beta, gamma). Additionally, the correlation between baseline power and age was assessed for each frequency band. Results indicated a significant interaction between time and age for alpha power, whereby older children exhibited a greater increase in alpha power over the course of the study ($F(1,20) = 6.62$, p < 0.05)and younger children remained stable. Additionally, there was a trend toward increased alpha power with age cross-sectionally at baseline ($F(1,20) = 4.17$, $p=0.06$). Baseline theta power was negatively correlated with age ($r = -0.36$, p<0.05), a finding consistent with typical development. In contrast, baseline gamma power showed a positive correlation with age ($r = 0.43$, p<0.05), contrary to the pattern observed in typical development. Together these findings may indicate differences in critical developmental windows for shifts in alpha (older children) and theta and gamma power (linearly beginning in young childhood) for children with FXS. These findings shed light on the developmental trajectories of resting-state power in children with FXS, with evidence for differing windows for developmental shifts in neural oscillatory dynamics that may suggest specific time ranges for ideal measurement of target engagement depending on molecular target and most strongly associated neural oscillatory behavior.

Disclosures: E. Auger: None. **E. Berry-Kravis:** 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.; Acadia, Biogen, BioMarin, Erydel, GeneTx/Ultragenyx, Ionis, Jaguar, Kisbee, Neuren, Neurogene, Orphazyme/Kempharm/Zevra, PTC Therapeutics, Roche, Taysha, Tetra/Shionogi, Yamo, Zynerba, Mallinckrodt Pharmaceuticals. **L. Ethridge:** 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.; Healx, Autifony, Tetra Therapeutics, Ultragenyx, Ovid.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.16/C32

Topic: A.07. Developmental Disorders

Support: NIH Grant 1U54 HD082008
USAMRDC Grant W81XWH-15-1-0436
USAMRDC Grant W81XWH-15-1-0434

NIH Grant 1F31NS117178-01
CIRM Award EDUC4-12752

Title: Astrocytes dysregulate GABA metabolism in Fragile X Syndrome.

Authors: *V. WAGNER[1], A. VARALLO[1], A. KULINICH[2], S. SUTLEY[4], M. RAIS[3], W. WOODWARD[1], X. SHUAI[3], J. KOKASH[5], T. P. PIEPPONEN[7], M. CASTRÉN[8], K. A. RAZAK[6], I. M. ETHELL[3];
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Abstract: Fragile X syndrome (FXS) is a leading genetic cause of autism-like symptoms, including sensory hypersensitivity and cortical hyperexcitability, resulting from epigenetic silencing of the Fragile X messenger ribonucleoprotein (*Fmr1*) gene. Recent observations in humans and *Fmr1* knockout (KO) animal models of FXS suggest symptoms are mediated by abnormal GABAergic signaling. As most studies have focused on neuronal mechanisms, the role of astrocytes in mediating defective inhibition in FXS is largely unknown. We found that human FXS astrocytes derived from patient-specific induced pluripotent stem cells (iPSCs) show ~7-fold increase in GABA levels compared to their control counterparts using high-performance liquid chromatography (HPLC). Similar to FXS human astrocytes *Fmr1* KO mouse astrocytes showed increased levels of GABA, potentially due to an up-regulation of GABA-synthesizing enzymes GAD65/67 assessed with western blotting and immunostaining. Finally, we found that astrocyte-specific *Fmr1* deletion during P14-P28 period affects connections between cortical excitatory neurons and PV cells, leading to long-term changes in cortical responses with impaired sound-evoked synchronization to gamma frequencies, but enhanced background gamma power and reduced habituation to sound using EEG recordings. We also performed behavioral tests to assess the effects of astrocyte-specific *Fmr1* deletion on mouse behaviors such as anxiety, hyperactivity and social novelty. Our findings suggest astrocytic FMRP has a key role in the development and function of inhibitory circuits and astrocytes are a valuable target for therapies to relieve FXS-associated phenotypes.

Disclosures: V. Wagner: None. **A. Varallo:** None. **A. Kulinich:** None. **S. Sutley:** None. **M. Rais:** None. **W. Woodward:** None. **X. Shuai:** None. **J. Kokash:** None. **T.P. Piepponen:** None. **M. Castrén:** None. **K.A. Razak:** None. **I.M. Ethell:** None.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.17/C33

Topic: A.07. Developmental Disorders

Support: CIHR
FRQS
QART

Title: Altered input/output properties of layer 5 pyramidal neurons in vivo in a mouse model of Fragile X syndrome

Authors: *D. MITCHELL[1], J. ZAKO[2], R. ARAYA[3];
[1]Univ. de Montréal, Montreal, QC, Canada; [2]Univ. de Montréal, Montréal, QC, Canada; [3]Univ. of Montreal, Montreal, QC, Canada

Abstract: Interacting with our environment requires our brain to combine sensory information with our internal representation of the world. Cortical layer 5 (L5) pyramidal neurons are believed to play an important role in this process since these neurons receive predictive and sensory inputs in a compartmentalized manner at their distal apical tufts and basal dendrites, respectively. The dendrites of L5 pyramidal neurons span all cortical layers, making them the main integrator of the cortical column projecting to other cortical and subcortical areas. Changes to the synaptic integration of excitatory inputs onto cortical neurons likely contributes to the behavioral phenotype associated with neurodevelopmental disorders such as Fragile X syndrome (FXS). FXS is the most frequent form of inherited intellectual disability and most common known cause of autism. We have recently shown that while subthreshold excitatory inputs integrate linearly in the basal dendrites of L5 pyramidal neurons of control animals, surprisingly those of FXS summate sublinearly, contradicting what would be expected of sensory hypersensitivity classically associated with autism spectrum disorders (Mitchell, Miranda-Rottmann et al., PNAS 2023). Here we aimed to test how this altered synaptic integration impacts the input/output properties of L5 pyramidal neurons in a mouse model of FXS, *Fmr1*-KO mice, *in vivo* during sensory stimulation. We utilized the rodent whisker sensorimotor system since it benefits from a well-characterized anatomical organization and stimuli that is easily manipulated. Thus, in one set of experiments, we imaged calcium levels in the distal dendrites of L5 pyramidal neurons from primary somatosensory cortex, while in another, we recorded the extracellular activity of these neurons, both during whisker puff stimulation. In both sets of experiments, we found that *Fmr1*-KO L5 pyramidal neurons exhibited less stimuli-driven responses and more non-stimuli-driven activity resulting in a lower signal-to-noise ratio. These results support our hypothesis that FXS is characterized by more than just a global cortical hypersensitivity, but rather a hyposensitivity of sensory inputs and hypersensitivity of predictive inputs onto cortical neurons. This work is funded by the CIHR, as well as FRQS and QART postdoctoral fellowships to DEM.

Disclosures: D. Mitchell: None. **J. Zako:** None. **R. Araya:** None.

Poster

PSTR520. Fragile X Syndrome

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR520.18/C34

Topic: A.07. Developmental Disorders

Support: US Army Medical Research grant W81XWH-15-1-0436

Title: Effects of sound repetition rate on auditory cortex development and behavior in young Fmr KO mice

Authors: *A. KULINICH, N. FAROOQ, K. CROOM, K. A. RAZAK, I. M. ETHELL; Univ. of California, Riverside, Riverside, CA

Abstract: Fragile X syndrome (FXS) is a leading genetic form of autism and intellectual disability. It is associated with a loss-of-function mutation in the Fragile X messenger ribonucleoprotein 1 (*Fmr1)* gene. The *Fmr1* knockout (KO) mouse model displays many aspects of FXS-related phenotypes and has been used as a major model to study FXS. Our previous studies have shown beneficial effects of developmental sound exposure, but not attenuation, on molecular, cellular, and functional properties in the auditory cortex (AuC) of *Fmr1* KO mice. However, it is unclear what specific sound properties had beneficial effects. Here, we studied the effects of sound trains with two different repetition rates on mouse auditory cortex development and FXS-associated behaviors in a mouse model of FXS. In this study, *Fmr1* KO and WT littermates were exposed to a 14 kHz tone with 1Hz or 5Hz repetition rate during postnatal (P)9-P21 developmental period. WT and *Fmr1* KO mice raised in a regular vivarium were used as control groups. Similar to our previous work, we found that PV cell density was lower in AuC of *Fmr* KO mice compared to WT, but was increased in layer (L)4 AuC of Fmr KO mice following the exposure to sounds with 1Hz and 5Hz repetition rates. However, PV protein level was higher in AuC of Fmr KO mice exposed to tone with 5Hz but not 1Hz repetition rates. Next, we analyzed baseline cortical activity using electroencephalography (EEG) recordings. Interestingly, enhanced resting state gamma power (both, low and high gamma) observed in the AuC of *Fmr1* KO mice was downregulated in mice exposed to sound trains with 5Hz but not 1Hz repetition rates. Analysis of event-related potentials (ERP) in response to broadband sound showed that increased ongoing responses and decreased habituation to sound were also corrected in AuC and frontal cortex (FC) of Fmr1 KO mice exposed to sound trains with 5Hz but not 1Hz repetition rates. Finally, behavior testing showed improvement of anxiety-like behaviors in Fmr1 KO mice exposed to sound trains with 5Hz but not 1Hz repetition rates, and Fmr1 KO mice spend the same amount of time in thigmotaxis as control WT mice and significantly less than control Fmr1 KO mice. Summarizing, our results show that developmental exposure of mice to sound trains with 5Hz but not 1Hz repetition rate had beneficial effects on PV cell development, functional cortical responses and behaviors in *Fmr1* KO mice. These findings have a significant impact on developing new approaches to alleviate FXS phenotypes and open new possibilities for combination of sound exposure with drug treatment which may offer a new therapeutic approach with a potential for long-lasting effects and reduced tolerance to drugs.

Disclosures: A. Kulinich: None. **N. Farooq:** None. **K. Croom:** None. **K.A. Razak:** None. **I.M. Ethell:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.01/C35

Topic: A.07. Developmental Disorders

Support: FONDECYT Postdoctorado 3220492
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Title: Tcf4 dysfunction differentially alters cortical neurogenesis in pitt-hopkins syndrome according to genotype status and sex

Authors: *F. ESPINOZA ROMERO[1], R. CARRAZANA[2], D. ÁVILA[3], E. RETAMAL[2], E. AEDO[1], C. ESCUDERO[4], A. ÁVILA[2];
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Abstract: Pitt-Hopkins Syndrome (PTHS) is a rare disease with an incidence close to 1:34.000. Patients might present cognitive delays, intellectual disability, lack of speech, and autistic behaviors. PTHS is caused by haploinsufficiency in the TCF4 transcription factor, different mutations in Tcf4 gen have been described affecting the DNA binding site. During embryonic development, TCF4 is highly expressed in the human and mice cerebral cortex. Moreover, a lack of TCF4 expression caused changes in cerebral cortex thickness, abnormalities in cortical lamination, and neuronal migration. In this study, we analyze cerebral cortex alterations during PTHS embryonic and postnatal development considering HET and KO genotypes, also sex differences.PTHS animals were analyzed at embryonic day 12 (E12), E15, and postnatal day 0 (P0). We characterize changes in apical and basal progenitors, upper layer (UL) neurons, and deep layer (DL) neurons populations using immunohistochemistry against TBR1, TBR2, PAX6, PHH6, SATB2, and CTIP2. In utero electroporation (IUE) was performed to induce TCF4 expression in the cerebral cortex of animals.
At the early stages of development (E12) we found an increase in TBR1/TBR2 neuronal-progenitor transition in Tcf4Het and Tcf4Ko animals, with a significant increase in PAX6+ apical progenitor population. This defect was related to a decrease in SATB2+ UL neurons and an increase in CTIP2+ DL neurons at P0. Interestingly, the alterations were more significant in the male than in the female genotype. We described alterations in cortical neurogenesis of PTHS animals, these changes were different between sex and genotypes. TCF4 embryonic reinsertion recovers alterations in UL and DL neurons.

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Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.02/C36

Topic: A.07. Developmental Disorders

Support: LouLou Foundation Grant

Title: Acetylated α-tubulin is a potential translational biomarker of disease progression in CDKL5 Deficiency Disorder (CDD)

Authors: A. M. THORNTON[1], C. CONNOLLY[2], ***C. DE PASQUALE**[3], A. FREEBURN[5], I. BARBIERO[6], D. DISHA[4], C. MCGURK[4], C. KILSTRUP-NIELSEN[7], M. TREVISANO[8], A. CONNOLLY[9], J. TELLO[4], C. K. CALLAGHAN[4], J. KEALY[4], M. BIANCHI[3];
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Abstract: CDKL5 Deficiency Disorder (CDD) it is an ultra-rare and highly debilitating neurodevelopmental disorder that affects 1/40-60,000 newborns, predominantly affecting females. CDD results from a mutation in the *CDKL5* gene on the X-chromosome and is associated with early-onset seizures and severe global developmental delay. CDKL5 protein interacts with microtubule associated proteins (MAPs) and CDKL5 is believed to directly influence microtubule dynamics by reducing dynamics. α-tubulin post-translational modifications (PTMs) drive microtubule dynamics which are in turn required for synaptic plasticity in neurons. Previously, we have shown in a *Cdkl5* KO mouse model that α-tubulin PTMs can be detected in plasma and that plasma levels of α-tubulin PTMs are reflective of changes seen in the brain with *Cdkl5* KO mice showing an α-tubulin PTM consistent with reduced microtubule dynamics. This suggests plasma levels of α-tubulin PTMs may be useful as a peripheral biomarker for CDD. Therefore, plasma was collected from CDD patients in Ireland, Italy and the USA (n=18; ages 6-27) along with control volunteers (n=16; ages 6-49) and analysed for α-tubulin PTMs along with other peripheral biomarkers (BDNF; NfL; and inflammatory cytokines). We found that Acet/Tot-Tub is overexpressed in the plasma of CDD patients, but there were no changes in Tyr/Glu-Tub, consistent with our *Cdkl5* KO mouse data. There were also significant reductions in BDNF and significant increases in NfL, indicating reduced neuronal health overall. Following a multiplex analysis of cytokines, we found a significant increase in the anti-inflammatory cytokine IL-10, potentially reflecting cytokine dysregulation in CDD. Based on this clinical study along with our previous preclinical data, plasma Acet/Tot-Tub represents a potential biomarker of disease progression and for drug development in CDD, due to its high potential as a translational biomarker. Its power is further increased by utilising a composite panel of biomarkers including those reflective of neuronal health and inflammatory status. We are further exploring this with a larger biomarker study in Italy, involving the Istituto Neurologico Carlo Besta and the Ospedale Borgo Trento in Verona, which has already begun recruitment. This broader investigation will provide a more

comprehensive understanding of the biomarker profile in CDD patients and contribute to the development of targeted therapies and drug interventions and we predict that results will confirm the data obtained in the presented study.

Disclosures: **A.M. Thornton:** None. **C. Connolly:** None. **C. De Pasquale:** None. **A. Freeburn:** None. **I. Barbiero:** None. **D. Disha:** None. **C. McGurk:** None. **C. Kilstrup-Nielsen:** None. **M. Trevisano:** None. **A. Connolly:** None. **J. Tello:** None. **C.K. Callaghan:** None. **J. Kealy:** None. **M. Bianchi:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.03/C37

Topic: A.07. Developmental Disorders

Title: Characterizing mICD mouse model of Angelman syndrome imprinting defects

Authors: *R. NARAYANAN[1], C. MILAZZO[2], S. BADILLO[3], E. VAN DER TOORN[2], Y. ELGERSMA[2], S. CHAMBERLAIN[4], T. KREMER[4];
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Abstract: Angelman Syndrome (AS) is a severe debilitating neurodevelopmental disorder with an estimated incidence of 1 in 20,000. Individuals with AS show strong deficits of fine and gross motor skills, absence of speech, intellectual disability and abnormal behavior. Additionally, 80% of the patients have epilepsy and problems with sleep. Currently, only symptomatic treatment is available, which is predominantly aimed at reducing seizures and improving sleep. AS is caused by the absence of functional maternally derived UBE3A protein. This is due to either (i) deletion in the 15q11-q13 region [DEL, >75% of the AS patients], (ii) imprinting defects affecting the AS-imprinting center (ICD), (iii) paternal uniparental disomy of chromosome 15 (UPD), and (iv) mutations specifically affecting the UBE3A gene. Current mouse models used in AS research are UBE3A-centric and do not address the expression changes of other genes in the 15q.11-13 locus on the pathophysiology of AS. This limits the potential to dissect differences in therapeutic responses for current UBE3A-targeting strategies and hampers identification of novel therapeutics/co-therapeutics. Here we studied a mouse line that harbors a mutation affecting the AS-PWS imprinting center (Lewis et al., 2019), hence modeling the mICD and UPD AS mutation. The mICD mice displayed robust deficits as previously reported for *Ube3a* mice (Jiang et al 1998; Sonzogni et al., 2019) such as increased body weight, reduced brain weight, impaired rotarod performance, reduced marble burying, nest building and increased immobility, hindlimb clasping in the tail suspension test. These behavioral abnormalities were accompanied by a loss of UBE3A protein in the cortex and bi-allelic expression of *Ube3a-ATS*, *Mkrn3-Snord115* gene cluster. In addition, proteomic analyses revealed larger changes in the mICD cortex when

compared to that of *Ube3a* mice, with significant alteration of the proteasome. Subsequently, the expression of UBE3A was reinstated in neonatal mICD mice by applying antisense oligonucleotides (ASOs) targeting *Ube3a-ATS*, which resulted in improvement in some of the behavioral phenotypes in adulthood. However, the behavioral domains improved were different when compared to a similar study done in *Ube3a* mice (Milazzo et al., 2021). Taken together, this study highlights potential AS subtype-specific differences at the molecular and behavioral level. Such a comprehensive analysis of mouse models covering all AS subtypes will enhance the success rate of translating pre-clinical findings to the clinic by assessing differences in dosing, efficacy and guiding non-Ube3a-targeting therapeutic strategies.

Disclosures: **R. Narayanan:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **C. Milazzo:** None. **S. Badillo:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **E. van der Toorn:** None. **Y. Elgersma:** None. **S. Chamberlain:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **T. Kremer:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.04/C38

Topic: A.07. Developmental Disorders

Support: R00NS112604

Title: In vitro characterization of an epilepsy associated kcnt1 variant in hipcs neurons and therapeutic recovery using an antisense oligonucleotide approach

Authors: K. SORIANO[1], S. S. GOLINSKI[1], T. W. YU[2], T. NAKAYAMA[2], *R. S. SMITH[1]; [1]Northwestern Univ., Chicago, IL; [2]Boston Children's Hosp., Boston, MA

Abstract: *KCNT1* is a gene that encodes for a sodium-activated potassium channel (more commonly known as Slack channels). *KCNT1* variants are associated with severe seizure-related conditions in children, such as early-onset epileptic encephalopathy, yet treatment options are limited. Previous studies have shown *KCNT1* variants result in increased potassium current and hyperexcitability, however, less in known about the pathophysiology in human neurons. Using NGN2 directed differentiation, we generated excitatory cortical neurons from patient-derived induced pluripotent stem cells (iPSCs) obtained from an individual with the R474H variant. In R474H NGN2-neurons, we performed patch clamp experiments and found increased amplitude potassium currents, hyperpolarized resting potentials, and increased peak amplitude of action potentials (AP), when comparing to control neurons. Upon treatment of R474H NGN2-neuurons with an antisense oligonucleotide (ASO) designed to knock down *KCNT1* expression via an RNase-H mechanism, patient neurons displayed decreased levels of potassium current, and normalized resting potential and AP amplitude, comparable to WT cells. However, ASO treated

patient-derived neurons also displayed increased afterhyperpolarization, a critical finding for future studies. These findings suggest the R474H *KCNT1* variant results in altered neurophysiology in human excitatory cortical neurons, and ASOs might offer a potential therapeutic benefit for patients.

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Poster

PSTR521. Angelman and Other Developmental Disorders

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.05/C39

Topic: A.07. Developmental Disorders

Support: Eagles Autism Foundation

Title: Characterization of PRKAR1B mutations associated with neurodegenerative and neurodevelopmental disorders

Authors: *A. GLEBOV-MCCLOUD[1], R. A. MERRILL[2], T. ABEL[2], S. STRACK[3]; [2]Univ. of Iowa, [1]Univ. of Iowa, Iowa City, IA; [3]Univ. Iowa Col. Med., Univ. Iowa, Iowa City, IA

Abstract: *PRKAR1B* encodes the protein kinase A (PKA) regulatory R1β subunit and has been linked to both neurodegenerative and neurodevelopmental disorders (NDNDDs). Unfortunately, our understanding of the underlying mechanisms that cause NDNDDs is limited. A novel neurodegenerative disorder characterized by dementia and parkinsonism is associated with the L50R R1β mutation. The L50 residue is in R1β's dimerization/docking domain, suggesting that the L50R mutation disrupts both R1β dimerization and R1β binding to A-kinase anchoring proteins (AKAPs), which bring PKA to specific subcellular compartments. *PRKAR1B* has also been linked to neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD) and Marbach-Schaaf neurodevelopmental syndrome (MASNS). Whole exome sequencing studies identified the *de novo* R243C R1β mutation in individuals with ASD. Moreover, the *de novo* Q167L, E196K, and R335W R1β mutations were discovered in individuals with MASNS. These mutated NDD residues are all in R1β's cyclic adenosine monophosphate (cAMP) binding regions, indicating that they disrupt PKA activation. These reports suggest that PKA is involved in both neurodegeneration and neurodevelopment. In this work, we want to determine how the R1β mutations affect PKA function. We used a NanoBiT split-luciferase assay (NanoBiT assay) to measure changes in regulatory subunit dimerization and the interaction between PKA regulatory and catalytic subunits. We also used a luciferase reporter assay to measure changes in transcriptional activity. To measure PKA catalytic subunit (PKAc) dissociation using the NanoBiT assay, cells were treated with forskolin and rolipram (F/R) and changes in luminescent signal over time were measured. To measure transcriptional activity in the luciferase assay, cells

were treated with 8-(4-Chlorophenylthio)-cAMP (8cpt-cAMP) or isoproterenol. We discovered that the L50R R1β subunit dimerizes with itself but does not dimerize with wild-type (WT) R1β. Moreover, L50R does not colocalize with AKAP1 or small membrane AKAP. We also found that PKAc does not dissociate from R335W R1β upon F/R stimulation. We showed that, upon treatment with isoproterenol, all R1β mutants notably decrease transcriptional activity, with R335W having the strongest effect. These findings reveal that the L50R mutation promotes neurodegeneration by impairing both PKA-mediated transcription and PKA localization. On the other hand, the ASD and MASNS mutants appear to hinder neurodevelopment solely by impairing PKA-mediated transcription. This suggests that PKA-mediated neurodegeneration and neurodevelopment proceed via distinct mechanisms.

Disclosures: A. Glebov-McCloud: None. **R.A. Merrill:** None. **T. Abel:** None. **S. Strack:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.06/C40

Topic: A.07. Developmental Disorders

Support: R35NS116843
R35NS097370

Title: Loss of function of HERC1 ubiquitin ligase in neurons leads to synaptic dysregulation

Authors: *Q. YANG[1], Y. HONG[1], J. L. R. LOPEZ[3], H. SONG[2], G.-L. MING[2];
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Abstract: Schizophrenia is a highly heritable psychiatric disorder. Despite the discovery of hundreds of common variants in schizophrenia patients, the causal connections among genetic mutations, gene function in brain development, and disease pathogenesis are challenging to study and poorly established. HERC1 is a rare, but high confident risk gene for schizophrenia discovered in a recent GWAS. HERC1 is an E3 ubiquitin ligase, while its role in human brain development is unknown. Here we explored the function of HERC1 in human iPSC-derived hippocampal neurons. We characterized neurons derived from human iPSCs carrying homozygous, heterozygous loss of function and patient-specific mutations under three different genetic backgrounds. We found that loss of function of HERC1 leads to increased synaptic density and activity. We further identified that this is caused by elevated PSD-95 protein levels. Our study reveals that psychiatric disorder relevant mutation causes synapse deficits and provides new insight into the molecular and synaptic etiopathology of schizophrenia.

Disclosures: Q. Yang: None. **Y. Hong:** None. **J.L.R. Lopez:** None. **H. Song:** None. **G. Ming:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

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Program #/Poster #: PSTR521.07/C41

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS104078
NIH Grant MH101703

Title: Rescue of abnormal dendritic spines of neurons from Angelman syndrome mice by wildtype neuron exosomes

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Abstract: Angelman syndrome (AS) is a rare neurogenetic disorder caused by deletion or mutations in the *UBE3A* gene, which leads to the deficiency of the UBE3A protein in neurons. UBE3A is crucial for normal neuronal communication, as it regulates the turnover of synaptic proteins and synaptic plasticity. Exosomes, small extracellular vesicles released by various cells, including neurons, play important roles in intercellular communication. Exosomes contain a variety of molecules, including proteins and nucleic acids, and can transfer these components between cells. Emerging evidence indicates that exosomes are critical for normal brain functions, and in recent years many studies have investigated the therapeutic potential of exosomes as vehicles for delivering therapeutic cargoes in various neurological disorders. In this study, we investigated the potential role of exosomes in regulating spine morphology in AS mice. Our results showed altered exosome release from neurons cultured from AS mice at different developmental stages when compared to that from wildtype (WT) mice, with lower level of secretion at early stage and increased secretion at more mature stages. Using synaptosomes prepared from adult mice, we showed that activation of the lysosomal calcium channel TRPML1 stimulated exosome release from WT synaptosomes, while it did not stimulate exosome release from AS synaptosomes. Intriguingly, UBE3A was present in exosomes secreted from WT mice. It has been previously shown that hippocampal neurons of AS mice exhibit fewer dendritic spines, as compared to those from WT mice. Incubation of cultured hippocampal neurons from AS mice with conditioned medium from cultured hippocampal neurons from WT mice rescued the number of dendritic spines. Exosomes from conditioned medium of WT neurons produced a similar rescue effect in cultured neurons from AS mice. The specificity of the exosomal preparation was demonstrated by western blot and immunolabeling approach. Whether and to what degree UBE3A present in WT exosomes participates in the rescue of AS neuronal morphology remains to be determined. Together, these results reveal both altered exosomal secretion and composition in Angelman Syndrome mice and, more importantly, open new therapeutical approaches using exosomes as a potential treatment for this disease.

Disclosures: E. Penna: None. **T. Reece:** None. **M. Baudry:** None. **X. Bi:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.08/C42

Topic: A.07. Developmental Disorders

Support: Cure LBSL

Title: Antisense oligonucleotide therapy in iPSC-derived neurons of patients with LBSL

Authors: *M. AMANAT[1], S. GUANG[2], A. SMITH FINE[4], A. FATEMI[5], C. L. NEMETH[3]; [2]Neurosci., [3]Kennedy Krieger Inst., [1]Kennedy Krieger Inst., Baltimore, MD; [4]Kennedy Krieger Inst. / Johns Hopkins, Kennedy Krieger Inst. / Johns Hopkins, Baltimore, MD; [5]Johns Hopkins Univ., Johns Hopkins Univ., Baltimore, MD

Abstract: Background: Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) is an extremely rare inherited white matter disorder characterized by progressive spastic gait, ataxia, and damage to the posterior spinal cord. LBSL is caused by mutations in DARS2, which disrupts the function of mitochondrial aspartyl-tRNA synthetase. In more than 90% of identified LBSL cases patients harbor compound heterozygote mutations consisting of a mutation in intron 2, affecting exon 3 splicing, and a missense mutation. It is hypothesized that enhancing expression of DARS2 mRNA by increasing exon 3 inclusion may improve protein function. Therefore, our objective was to develop an antisense oligonucleotide (ASO) that targets DARS2 mRNA, aiming to promote exon 3 inclusion and restore gene expression. **Methods:** We designed fifteen ASOs, each consisting of 20 base pairs, to target potential intronic splicing silencers located upstream of exon 3. All nucleotides were modified with 2′-O-methoxyethyl (2′-O-MOE) and phosphorothioate (PS) to enhance ASO stability and prevent RNase-H activity. Induced pluripotent stem cells (iPSCs) were generated from blood cells of three LBSL patients and one healthy individual. Mutations in two LBSL cell lines were corrected to generate isogenic iPSCs. Patient iPSCs were transfected with ASOs to find the most effective ASO to increase exon 3 inclusion by RT-qPCR. This ASO was then tested at two different doses (50 nM and 100 nM) on iPSC-derived neural progenitor cells (NPCs). In addition to assessing exon 3 inclusion through RT-qPCR, we measured neurite outgrowth using confocal high content screening, as well as lactate levels in the cultured media of iPSC-derived motor neurons using the Lactate Reagent Set from Pointe Scientific. **Results:** The ASO targeting the region 180 to 200 nucleotides upstream of DARS2 exon 3 displayed the greatest increase in exon 3 inclusion compared to other designed ASOs and untreated iPSCs. RT-qPCR analysis of NPCs transfected with this ASO demonstrated elevated levels of DARS2 exon 3 in all LBSL cell lines, with a dose-dependent response. Additionally, higher outgrowth, processes, and branches per cell were noted in treated NPCs compared to untreated after 72 hours of ASO administration. Motor neurons treated with the ASO for one week also exhibited lower lactate levels compared to baseline lactate levels of LBSL neurons. **Conclusions:** ASO therapy holds promise for restoring gene expression and improving neurite outgrowth in iPSC-derived NPCs obtained from

LBSL patients. The observed effect of ASO treatment on lactate levels in LBSL neurons suggests an improvement in mitochondrial function.

Disclosures: **M. Amanat:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. **S. Guang:** None. **A. Smith Fine:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. **A. Fatemi:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute. **C.L. Nemeth:** A. Employment/Salary (full or part-time):; Kennedy Krieger Institute.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.09/C43

Topic: A.07. Developmental Disorders

Support: 1R01NS107428

Title: Biallelic and de novo variants in SLC8A3 cause neurodevelopmental disorder, microcephaly, and skeletal defects in human

Authors: *A. A. ZAIB[1,2], A. GHAFFAR[3], E. V. NIEUWENHOVE[4], G. LESCA[5], K. JANSEN[4], M. T. WARDE[5], L. LAGAE[6], Z. M. AHMED[7], S. RIAZUDDIN[8,2], S. RIAZUDDIN[7]; [1]Dept. of Otolaryngology, Univ. of Maryalnd, Baltimore, MD; [2]Ctr. of Excellence in Mol. Biol. (CEMB), Univ. of the Punjab, Lahore, Pakistan; [3]Otolaryngology Dept., Univ. of Maryland Baltimore, Baltimore, MD; [4]VIB-KU Leuven Ctr. for Brain & Dis. Res., Leuven, Belgium; [5]Univ. Hosp. of Lyon HCL, Lyon, France; [6]Dept. of Develop. and Regeneration, Univ. Hosp. Leuven Belgium, Leuven, Belgium; [7]Dept. of Otolaryngology, Univ. of Maryland, Baltimore, MD; [8]Jinnah Burn and Reconstructive Surgery Ctr., Allama Iqbal Med. Col., Lahore, Pakistan

Abstract: Genetics of neurodevelopmental disorder (NDD) is expanding at full tilt despite its association with approximately 13% of human protein-coding genes the number is on the rise with advancement in diagnostics, helping to understand and characterize the etiology of this heterogenous disorder. Hereby, we report a *de novo* (c.2645G>A) and 3 biallelic (c.1462 C>T; c.1636G>A; c.2461A>G) missense variants in the *SLC8A3* gene (MIM: 607991) as a novel candidate for NDD together with intellectual disability (ID), cerebral and cerebellar atrophy, epilepsy, microcephaly, motor delay, skeletal abnormalities, facial dysmorphism, and behavioral anomalies in five individuals from three families of different ethnicities. *SLC8A3* encodes sodium-calcium exchanger 3 (NCX3) which is an ATP-independent, reversible, membrane transporter critical for maintaining intracellular Ca^{+2}/Na^{+} homeostasis. This study aims to characterize the functional consequences of disease-causing variants in *SLC8A3* identified using a whole exome sequencing approach. Computational studies suggest the loss of function impact of variants on protein. *In vitro* studies in dermal fibroblasts isolated from an affected individual (c.2461A>G) showed significantly reduced mRNA and protein steady-state levels. We observed altered intracellular Ca^{+2} concentration and electrophysiological measurements in mutant

fibroblasts in preliminary studies. Overexpression of SLC8A3 variants in fibroblasts and COS7-cells showed significantly reduced protein levels in c.1462C>T and c.2461A>G expressing cells. Morpholino-based knockdown of *slc8a3* in *neurod1-GFP* transgenic zebrafish at the 1-2 cell stage resulted in developmental delay, microcephaly, and skeletal deficits. Spontaneous motility assay performed on 5dpf morphants showed reduced to no movements in both light and dark. Cartilage staining of morphants showed mandibular prognathism comparable to affected human subjects in this study. Whole mount confocal imaging of *slc8a3* morphants depicted reduced neuronal population and disrupted parallel fibers in the cerebellum, motor coordinating center of the brain, giving compelling evidence of direct involvement of *slc8a3* in neuronal development. Human WT *SLC8A3* mRNA micro-injections restored the behavioral, skeletal, and neuronal phenotype of slc8a3 morphants suggesting its evolutionary importance. However, none of the ID-associated *SLC8A3* variants were able to restore the phenotype. Taken together, our *in vitro* and in *vivo studies* on *SLC8A3* suggest its importance in early development and potential pathogenicity of all 4 ID-associated missense variants in humans and zebrafish.

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Poster

PSTR521. Angelman and Other Developmental Disorders

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.10/C44

Topic: A.07. Developmental Disorders

Support: Beijing Natural Science Foundation L222077

Title: Gene Replacement Therapy in A Rat Cri du Chat Syndrome Model

Authors: J. SHEN[1], Y. WANG[1], Y. LIU[2], J. LAN[2], X. LI[2], Y. WANG[2], S. WANG[1], ***F. YANG**[2];
[1]Inst. of Neurosci., Chongqing Med. Univ., Chongqing, China; [2]Beijing Tiantan Hospital, Capital Med. Univ., Beijing, China

Abstract: The Cri du Chat syndrome is a devastating genetic disorder for which there is currently no effective treatment. It is caused by a deletion of variable size occurring on the short arm of chromosome 5, which ranges from the entire short arm to the region 5p15. The most important clinical features are a high-pitched cat-like cry, distinct facial dysmorphism, microcephaly and severe psychomotor and mental retardation. In a rat model of a common genetic of Cri du Chat syndrome - a copy number variation on chromosome 5p15 - genetic deletion of the syntenic region from 2q22 induces deficits in social behavior and cognitive impairment. Here we describe a gene replacement approach through intravenous injection of an adeno-associated virus vector with brain-wide expressing *Ctnnd2* in the rat Cri du Chat

syndrome model. We demonstrated that a single injection of AAV-Ctnnd2 crossed the blood brain barrier dramatically rescued the deficits both social behavior and cognitive function of the Cri du Chat syndrome-like rat. Our results opened up a venue of gene replacement therapy in the Cri du Chat Syndrome.

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Poster

PSTR521. Angelman and Other Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NSFC Grant 82271908
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Title: Mutations of MAN1B1 cause intellectual disability in MAN1B1-CDG patients through impaired cortex neuron development

Authors: *Q. TIAN, L. ZANG, A. AHMED, K. XIA, Z. HU;
Central South Univ., Changsha, China

Abstract: MAN1B1-CDG, a neurodevelopmental syndrome mainly characterized by intellectual disability (ID), is an autosomal recessive inherited N-linked glycosylation disorder caused by *MAN1B1* gene mutation. Enormous variants on *MAN1B1* were found to cause MAN1B1-CDG, but its function on neuronal development was unclear. We use combined homozygosity mapping and exome sequencing to identify the genetic basis of a consanguinity MAN1B1-CDG family from Pakistan. The temporal expression pattern of *Man1b1* was analyzed by qPCR of the mouse cortex. To characterize progenitor proliferation and cortex neuron stratification, we delivered *Man1b1*^{shRNA} to the mouse ventricular zone (VZ) by in-utero electroporation (N=3). We then assessed progenitor proliferation and neuronal migration by BrdU labeling and immunofluorescent (IF) staining. Finally, we introduced *Man1b1*^{shRNA}, *Man1b1*^{WT}, and *Man1b1*^{MUT} plasmids to primarily cultured embryo pyramidal neurons to depict their role in neuron maturation using IF. We found that a novel frameshift variation c.772_775del (p.L258Mfs*16) on *MAN1B1* was co-segregated with ID in the family. *Man1b1* was expressed embryonically as early as E12.5 and increased to its peak level from P7 to P20, indicating its role in neuron development. Knockdown (KD) the *Man1b1* expression in E13.5 mouse VZ increased the BrdU (Control: 18.55%±2.0, mean ± SEM; KD: 30.70%±2.80) incorporation and Ki67^{+} (Control: 5.8%±1.3; KD: 10.0%±1.7) cell proportion indicating elevated cell proliferation; however, the BrdU^{+}/Ki67^{-} (Control: 88.00%±2.3; KD: 75.00%±4.1) cells were decreased in the KD group reflect the attenuated cell cycle exit or differentiation. The neuron migration was not

affected in the KD group (P>0.05). When KD the *Man1b1* in primarily cultured pyramidal neurons, the axon (Control: 730.2±36.7μm; KD: 453.6±39.13μm) and neurites (Control: 1043±44.19μm; KD: 703.3±59.18μm) length were reduced compared with control. The overexpression of *Man1b1*^{WT} can rescue the shorted axon (554.6±27.5μm) and neurites (890.1±49.14μm) length but *Man1b1*^{MUT} (445±24.22μm and 757.8±50.92μm) cannot. In addition, we find the dendrites' complexity was lower in KD compared with control (P<0.0001), and can be rescued by *Man1b1*^{WT} but not *Man1b1*^{MUT}. Finally, the increased immature spine was noticed in the *Man1b1* KD group (Control: 0.636±0.034/μm; KD: 0.747±0.015/μm) and can be rescued by *Man1b1*^{WT} (0.517±0.03/μm) but not *Man1b1*^{MUT} (0.758±0.014/μm), reflect its role in the promoting spine maturation during neuronal development. In conclusion, mutations on *MAN1B1* may be causing ID by inhibiting neuron differentiation and maturation during neuronal development.

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Poster

PSTR521. Angelman and Other Developmental Disorders

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Program #/Poster #: PSTR521.12/C45

Topic: A.07. Developmental Disorders

Support: NIH Grant R00EY028964
ASF20210504

Title: Validating behavioral phenotypes in a mouse model of Angelman syndrome using multidimensional analysis

Authors: *D. D. KERR[1], C. D. GUOYNES[1], A. RAI[1], M. S. SIDOROV[1,2];
[1]Ctr. for Neurosci., Children's Natl. - DC, Washington, DC; [2]Pediatrics and Pharmacol. & Physiol., George Washington Univ. Sch. of Med., Washington, DC

Abstract: Angelman syndrome (AS) is a neurodevelopmental disorder that affects 1 out of 20,000 individuals and is caused by loss of function mutations in the maternal copy of the *UBE3A* gene. AS is a multifaceted disorder associated with a lack of speech, motor, and cognitive impairments, disrupted sleep, and seizures. The *Ube3a*$^{m-/p+}$ mouse model for AS has high construct and face validity across several behavioral domains, including seizures, motor, and sleep. The AS research community widely uses a "gold standard" behavioral battery for *Ube3a*$^{m-/p+}$ mice (Sonzogni et al., *Molecular Autism*, 2018) to assess behavioral improvement following various treatment approaches in development. Our prior work (Tanas et al., *Translational Psychiatry*, 2022) demonstrated that multidimensional analysis (principal component analysis + k-means clustering) could simplify this gold standard behavioral battery to a single number that represents overall behavioral severity and is sensitive to treatment. However, our prior approach had two significant limits: (1) hard-coding multidimensional

analysis is cumbersome and not easily adaptable to new datasets, and (2) the “gold standard” behavioral battery, while highly reliable, measures only a limited range of tests. Here, we present a MATLAB-based graphical user interface called PUMBAA (Phenotyping Using a Multidimensional Behavioral Analysis Algorithm) that enables user-friendly multidimensional analysis of diverse datasets. PUMBAA enables users to customize analysis while providing detailed outputs related to multidimensional analysis. We then used PUMBAA to evaluate the reliability of abnormal operant extinction in *Ube3a*$^{m-/p+}$ mice. Prefrontal circuits partly drive operant extinction, and we previously reported abnormal operant extinction in *Ube3a*$^{m-/p+}$ mice (Sidorov et al., *J Neurosci*, 2018). Here, we replicated prior operant extinction results separately in male and female mice and two independent cohorts (total n =30 WT mice, 26 *Ube3a*$^{m-/p+}$). We found that extinction may be performed in series with other behavioral tests to expand the range of phenotypes modeled reliably in *Ube3a*$^{m-/p+}$ mice.

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Poster

PSTR521. Angelman and Other Developmental Disorders

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Program #/Poster #: PSTR521.13/C46

Topic: A.07. Developmental Disorders

Support: MH101703

Title: Impairment of neuronal morphology, synaptic plasticity and behavior in Angelman Syndrome mouse model: rescue by serotonin receptor 7 stimulation

Authors: *A. PIZZELLA[1,3], E. PENNA[3], Y. LIU[3], E. LACIVITA[4], M. LEOPOLDO[4], C. PERRONE-CAPANO[5], X. BI[3], M. BAUDRY[3], M. CRISPINO[2];
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Abstract: Angelman Syndrome (AS) is a rare incurable neurodevelopmental disorder characterized by speech impairment, motor dysfunctions, seizures activities and intellectual disability. AS has a high comorbidity with Autism Spectrum Disorder (ASD). ASD, depression and schizophrenia are some of the disorders associated with altered serotonin receptor 7 (5-HT7R). 5-HT7R is a G-protein coupled receptor involved in various forms of synaptic plasticity. Thus, our aim was to investigate whether stimulation of 5-HT7R could reverse the various molecular, cellular and behavioural impairments in the AS mouse model. Our results first showed that 5-HT7R stimulation by acute systemic injection of LP-211, a potent and selective agonist, rescued learning impairment, as assessed by fear conditioning testing, in AS mice. 5-HT7R stimulation was also beneficial for synaptic plasticity in AS mice. Treatment of acute hippocampal slices with LP-211 reversed the LTP impairment observed in slices from AS mice,

while it had no effect in slices from wildtype mice. These results strongly indicated the involvement of 5-HT7R in AS pathogenesis. We then investigated the effect of 5-HT7R activation on neuronal morphology of cultured hippocampal neurons from AS and WT mice. It was previously demonstrated that the density of dendritic spines was reduced in neurons from AS mice as compared to WT mice. Chronic stimulation with LP-211 for 3 days restored spine density to the level found in WT mice neurons. To investigate in more details the effects of 5-HT7R stimulation on synapses, we isolated synaptosomes from cortex of AS and WT mice, to study local protein synthesis. We found that synaptic protein synthesis was reduced in synaptosomes from AS mice, as compared to WT, indicating for the first time that this local translation system is one of the cellular machineries altered in AS. Moreover, we demonstrated that the decrease in synaptic protein synthesis in cortex of AS mice was completely rescued by acute stimulation with LP-211. Overall, our study demonstrates that activation of 5-HT7R can rescue multiple phenotypes in AS mice, many of them related to synaptic plasticity mechanisms. These results provide a new perspective for therapeutical approaches of the disease, using 5-HT7R as a target.

Disclosures: A. Pizzella: None. **E. Penna:** None. **Y. Liu:** None. **E. Lacivita:** None. **M. Leopoldo:** None. **C. Perrone-Capano:** None. **X. Bi:** None. **M. Baudry:** None. **M. Crispino:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: CIHR PJT-166165

Title: Pain experience of children with Christianson Syndrome

Authors: *S. PREMACHANDRAN, D. D. OCAY, D.-L. YE, L. S. MIRAUCOURT, J. ORLOWSKI, R. SHARIF NAEINI, C. E. FERLAND;
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Abstract: Children with severe cognitive impairments express pain differently due to difficulties with communication and are unable to self-report pain intensities. This has led to the assumption that children with disabilities/verbal impairments have a higher pain tolerance threshold. To explore this possibility, we recruited fourteen young male participants with Christianson Syndrome (CS) for this study. This X-linked neurodevelopmental disorder is caused by a loss-of-function mutation in the *SLC9A6* gene encoding the cation/proton exchanger NHE6. It is associated with autism-spectrum disorder-like symptoms, including mutism and hyposensitivity to pain. Using a mouse model of CS, we observed hyposensitivity to noxious thermal and chemical stimuli as well as an increased number of aversive responses to dynamic mechanical

stimuli in our NHE6 KO mice, compared to control mice. Following the results observed in the NHE6 KO mice, children with CS were subjected to a novel observational tool, the Pain Sensory and Painful Situations Questionnaire (PSQ) which takes multiple painful situations into account to broaden the description of pain expression. Using social expressive behaviours of pain, the PSQ documented two of the participants likely experienced moderate to severe pain most of the time. Similar to our mouse model of CS, we observed hyposensitivity to a number of different painful sensations and an increased number of aversive responses to innocuous mechanical stimuli in our patient cohort. About 30-50% of these patients exhibited an aversion to normally innocuous stimulation like light touch, and getting in contact with gusts of air or smooth surfaces. Despite that hyposensitivity to different painful situations was present and vocal expression of pain was less prominent in our sample of CS children, our work suggests they may experience chronic or recurrent pain which importantly calls for treatment.

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Poster

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Topic: A.07. Developmental Disorders

Support: NIMH grant MH065635
FAST Italia

Title: A new prodrug reverses core symptoms of Angelman syndrome modeled in mice and enhances memory in healthy mice

Authors: *F. ARIA[1], C. ARP[2], K. PANDEY[3], L. MUNARI[3], *F. ARIA[3], D. TRAUNER[2], C. M. ALBERINI[3];
[2]Dept. of Chem., [3]Ctr. for Neural Sci., [1]New York Univ., New York City, NY

Abstract: Angelman syndrome (AS) is a rear genetic neurodevelopmental disorder that affects approximately 1 on 12,000 children. AS is caused by genetic alterations of the 15q11.2-q13.1 chromosome region, which leads to several neuropsychiatric symptoms, including cognitive impairments and motor deficits. To date, the treatments available for AS patients are limited to symptomatic management. Our previous works on a mouse model of AS showed that the insulin like growth factor 2 receptor (IGF2R) ligands IGF2 and mannose 6 phosphate (M6P) reverse several behavioral defects of AS mice. Here we identified and synthesized a new small molecule compound (PMP1), designed as a prodrug derivative of M6P, that could provide long-lasting behavioral effects and could be administered orally. We tested PMP1 by subcutaneous (s.c.) injection into healthy mice (WT mice) and found that a single injection results in significant memory enhancement, tested with both aversive and non-aversive memory tasks. We also found

that PMP1 reversed memory and motor impairment in AS mice. PMP1 compared to IGF2 and M6P, in both WT and AS mice, emerged as more effective as memory enhancer in WT mice and in reversing memory and motor deficits in AS mice. We also found that PMP1 works effectively via oral administration in both WT and AS mice, and the effects are long lasting. Finally, we determined that the effect of PMP1 in memory enhancement requires IGF2R. Together, these data lead to the conclusion that PMP1 represents a new potential molecule for the treatment of AS and potentially of other diseases of cognitive impairments.

Disclosures: F. Aria: None. **C. Arp:** None. **K. Pandey:** None. **L. Munari:** None. **F. Aria:** None. **D. Trauner:** None. **C.M. Alberini:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.16/C49

Topic: A.07. Developmental Disorders

Support: MOST Grant 111-2423-H-002-009
MOST Grant 110-2410-H-002-235-MY3
NSTC Grant 112-2321-B-002-022

Title: Snord116 deletion mouse model for Prader-Willi syndrome reveals attention deficits and abnormal neuronal properties

Authors: *P.-H. TSAI[1], C.-Y. CHANG[4], W.-S. LAI[1,2,3];
[1]Dept. of Psychology, [2]Grad. Inst. of Brain and Mind Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Dept. of Psychiatry, Columbia Univ., New York, NY

Abstract: Prader-Willi syndrome (PWS) is a neurodevelopmental disorder that causes a considerable burden on more than 350,000 people worldwide. People with PWS are characterized by displaying hypotonia during their neonatal period, followed by hyperphagia, obesity, developmental delay, inattention, cognitive impairment, and other severe behavioral problems in their later life. Nowadays, PWS is assumed to be caused by defective expression of paternal genes in chromosome 15q11-q13. Within this region, accumulating data indicates that the lack of expression of imprinted small nucleolar RNA cluster *SNORD116* might contribute the pathogenesis of PWS. However, the precise role of *SNORD116* in the etiology of PWS remains elusive. This study aims to investigate the influence of *Snord116* deletion in various behaviors related to the clinical-characteristic features of PWS using *Snord116* $^{+/-}$ mice as a model. A battery of behavioral tests (including open field, y-maze, three-chamber social test, object-based attention test, gait analysis, rotarod, elevated plus maze, nesting building, and marble burying test) was conducted to assess motor, memory, social behavior, attention, and other cognitive functions in adult *Snord116* $^{+/-}$ mice and their wild-type littermate controls (WT). Our results reveal that *Snord116* $^{+/-}$ mice displayed object-based attention deficit compared to WT while

remaining normal in motor abilities, spatial memory, anxiety level, social interaction, and other behavioral aspects. In order to examine the potential cause of attention deficit, primary cortical neurons were isolated from E15.5 *Snord116* $^{+/-}$ mice embryos to further investigate whether *Snord116* deletion would result in neuromorphological and neuroelectrophysiological alterations during early development. Compared to WT cortical neurons, the neurons derived from *Snord116* $^{+/-}$ embryos displayed decreased dendritic complexity at 6 days *in vitro* (DIV) while showing an increased mean firing rate at DIV 20. Further investigation of *Snord116* $^{+/-}$mice is needed to elucidate the importance and underlying mechanism of *Snord116* in PWS-related cognitive deficits.

Disclosures: P. Tsai: None. **C. Chang:** None. **W. Lai:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

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Program #/Poster #: PSTR521.17/C50

Topic: A.07. Developmental Disorders

Support: Foundation for Angelman Syndrome Therapeutics

Title: Evaluating peak alpha frequency as an EEG biomarker for Angelman syndrome

Authors: *M. S. BOWEN-KAUTH[1], A. E. YOUNGKIN[1], C. A. MCNAIR[1], D. P. RYAN[2], J. J. SHIDE[1], A. H. DICKINSON[2], M. S. SIDOROV[1,3];
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Abstract: Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe intellectual disability, motor impairments, epilepsy, and sleep disturbances. AS is caused by the loss of function of the maternally expressed *UBE3A* gene. Clinical trials aiming to reinstate the silenced paternal *UBE3A* allele in neurons are currently underway. Biomarkers are needed for these trials that are safe, quantifiable, robust, and linked to clinically meaningful outcomes. Electroencephalography (EEG) is used to safely monitor brain rhythms in children with AS. While we have previously demonstrated that delta EEG rhythms are robustly increased in children with AS, other biomarkers are needed to assess neural activity throughout a broader age range. Peak alpha frequency (PAF), the frequency where oscillations in the alpha range are the strongest, increases across development in neurotypical children. Our prior work demonstrated that PAF does not develop normally in children with autism. Here, we test the hypothesis that PAF does not develop normally in AS and evaluate its potential as a biomarker. We quantified over 160 EEGs from male and female individuals with AS gathered via the AS Natural History Study and age-matched neurotypical controls ranging from 6 months to 14 years. In a preliminary subset of ~60 EEGs, PAF development did not follow a typical trajectory in children

with AS. While PAF increased across development in the neurotypical population, PAF did not increase with age in children with AS. Furthermore, PAF was more difficult to detect in EEGs from children with AS. Our preliminary work suggests that PAF may be a potential biomarker for AS. Ongoing work seeks to correlate PAF with clinical severity.

Disclosures: **M.S. Bowen-Kauth:** None. **A.E. Youngkin:** None. **C.A. McNair:** None. **D.P. Ryan:** None. **J.J. Shide:** None. **A.H. Dickinson:** None. **M.S. Sidorov:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.18/C51

Topic: A.07. Developmental Disorders

Support: GR-2017–02364378/Italian Ministry of Health
GGP19177/ Telethon

Title: Gene replacement therapy for the cure of Creatine Transporter Deficiency Syndrome

Authors: *L. IOVINO[1,2], F. DI VETTA[1,3], L. DADÀ[1], C. MONTANI[4], E. GHIRARDINI[1], F. CALUGI[1,5], G. SAGONA[1], T. PIZZORUSSO[1,5], A. GOZZI[4], L. BARONCELLI[1,2]; [1]Neurosci. Inst., Italian Natl. Res. Council, PISA, Italy; [2]IRCCS Stella Maris Fndn., Pisa, Italy; [3]Univ. of Pisa, Pisa, Italy; [4]Functional Neuroimaging Lab., Italian Inst. of Technol., Rovereto, Italy; [5]Scuola Normale Superiore, Pisa, Italy

Abstract: Creatine Transporter Deficiency (CTD) is an X-linked metabolic disorder originating from mutations of the solute carrier family 6-member 8 (SLC6A8) gene. SLC6A8 encodes for the transporter responsible of cellular creatine (Cr) uptake (creatine transporter, CRT). Depletion of brain Cr causes a predominantly neurological clinical picture including intellectual disability, psycho-motor impairment, autistic-like behavior and seizures. CTD is a rare but probably underdiagnosed disorder, representing a major issue in health care due to the large impact on patient quality of life and assistance costs. There is still no effective cure for this disorder and the current standard of care includes the palliative treatment of epilepsy and behavioural problems. To understand whether gene therapy might be a potential disease-modifying treatment for CTD, we developed an adeno-associated viral vector (AAV9) carrying a functional copy of the human *SLC6A8* gene driven by a small synthetic promoter (AAV-SLC6A8). We found that a single intraventricular infusion of AAV-SLC6A8 in newborn wild-type and *Slc6a8* mutant mice is sufficient to induce a high expression and widespread distribution of the transgene in the brain, associated with a significant increase in cerebral Cr levels. The postnatal reinstatement of CRT function leads to the rescue of functional hypoconnectivity in the mutant brain and the improvement of autistic-like stereotyped behavior. In contrast, AAV-mediated delivery of SLC6A8 did not ameliorate cognitive function in mutant animals and we observed a deterioration of mnemonic performance in treated wild-type mice. These results prompted us to

devise a second generation gene therapy cassette with the aim to maximise the beneficial effects of treatment and mitigate potential safety issues. As an alternative to the use of non-native promoters for expressing *SLC6A8*, we are now testing a portion of its endogenous regulatory sequence. Using in vitro models, we found that this strategy reduces transgene expression, potentially better mimicking the physiological levels of CRT protein and improving its intracellular trafficking. In summary, our results provide proof-of-concept evidence that gene therapy has potential applications for treating CTD and suggest that further steps of vector engineering to finely tune CRT expression are crucial for optimising efficacy.

Disclosures: **L. Iovino:** None. **F. Di Vetta:** None. **L. Dadà:** None. **C. Montani:** None. **E. Ghirardini:** None. **F. Calugi:** None. **G. Sagona:** None. **T. Pizzorusso:** None. **A. Gozzi:** None. **L. Baroncelli:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

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Program #/Poster #: PSTR521.19/C52

Topic: A.07. Developmental Disorders

Support: NIH Grant ROIMH109719
JGA/UCD Subaward: A19-3376-S006

Title: The critical in vivo role of neuronal PP1β and its potential implications in disease

Authors: ***C. MCKEE**[1], K. FOLEY[1], L. CISCO[2], O. MAROLA[3], P. SHRAGER[1], J. LUECK[2], H. XIA[2];
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Abstract: Protein Phosphatase 1 (PP1) is a major Ser/Thr phosphatase expressed throughout the brain. Although correlations between PP1 and various neurodevelopmental/neurodegenerative diseases have been suggested, a causative role for PP1 in many of these contexts has yet to be established. A limitation in the field is a failure to distinguish between the 3 major PP1 isoforms, α, β, and γ, which can have differing and, even opposing functions. While PP1α and PP1γ have been studied for their role in synaptic plasticity, less is known about the neuronal function of PP1β. This study seeks to investigate the neuronal role of PP1β *in vivo,* and to uncover potential mechanisms by which PP1β may influence disease pathophysiology. Floxed alleles of *Ppp1cb* were conditionally recombined in neurons using Thy1-Cre (PP1β cKO), allowing us to investigate neuronal PP1β function in the developing mouse brain. These mice exhibit a failure to thrive and typically die by 2-3 postnatal weeks. These mice also demonstrate increased paired-pulse facilitation within the hippocampus, suggesting impaired neurotransmitter release. In agreement with studies suggesting activity influences myelination within specific brain regions, we found a significant decrease in myelin basic protein in the cortex. Furthermore, to assess the

influence of PP1β on functional myelination in an activity-independent context, we measured compound action potentials (CAPs) along the optic nerve. Decreased rapid peak one amplitudes from these recordings suggest impaired optic nerve myelination. However, electron micrograph analyses failed to detect a significant deficit in myelinated axons. Altered nodes of Ranvier could influence CAP recordings, and indeed we found a significant decrease in the number of intact nodes in the optic nerves of PP1β cKO mice, suggesting a potential role for PP1β in nodal structure. Next, we generated a neuron specific inducible PP1β KO mouse line (iKO) to study PP1β function in adolescent mice. These iKO mice exhibit significantly impaired hind limb mobility and respiratory patterns 3 weeks post recombination. These data support the hypothesis that PP1β alters action potential propagation in a way that abrogates downstream functionality. These results shed light on the isoform specific role of PP1β and potential mechanisms that could be disrupted by PP1β in neurodevelopmental/neurodegenerative diseases. Future studies will seek to uncover the molecular substrates underlining these isoform specific effects and provide potential therapeutic targets for diseases in which PP1β functionality is altered.

Disclosures: **C. McKee:** None. **K. Foley:** None. **L. Cisco:** None. **O. Marola:** None. **P. Shrager:** None. **J. Lueck:** None. **H. Xia:** None.

Poster

PSTR521. Angelman and Other Developmental Disorders

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Program #/Poster #: PSTR521.20/C53

Topic: A.07. Developmental Disorders

Support: KNIH project No.2021ER040200
NRF-15 2022R1A2C2091689

Title: Phenotypic characterization of CSNK2A1-Related Neurodevelopmental Syndrome

Authors: *B. LEE;
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Abstract: CSNK2A1-Related Neurodevelopmental Syndrome (Okur-Chung syndrome) is an extremely rare genetic disorder, characterized by variable dysmorphic features, hypotonia, stunted growth and developmental delay/intellectual disability/behavioral abnormalities. Only more than 50 patients have been reported worldwide. In the current study, we report 6 unrelated Korean patients with CSNK2A1-Related Neurodevelopmental Syndrome. The genetic diagnosis was confirmed either by whole exome or genome sequencing. Six different *CSNK2A1* mutations have been identified. The median age at evaluation was 4.1 years (2.3-10.4 years). All patients were born as appropriate for gestational age, but post-natal growth retardation was noted in 3 patients. Of note, one patient showed overgrowth with obesity and precocious puberty. Variable facial dysmorphisms were noted in all the patients; micrognathia (2 pts), low set ears (3pts), eye abnormalities (hypertelorism (4 pts), epicanthal folds (3 pts), and synorphis (2pts)), midfacial

hypoplasia (3 pts), round face/full cheek (1 pt), broad nasal bridge (3 pts), and thin upper lip (3 pts). Cardiac and renal anomaly was found in 2 and 1 patients, respectively. Joint hyperextensibility (2 pts) and clinodactyly (4 pts) were found as well. All the patients showed hypotonicity in their infantile period and global development delay with speech delay. Variable behavioral abnormalities were noted as tantrums (1 pt) and attention deficient hyperactivity disorder (2 pts). Variable brain abnormalities such as symmetric FLAIR hyperintensity in the peritrigonal or bilateral cerebral white matter, pachygyria, thin corpus callosum or brain stem, small optic nerve, pons, cerebellar vermis or small pituitary gland were found in 4 patients. Febrile convulsion (3 pts) and neonatal seizure (1 pt) were also noted.The results of our study expand the phenotypic spectrum of this extremely rare condition and indicate the broad multi-systemic surveillance would be suggested for patient care.

Disclosures: B. Lee: None.

Poster

PSTR521. Angelman and Other Developmental Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR521.21/C54

Topic: A.07. Developmental Disorders

Title: New insights from old assays: Revisiting rodent beam walking with machine learning-based analytics

Authors: F. TOZZI[1], R. NARAYANAN[1], D. ROQUEIRO[1], Y.-P. ZHANG[2], ***E. C. O'CONNOR**[1];
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Abstract: Beam walking was first deployed in the 1970s for rodents as a test of motor coordination and balance, and remains widely used today in assessing disease model phenotypes and the effects of CNS active substances. However, while the task has ethological validity, the main endpoints of ‘foot-slip counts’ and ‘time to cross’ are prone to between-rater variability and are limited in their sensitivity and specificity to detect biological effects under different test conditions. Here we asked whether deep and machine learning-based methods of data capture and analytics could reveal previously hidden, but biologically relevant, features in the beam walking task. The test conditions included mouse models for neurodevelopmental disorders (including Angelman syndrome) and pharmacological challenge (including diazepam) in wild-type mice. Markerless pose estimation, using DeepLabCut, was deployed to label 13 body parts of mice video-recorded in the sagittal plane, while traversing one of three, 1 meter long beams. A total of 394 features were extracted from the labeled body parts. A simple classifier was developed to automatically detect foot-slips, which achieved a high degree of recall (>90%) and precision (>85%). Using the 394-feature set, a random-forest classifier was deployed to predict group differences in the different experimental conditions. The classifier revealed and differentiated effects in disease models and with pharmacological challenges that were not seen

with classical endpoints of ‘foot-slips’ and ‘time to cross’, and with greater sensitivity. Taken together, our work illustrates how implementation of deep and machine learning-based data capture and analytics supports the revisiting of ethologically-valid behavioral tasks in rodents to bring new scientific insights. Ultimately, such work strengthens the understanding of how brain function generates behavior in health and disease states.

Disclosures: **F. Tozzi:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **R. Narayanan:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **D. Roqueiro:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **Y. Zhang:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **E.C. O'Connor:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.01/C55

Topic: B.03. Ion Channels

Support: NIH Grant 5U01DA051373

Title: High-dose d-cysteine ethyl ester but not naloxone reverses fentanyl-mediated inhibition of intrinsic Ca^{2+} activity in neurons of the superior cervical ganglion

Authors: *T. NAKASHE[1], Z. T. KNAUSS[2], A. C. BEARD[3], D. MUELLER[1], S. J. LEWIS, 44120[4], D. S. DAMRON[1];
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Abstract: The opioid epidemic is a major health crisis in the U.S., resulting in an estimated 109,000 deaths in 2021, with an estimated 70% increase in emergency room visits for overdose treatment. Overdose results in opioid-induced respiratory depression (**OIRD**) which can be treated by the administration of competitive opioid receptor antagonists (e.g., naloxone). However, OIRD resulting from highly potent synthetic opioids (e.g., fentanyl) are longer lasting, and can induce wooden chest syndrome (**WCS**) which is highly resistant to reversal. As such the current medical standard care response is to administer multiple doses of naloxone. However, few studies have examined the effects of high-dose naloxone on the function of peripheral regulatory centers of respiratory and cardiovascular drive such as the superior cervical ganglion (**SCG**) in the absence or presence of synthetic opioids. The thioester drug, D-cysteine ethylester (**D-CYSee**) delivers rapid and long-lasting recovery of respiratory function from OIRD in male and rats with minimal effect on analgesic efficacy. Thus, we assessed and compared the effects of naloxone and D-CYSee in the absence and presence of fentanyl on intrinsic Ca^{2+} activity in heterogeneous cell cultures of the SCG of Sprague-Dawley rat pups (N = 21, 7/culture). Cells

were cultured for 12-days prior to loading with the fluorescent Ca^{2+} probe, Cal-520 AM, and imaged on an inverted microscope. We assessed changes in intrinsic unstimulated intracellular Ca^{2+} (iCa^{2+}) activity over 25-minutes in which cells were perfused under one of four treatments preceded by a control period: **1)** naloxone (1, 10 and 100 uM), **2)** fentanyl (100 nM) + naloxone (100 uM), **3)** D-CYSee (1, 10 and 100 uM), and **4)** fentanyl + D-CYSee (100 uM) all conditions were followed by a drug washout. Under control conditions, cells displayed intrinsic Ca^{2+} activity at 1.2Hz. The administration of naloxone at (1,10 and 100 uM) produced a dose-dependent inhibition of intrinsic iCa^{2+} activity ($p<0.05$). Administration of D-CYSee (1, 10 and 100 uM) failed to induce any change in amplitude or frequency of intrinsic $_{i}Ca^{2+}$ activity ($p>0.05$). Administration of fentanyl inhibited intrinsic iCa^{2+} activity ($p<0.05$) that was reversed during co-administration of D-CYSee (100 uM, $p<0.05$) and not naloxone ($p>0.05$). Drug washout resulted in a prompt return to baseline intrinsic iCa^{2+} activity ($p>0.05$). This study provides the first evidence that high-dose naloxone, but not D-CYSee, can inhibit activity of the SCG. These findings suggest that repeated administration of naloxone may negatively impact patient recovery from OIRD and that D-CYSee is effective at recovering fentanyl disrupted SCG activity.

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Poster

PSTR522. Calcium Channels

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.02/C56

Topic: B.03. Ion Channels

Support: R01NS128403

Title: Stim2 is a key regulator of pruritus in the spinal dorsal horn via grpr signaling

Authors: *F. HARERAM, Y. MEI, A. YI, F. WANG, A. BEKKER, H. HU;
Anesthesiol., New Jersey Med. Sch., Newark, NJ

Abstract: Chronic pruritus is a debilitating condition and represents a clinical challenge**.** Previous studies have demonstrated that neuronal signaling pathways in the spinal cord play a crucial role in pruritus. Although several molecules have been identified as key itch modulators, the molecular mechanisms underlying pruritus remain incompletely understood. We have shown that the SOC family members STIM1 and STIM2 (endoplasmic reticulum calcium sensors) are expressed in dorsal horn neurons, but their functional significance is still elusive. Here we show that deletion of STIM1 in excitatory neurons attenuates nociception, but does not affect pruritogen-induced scratches. In contrast, ablation of STIM2 in excitatory neurons markedly attenuates pruritogen-induced scratches but has no effect on nociception. Inhibition of STIMs by intrathecal injection decreases histamine (His)- and chloroquine (CQ)-induced itch behavior.

Moreover, inhibition or deletion of excitatory neuronal STIM2 reduces fluorescein isothiocyanate (FITC)- and 1-fluoro-2, 4-dinitrobenzene (DNFB)-induced chronic itch, suggesting that neuronal STIM2 plays a role in both acute and chronic itch. Importantly, STIM2 expression is increased in the dorsal horn from diphenylcyclopropenone (DCP)- and DNFB-induced mouse models of chronic itch. Calcium imaging data reveal that gastrin release peptide (GRP)-induced calcium response is largely decreased in dorsal horn neurons from STIM2 KO mice. Consistently, STIM2 KO mice show much less GRP-induced itch behavior. These data clearly demonstrate that STIM2 plays an important role in pruritus. Our findings establish for the first time a link between STIM2 and GRPR.

Disclosures: F. Hareram: None. **Y. Mei:** None. **A. Yi:** None. **F. Wang:** None. **A. Bekker:** None. **H. Hu:** None.

Poster

PSTR522. Calcium Channels

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Program #/Poster #: PSTR522.03/C57

Topic: B.03. Ion Channels

Support: NIH NINDS R00NS116123
Warren Alpert Foundation

Title: Cell-specific alternative splicing of calcium-dependent secretion activator and coupling to voltage gated Ca_V calcium channels

Authors: *E. MUSTAFA[1], M. DAESCHNER[2], Q. MOSLEY[2], E. LOPEZ SOTO[2];
[2]Mol. Biomed. Sci., [1]North Carolina State Univ., Raleigh, NC

Abstract: Calcium signals at the nerve terminal are critical for the initiation of transmitter release. Voltage-gated calcium Ca_V channels are the main source of excitation-dependent calcium increase in nociceptors and Ca_V channels regulate vesicle exocytosis and release of glutamate and neuropeptides. Interactions of Ca_V channels and synaptic proteins including the soluble N-ethyl-maleimide-sensitive factor attachment protein receptor (SNAREs) complex have been shown to ensure triggering of vesicle exocytosis and transmitter release at central synapses. With few exceptions, studies on the regulation of Ca_V channels by synaptic proteins focus on the role of Syntaxin, Munc18 and SNAP-25, while overlooking the potential relevance of the calcium-dependent activator protein for secretion 1, CAPS1. CAPS1 has been shown to regulate large dense core vesicle exocytosis and it is associated with calcium-dependent release of neuropeptides in nociceptors. Here, we present evidence that CAPS1 regulates Ca_V channels. We used whole-cell patch clamp in voltage clamp mode with 1 mM Ca^{2+} or 5 mM Ba^{2+} as charge carrier. We recorded Ca_V macroscopic currents from tsA201 cells overexpressing $Ca_V\alpha_1$ subunits with required auxiliary subunits β_3, $\alpha_2\delta_1$, and eGFP with or without CAPS1. The experimenter was blinded to conditions until post-analyses. We found that $Ca_V2.2$ and $Ca_V3.2$ currents, but not

Cav1.2 currents, were ~66% and ~40% upregulated when CAPS1 was co-expressed, in comparison with control conditions without CAPS1. These findings suggest a role for CAPS1 in coupling with Cav channels to contribute to calcium-dependent vesicle exocytosis. We therefore characterized CAPS1 expression by performing transcriptomic and western blot analysis of genetically identified sensory neurons from mice and we found cell-specific spliced CAPS1 isoforms in nociceptors. Spliced CAPS1 isoforms containing exon 16a are enriched in peptidergic nociceptors (50.75 ± 2.37%, p=0.0001) in comparison to non-peptidergic nociceptors (24.05 ± 0.64%) and low-threshold mechanoreceptors (1.62 ± 0.64%). Having established the precise spliced CAPS1 isoforms expressed in nociceptors, we are analyzing CAPS isoform and Cav channel interactions to link these analyses directly to transmitter release. Given the impact of alternative splicing of synaptic proteins in the brain, we argue that cell-specific spliced CAPS1 isoforms regulate cell-specific Cav channel function for neuropeptide release in nociceptors.

Disclosures: E. Mustafa: None. **M. Daeschner:** None. **Q. Mosley:** None. **E. Lopez Soto:** None.

Poster

PSTR522. Calcium Channels

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Topic: B.03. Ion Channels

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Flag ERA JTC 2019 (MILEDI project)
Italian National Recovery and Resilience Plan (PNRR), M4C2, funded by the European Union – NextGenerationEU (Project IR0000011, CUP B51E22000150006, "EBRAINS-Italy")

Title: Dendritic BK channels activation by N-type voltage-gated Ca^{2+} channels in neocortical layer-5 pyramidal neurons

Authors: *L. BLÖMER[1,2], E. GIACALONE[3], F. ABBAS[4], M. MIGLIORE[3], M. CANEPARI[1,5];
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Abstract: The action potential (AP) generated in the axon actively back-propagates into the dendrites leading to activation and deactivation of diverse ion channels, including voltage-gated Ca^{2+} channels (VGCCs). The full understanding of the role of each VGCC remains elusive.

Here, using ultrafast membrane potential (V_m) and Ca^{2+} imaging, we show that dendritic N-type VGCCs, activated by the back-propagating AP (bAP) in layer-5 neocortical pyramidal neurons from brain slices, selectively target large-conductance Ca^{2+}-activated K^+ channels (BK CAKCs). We show that this coupling between N-type VGCCs and BK CAKCs occurs within 500 µs following the AP peak, i.e. before the peak of the Ca^{2+} current elicited by the AP. As a consequence, when N-type VGCCs are inhibited, the early widening of the AP shape boosts the other activated VGCCs increasing the total Ca^{2+} influx associated with bAP. We present a realistic NEURON model showing that the physical coupling between N-type and BK channels is necessary to reproduce the experimental results. In addition, we show how the timely activation of BK CAKCs during a bAP can regulate dendritic integration when incoming synaptic inputs are occurring. These results indicate a precise functional role of dendritic N-type VGCCs activated by bAPs.

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Poster

PSTR522. Calcium Channels

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Program #/Poster #: PSTR522.05/C59

Topic: B.03. Ion Channels

Support: DFG Grant HE3604/11-1

Title: Bassoon phase separation directs Cav2.1 calcium channels presynaptic organization

Authors: *M. BORGHI[1], C. AMARAL[1], A. EL KHALLOUQI[1], A. FEJTOVA[2], M. HEINE[1]; [1]Inst. for Developmental Biol. and Neurobio., Johannes Gutenberg Univ. Mainz, Mainz, Germany; [2]Dept. of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Abstract: In presynaptic nerve terminals, Cav2.1 calcium channels are essential for fast neurotransmitter release and synaptic plasticity. Together with synaptic vesicles and a wide array of scaffolding proteins, they form the release machinery of the presynaptic active zone. Albeit efforts in the past have uncovered many peculiarities of the release machinery's structure, how Cav2.1 channels are specifically arranged and their dynamic movement is orchestrated in concert with active zone rearrangements remains poorly understood. Indeed, presynaptic proteins such as RIM, RBP2, and Bassoon mediate tethering of Cav2.1 channels close to synaptic vesicles through specific protein-protein binding domains. However, deletion of individual binding domains results in mild dysregulation of Cav2.1 channels localization and vesicle release. Moreover, previous studies have shown that RIM and RBP2 can assemble in phase-separated condensates, however the ability of calcium channels to sequester in these condensates is still elusive. Thus, we hypothesize that the mechanisms by which Cav2.1 channels dynamically

rearrange inside release sites could be mediated either by direct interaction with scaffolding proteins or through a general trapping in a phase-separated compartment. In HEK293T cells, we found Bassoon to have the ability to undergo liquid-liquid phase separation. Computational modelling further supported this observation by predicting the presence of several intrinsically disordered regions in the Bassoon structure that can act as means for the protein to organize in highly condensed biological assemblies (also known as membraneless compartments). In primary hippocampal neurons, single particle tracking revealed that Bassoon condensates influence Cav2.1 surface mobility and confinement. Taken together, our results report a critical role of Bassoon phase separation in governing Cav2.1 dynamics in the presynaptic active zone.

Disclosures: **M. Borghi:** None. **C. Amaral:** None. **A. El Khallouqi:** None. **A. Fejtova:** None. **M. Heine:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.06/C60

Topic: B.03. Ion Channels

Support: R01DC016324

Title: Presynaptic T-type calcium channel $Ca_V3.2$ in the calyx of Held

Authors: ***Y. DARWISH**, T. XIAO, T. WU, H. HUANG;
cell and molecular biology, Tulane Univ., New Orleans, LA

Abstract: At chemical synapses, action potentials trigger neurotransmission while subthreshold depolarization of membrane potential profoundly facilitates subsequent spike-evoked neurotransmitter release probability and enhances synaptic strength. Synaptic transmission thus relies on a hybrid between ‘analog’ resting membrane potential and ‘digital’ action potential. In recordings made from the mouse calyx of Held, a giant mammalian glutamatergic terminal, we found that the T-type Ca^{2+} channel $Ca_V3.2$ is responsible for depolarization-dependent regulation of release. Two-photon Ca^{2+} imaging revealed that low voltage depolarizations evoke a Ca^{2+} increase, which is blocked by TTA-P2 (T-type blocker) and ascorbic acid (a nonspecific inhibitor of $Ca_V3.2$) and is absent in $Ca_V3.2$ knockout mice. Immunohistochemistry confirmed the expression of $Ca_V3.2$ in the calyx of Held terminals. The presynaptic $Ca_V3.2$ activates just below the resting potential. As a result, blockers of $Ca_V3.2$ hyperpolarizes nerve terminals and reduces mEPSC frequency as well as EPSC amplitude. In paired pre- and postsynaptic recordings, a brief depolarization of the terminal activates $Ca_V3.2$, which increases presynaptic cytosolic Ca^{2+}, and enhances glutamate release. However, in the presence of TTA-P2 or ascorbic acid, a stronger depolarization (> -60 mV) is required to facilitate synaptic transmission. In conclusion, we show that $Ca_V3.2$ is expressed in the calyx of Held, that it contributes to resting membrane properties and mediates the depolarization-induced facilitation of neurotransmitter release.

Disclosures: **Y. Darwish:** None. **T. Xiao:** None. **T. Wu:** None. **H. Huang:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.07/C61

Topic: B.03. Ion Channels

Support: Penn State College of Medicine's Comprehensive Health Studies Program
NIH Grant NS105987

Title: Investigating store-operated calcium entry as a novel mechanism for shaping calcium homeostasis in nodose ganglia neurons

Authors: *S. L. STELLA, Jr.[1], H. J. GOUDSWARD[2], G. M. HOLMES[3];
[1]Neural and Behavioral Sci., Penn State Univ. Hershey-College of Med., Hershey, PA; [2]Penn State Col. of Med. Neurosci. Grad. Program, Hershey, PA; [3]Neural & Behavioral Sci., Penn State Univ. Col. of Med., Hershey, PA

Abstract: Nodose ganglia neurons play a crucial role in transmitting sensory information from the viscera to the central nervous system, contributing to the regulation of vital physiological processes. Calcium ions are integral to neuronal signaling and transmitter release, and disruptions in calcium homeostasis can impact neuronal function. In this study, we investigated the presence of store-operated calcium entry (SOCE) in nodose ganglia neurons and its potential role in influencing calcium homeostasis. To explore SOCE we measured $[Ca^{2+}]_i$ changes in isolated nodose ganglia neurons using the Ca^{2+}-sensitive dye, Calbryte 520, and monitored calcium entry following store depletion with 2,5-di-t-butyl-1,4-benzohydroquinone (BHQ). Additionally, immunocytochemistry was performed on nodose ganglia with specific antibodies to the STIM and Orai proteins to determine the localization and expression levels. Cell viability was confirmed by measuring $[Ca^{2+}]_i$ increases following KCl-evoked depolarization of nodose ganglia neurons and stimulation of P2X and P2Y receptors with 100 µM ATP. Both STIM1 and Orai1 expression was observed in nodose ganglia neurons. Store depletion using 10 µM BHQ in a Ca^{2+}-free superfusate initially increased cytoplasmic Ca^{2+} levels, which gradually decreased over time. After the initial rise induced by BHQ, the calcium stores remained depleted during the application of a Ca^{2+}-free superfusate, presumably detected by the Ca^{2+} sensor STIM1. Switching to a superfusate with 2 mM Ca^{2+} elicited an immediate rise in cytoplasmic Ca^{2+} due to SOCE, which was effectively inhibited by 30 µM 2-APB. This is the first report of SOCE entry in nodose ganglia, and reveals a novel mechanism with substantial implications for the regulation of calcium in nodose ganglia, underscoring its potential to influence neurotransmission from these sensory neurons.

Disclosures: **S.L. Stella:** None. **H.J. Goudsward:** None. **G.M. Holmes:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.08/C62

Topic: B.03. Ion Channels

Support: MSCRFD-5633
T32GM008181

Title: Using iPSC-derived neurons to investigate the role of $Ca_V1.2$ dysfunction in the pathogenesis of autism spectrum disorder

Authors: *K. HEROLD, M. BAMGBOYE, D. C. O. VIEIRA, D. DISILVESTRE, S. BROWN, J. HUSSEY, J. O. OWOYEMI, I. E. DICK;
Univ. Maryland, Baltimore, Baltimore, MD

Abstract: There is a significant connection between autism spectrum disorder (ASD) and the dysregulation of Ca^{2+} signaling as determined through pathway and genetic analyses. Additionally, L-type Ca^{2+} calcium channels (LTCCs) have been identified as associating with ASD in a number of GWAS and functional studies and it has been well documented that these channels are important for the normal functioning of the brain. Timothy syndrome (TS), a multi-system disorder known to produce both cardiac and neurological symptoms, is the result of a *de novo* point mutation in the LTCC $Ca_V1.2$ and TS patients exhibit developmental delay and ASD. The high penetrance of ASD in TS patients make it an ideal model system for understanding the role the LTCC $Ca_V1.2$ has in ASD pathogenesis and calcium dysfunction as it relates to ASD. Previous work has indicated that there may be a link between enhanced LTCC channel activation, which is seen in some TS mutations, and the development of the neurological features associated with TS, including ASD. In order to study how changes in $Ca_V1.2$ gating, such as enhanced $Ca_V1.2$ activation, impact neuronal function in the context of a human neuron, we utilized human induced pluripotent stem cell (iPSC)-derived excitatory neurons harboring select $Ca_V1.2$ TS mutations. Initial voltage clamp recordings from these neurons demonstrate that there are marked differences in the biophysical properties of the $Ca_V1.2$ channels harboring the TS mutation compared to the wildtype channel. In addition, current clamp recordings reveal that there are differences in action potential properties between distinct neuronal populations, demonstrating an effect of the TS mutations at the level of a single neuron. Overall, the study of rare mutations like TS through these iPSC-derived neurons can provide us with a better understanding of ASD pathogenesis, particularly since Ca^{2+} disruption may be a recurrent characteristic of ASD.

Disclosures: K. Herold: None. **M. Bamgboye:** None. **D.C.O. Vieira:** None. **D. Disilvestre:** None. **S. Brown:** None. **J. Hussey:** None. **J.O. Owoyemi:** None. **I.E. Dick:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.09/C63

Topic: B.03. Ion Channels

Support: T32AR007592
MSCRF Post-Doctoral Fellowship

Title: Elucidating the mechanisms underlying novel mutations in CACNA1A rare disease.

Authors: *A. A. KRAMER[1], E. G. GUDMUNDSSON[2], K. W. BARANANO[3], R. A. BANNISTER[4], I. E. DICK[1];
[1]Univ. of Maryland Baltimore, Baltimore, MD; [2]Morgan State Univ., Baltimore, MD; [3]Johns Hopkins, Baltimore, MD; [4]Natl. Inst. on Aging, Bethesda, MD

Abstract: *CACNA1A* encodes the voltage-gated calcium channel $Ca_V2.1$ which is highly expressed in Purkinje neurons and at the neuromuscular junction, where these channels are critical for controlling the release of neurotransmitters. Individuals harboring mutations in this gene present with a wide array of symptoms including Familial hemiplegic migraine type 1, epilepsy, Episodic ataxia type 2, neuromuscular eye disorders and hypotonia. These mutations have historically been classified as gain-of-function (GOF) or loss-of-function (LOF), but recent reports have demonstrated that a variety of distinct biophysical changes may occur in response to different mutations, which is likely representative of the increasing diversity of *CACNA1A* disease presentations. This diversity of functional effects underscores the need to fully investigate the impact of novel mutations on distinct aspects of channel gating in order to move forward both the basic understanding of $Ca_V2.1$ pathogenesis and also to maximize the translational/therapeutical potential. Here, we use biophysical approaches to evaluate previously uncharacterized mutations in *CACNA1A* which were identified in patients presenting with complex clinical phenotypes including congenital ataxia, hemiplegic migraine and epilepsy. We find that these mutations exhibit diverse effects on distinct properties of channel gating, including voltage dependence of activation, inactivation and current density. By studyivng these mutations and correlating the results with patient presentations we hope to gain new insights into pathological mechanisms underlying *CACNA1A* rare disease.

Disclosures: A.A. Kramer: None. **E.G. Gudmundsson:** None. **K.W. Baranano:** None. **R.A. Bannister:** None. **I.E. Dick:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.10/D1

Topic: B.03. Ion Channels

Support: NIH Grant R01NS055251

Title: Cell-specific expression of a Cav2.1 channel splice isoform that regulate CDF is regulated by CTCF binding to the Cacna1a gene

Authors: *M. S. SISTI[1], R. MEIR[2], D. LIPSCOMBE[1];
[1]Dept. of Neurosci., [2]Neurosci., Brown Univ., Providence, RI

Abstract: Cell-specific expression of a CaV2.1 channel splice isoform that regulates CDF is controlled by CTCF binding to the *Cacna1a* gene
M. S. Sisti, R. Meir and D. Lipscombe; Neuroscience Department, Brown University,Providence, RI.
Disclosures M. S. Sisti: None. R. Meir: None. D. Lipscombe: None.
CaV2.1 voltage gated calcium channels dominate in controlling transmitter release from mammalian synapses. *Cacna1a* gene encodes the CaV2.1 α1 subunit and it contains a number of alternatively spliced exons that are tissue specific. We previously identified the mechanism of tissue-specific alternative splicing that controls the expression of a functionally important exon pair, e37a and e37b, in the closely related *Cacna1b* gene (Lopez-Soto & Lipscombe, 2020). Here we test if cell-specific splicing of homologous e37a of *Cacna1a* share a common regulation mechanism with *Cacna1b*, even though the cell-specific expression patterns of e37a exons from *Cacna1a* and *Cacna1b* are different. We and others have shown that CTCF, the DNA binding protein CCCTC-binding factor and a master regulator of gene expression, that might regulate alternative splicing via integrating chromatin looping and co-transcriptional factors. (Shukla et al., 2011; Lopez-Soto & Lipscombe, 2020). In *Cacna1b*, cell-specific hypomethylation within the CTCF binding motif of e37a promotes CTCF binding and exon recognition/inclusion. We have now shown that e37a of *Cacna1a*, like e37a of *Cacna1b*, binds CTCF. Using the electrophoretic mobility shift assay we show direct, specific and saturable binding of CTCF to *Cacna1a* e37a, but not to *Cacna1a* e37b. *Cacna1a* e37a dominates across DRG sensory neurons (Zheng et al., 2019) and it is expressed in various neurons in brain. For example, RNA-seq data show *Cacna1a*-e37a splice isoforms dominate in *Scnn1*, PV andSST cells of cerebellar cortex while *Cacna1a*-e37b dominates in the hippocampus. This pattern of expression is very different from *Cacna1b* e37a which is enriched in *Trpv1* nociceptors but not in most other regions of the nervous system. We test the hypothesis that the *de novo* DNA methyltransferase DNMT3a accessibility to CpG sites in *Cacna1a* e37a and *Cacna1b* e37a controls the methylation state of e37a. Specifically, that DNMT3a has restricted access to e37a in neurons where its expression is high resultingin hypomethylation, CTCF binding, and e37a inclusion. CaV2.1 channels containing the e37a encoded motif exhibit strong calcium-dependent facilitation (CDF), a feature that is absent in CaV2.1-e37b channels. Elucidating the mechanism that controls *Cacna1a*-e37a expression will give insight into the cell-specific origins of CDF-dependent synaptic facilitation.

Disclosures: M.S. Sisti: None. **R. Meir:** None. **D. Lipscombe:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.11/D2

Topic: B.03. Ion Channels

Title: Computational Modelling of the Dopamine Neuron Reveals Cocaine Exposure Modulates the Firing Patterns by Inhibiting Small Conductance CalciumActivated Potassium Current

Authors: *C. MAHAPATRA;
Indian Inst. of Technol. Bombay, Mumbai, India

Abstract: Objective The neurological reason behind the loss of dopamine neurons causing Parkinson's disease (PD) is an unsolved mystery. Background As PD isidentified as a classic disorder of "brain arrhythmias", several pharmacological targets are under clinical trial to rejuvenate neurons for evoking normal firingpatterns. With experimental evidence, this in silico study investigates action potential (AP) oscillation patterns of dopamine neurons towards cocaine exposure.Methods This single compartmental in silico model comprises the inward rectifier ion channels, voltage-gated sodium channel, voltage-gated potassiumchannel, L-type calcium channel, large-conductance calcium-dependent potassium (BK) channel, small conductance calcium-dependent potassium (SK) channel,and calcium diffusion mechanisms. All ion channels are expressed by the conventional Hodgkin-Huxley formalism. Cocaine exposure (1mg/kg to 10mg/kg)profile is reflected as the conductance of SK channel is mimicked by changing the maximum conductance of SK channel in dopamine neuron. Results Afterinjecting a current stimulus (Istim) of varying magnitude (0.1-0.6nA) and duration (1-5ms), APs are reproduced by the whole-cell model. The modulating effectsof cocaine exposure on dopamine neurons' electrophysiological properties are investigated in two folds. First, we simulated the current-voltage profile of the SKion channel with respect to multiple doses of cocaine under the voltage clamp protocol. It showed the continuous decrease of outward current because ofmultiple doses of cocaine from 1mg/kg to 10mg/kg. Then, the altered SK ion channel outward current is incorporated into the whole-cell model to investigatethe AP firing patterns. The frequency of the firing patterns is elevated for the cocaine dose of 10mg/kg when the cell in injected by the current stimulus.Conclusions Cocaine works on a membrane receptor pathway to inhibit the extracellular calcium entry into the cell. As a result, a few SK ion channels areactivated across the membrane and it reduces the whole-cell outward current. The reduced outward current elevates the cell's excitability for AP generation. Ourin-silico study interprets a sub-cellular mechanism linking cocaine-evoked altered ion channel activity to neuronal firing patterns, shedding light on novelpharmacological targets for PD.

Disclosures: C. Mahapatra: None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.12/D3

Topic: B.03. Ion Channels

Support: DoD W81XWH-21-1-0544

Title: Inhibition of endocannabinoid catabolizing enzyme fatty acid amide hydrolase to reduce chronic neuropathic pain following spinal cord injury

Authors: *E. SIPPLE[1,2], K. GUNARATNA[3], H. LIU[3], J. LAUZADIS[2], M. KACZOCHA[3], M. PUOPOLO[3];
[1]Anesthesiol., Med. Scientist Training Program, Stony Brook, NY; [3]Anesthesiol., [2]Stony Brook Med., Stony Brook, NY

Abstract: Chronic neuropathic pain is a secondary complication affecting up to 60-70% of people with spinal cord injury (SCI). SCI-induced neuropathic pain (SCI-NP) is often lifelong and therapeutically intractable, resulting in a severe decline in quality of life and increased risk for depression, anxiety, and addiction. Anticonvulsant and antidepressant drugs are first-line analgesics used to treat SCI-NP, but their efficacy is very limited, resulting in many cases in a decrease of only 20-30% in pain intensity. Data from our laboratory showed that the increased activity of T-type calcium channels induced by the injury is responsible for driving nociceptors' hyperexcitability and for promoting the development/maintenance of SCI-induced neuropathic pain (SCI-pain) (Lauzadis et al., J Neurosci. 2020 Sep 16;40(38):7229-7240). The endocannabinoid anandamide (AEA) has been shown to directly inhibit T-type calcium channels, suggesting that inhibition of endocannabinoid catabolizing enzyme fatty acid amide hydrolase (FAAH) (with subsequent increase in the levels of AEA and inhibition of T-type channels) may represent a therapeutic strategy to reduce SCI-nociceptors' hyperexcitability and SCI-NP. SD rats (300-350 g) were used in this study. SCI was performed by a midline spinal cord contusion at T10 by using an Infinite Horizon Impactor (150 kilodynes, 1s dwelling time). The mechanical allodynia was measured with the von Frey filaments and the up-down method with the 50% threshold. Spontaneous pain was measured with the conditioned place preference (CPP) paradigm. The action potential clamp technique was used in dissociated dorsal root ganglia (DRG) neurons isolated from SCI and sham rats to measure the T-type calcium charge (sensitive to 1 µM TTA-P2) during the interspike interval from -80 mV to -50 mV. In SCI rats, the 50% mechanical threshold dropped from 20.1±1.9 g (pre-injury) to 12.4±1.6 g (post-SCI) and to 12.9±1.5 g following vehicle injection. PF3845 (10 mg/kg, FAAH inhibitor) increased the 50% mechanical threshold to 21.0±2.6 g at 1-hour post-injection and to 20.4±2.9 g at 3-hour post-injection (n=9). SCI rats subjected to the CPP paradigm showed an increase in the PF3845-paired chamber of 55±32 s and a decrease of -40±37 s in the vehicle-paired chamber (n=9). In voltage clamp experiments in SCI-nociceptors, the interspike T-type calcium charge dropped from 56±18 pC/pF (n=12) in control to 10±3 pC/pF (n=12) in the presence of 5 µM PF3845. Taken together, our data suggest that inhibition of FAAH reduces the activity of T-type calcium channels in SCI-nociceptors and reduces both mechanical hypersensitivity and spontaneous pain following SCI.

Disclosures: E. Sipple: None. **K. Gunaratna:** None. **H. Liu:** None. **J. Lauzadis:** None. **M. Kaczocha:** F. Consulting Fees (e.g., advisory boards); Artelo Biosciences. **M. Puopolo:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.13/D4

Topic: B.03. Ion Channels

Support: NHMRC Grant APP1188169
UQ PhD Scholarship
University of Queensland (UQ) internal funds

Title: Environmental regulation of transmitter release by non-uniformly distributed voltage-gated calcium channel subtypes at amphibia neuromuscular junctions

Authors: *V. J. CHEN, P. G. NOAKES, N. A. LAVIDIS;
Sch. of Biomed. Sci., Univ. of Queensland, Brisbane, Australia

Abstract: We have previously reported that quantal transmitter release from Toad (*Bufo marinus*) neuromuscular junctions (NMJs) is reduced during the dry season and upregulated during the wet season. Additionally, the morphology of the neuromuscular junction does not significantly change between the seasons, we showed that the NMJs are more sensitive to extracellular calcium during the wet season (Ge & Lavidis 2017, 2018). This suggests that the change in synaptic transmission across seasons is achieved by the regulation of voltage-gated calcium channels (VGCCs). Here, we examined which VGCCs are responsible for quantal neuro-transmitter release during the dry and wet seasons. Toads between 45mm to 55mm in length were collected and euthanised by double pithing, as approved by the University of Queensland Animal Ethics Committee. The *iliofibularis* muscle with its motor nerve supply was dissected and prepared for neuromuscular electrophysiology. In brief, the muscle was pinned out in a recording bath perfused with modified Krebs solution gassed with O_2 95%, CO_2 5% (pH7.4) and contained 0.4 mM $CaCl_2$ at room temperature (18 ± 2 C^o). Quantal neuro-transmitter release from motor nerve terminals was examined using intracellular and focal extracellular microelectrode electrophysiology, using similar stimulating, and recording parameters to our previous papers (Ge and Lavidis, 2017, 2018). Motor nerve terminal branches for recordings were located using DiOC2 (5)-fluorescent imaging (Ge & Lavidis, 2017). To determine the specific VGCC contribution to synaptic transmission, we used ω-conotoxin GVIA (0.5mM) to identify N-type VGCCs and ω-agatoxin IVA (100μM) to identify P/Q-type VGCCs. 25 NMJs from dry season were compared to 17 NMJs from wet season toads. We found that N-type VGCCs are responsible for 88.7%±8.8% of the quantal release during the dry season and 70.2%±34.8%% during the wet season. P/Q-type VGCCs involvement in quantal release increased from 18.9%±11.7% in the dry season to 36.3%±18.9% during the wet season. Following $DiOC_2$(5)-fluorescence to aid in focal extracellular examination of quantal release, our results indicated a non-uniform distribution of P/Q-type channels. Our present study indicates that the upregulation quantal transmitter release during the wet season is due to P/Q-type VGCCs.

Ge & Lavidis 2017. Doi: 10.1152/ajpregu.00070.2017 Ge & Lavidis 2018. Doi: 10.1152/ajpregu.00263.2017.

Disclosures: V.J. Chen: None. **P.G. Noakes:** None. **N.A. Lavidis:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.14/D5

Topic: B.03. Ion Channels

Support: 5T32 NS077889

Title: Age and Sex Differences in Spontaneous Neuronal Firing in the Hippocampus: Insights from Calcium imaging using a Synapsin-CreGCAMP6s mouse model

Authors: *T. UJAS, N. TAVAKOLI, S. G. MALONE, T. L. ANDERSON, J. TURCHAN-CHOLEWO, P. I. ORTINSKI, A. M. STOWE;
Univ. of Kentucky, Lexington, KY

Abstract: Background: Calcium imaging has proven a key tool that provides an indirect but accurate measure of action-potential generation within neurons. The present study focuses on investigating age and sex differences in spontaneous neuronal firing in the hippocampus of the transgenic C57BL/6 synapsin-Cre/GCaMP6S$^{+/-}$ mouse strain. Methods: C57BL/6 synapsin-Cre/GCaMP6S$^{+/-}$ adult (4-8mo; n=6) and aged adult (10-18mo; n=6) mice were used. 7 animals were male and 5 were female, these were split between the adult and aged adult groups. Brains were extracted and sectioned into 300µm slices, oxygenated, and kept at 37ºC in aCSF, using an NMDG (N-methyl-D-glucamine) solution to improve cell health. Spontaneous neuronal activity in the CA1 and Dentate Gyrus (DG) of the hippocampus was recorded using wide-field calcium imaging. Data from 453 CA1 cells and 668 DG cells were recorded in total. For each cell, a fluorescence trace was generated by averaging all pixels within ROI outline in each recording frame. The associated background signal was removed from each cell's fluorescence trace. Calcium transients were identified using a wavelet ridgewalking algorithm. Statistical analyses were performed in GraphPad Prism. Results: We found that the aged males had significantly higher DG amplitudes over other groups (Kruskal-Wallis Test, $p = 0.0119$). Additionally, we saw more significance differences in amplitude in the DG versus the CA1. When comparing age groups, we saw that amplitude was significant in the CA1 (Mann-Whitney Test, $p = 0.0289$ for CA1). Aged adults showed higher amplitudes. When looking at sex differences we observed a strong difference in the duration of calcium signals in DG in Male v. Female groups (Mann-Whitney Test, $p<0.0001$). Females had a longer signal time over males. Finally, event frequency was only significant in the DG between various groups, but not in the CA1 (Kruskal-Wallis Test, $p <0.0001$). The key takeaway from the results show that aged males had the highest amplitudes in the DG, while female groups had the longest duration of signals in the DG. Conclusions: Our

experimental optimizations on this transgenic line proved successful and valuable in establishing essential data for comparing the hippocampal signaling of naïve animals to that of post-stroke animals in our ongoing investigation.

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Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.15/D6

Topic: B.03. Ion Channels

Support: Italian Ministry of University and Research Grant PRIN 2017ANP5L

Title: Voltage-gated calcium channels and NMDA glutamate receptors are both necessary for initiation of cortical spreading depression

Authors: M. VITALE[1], A. TOTTENE[1], M. ZARIN ZADEH[1], K. BRENNAN[2], *D. PIETROBON[1];
[1]Univ. Padova, Padova 35100, Italy; [2]Univ. of Utah Sch. of Med., Salt Lake City, UT

Abstract: There is increasing evidence from human and animal studies that cortical spreading depression (CSD) is the neurophysiological correlate of migraine aura and a trigger of migraine pain mechanisms. The mechanisms of initiation of CSD in the brain of migraineurs remain unknown, and the mechanisms of initiation of experimentally induced CSD in normally metabolizing brain tissue remain incompletely understood and controversial. Here, we investigated the mechanisms of CSD initiation by focal application of KCl in mouse cerebral cortex slices. High KCl puffs of increasing duration up to the threshold duration eliciting a CSD were applied on layer 2/3 whilst the membrane potential of a pyramidal neuron located near the site of CSD induction and the intrinsic optic signal were simultaneously recorded. This was done before and after the application of a specific blocker of either NMDA or AMPA glutamate receptors (NMDARs, AMPARs) or voltage-gated Ca^{2+} (Ca_V) channels. If the drug blocked CSD, stimuli up to 12-15 times threshold were applied. Blocking either NMDARs with MK-801 or Ca_V channels with Ni^{2+} completely inhibited CSD initiation by both CSD threshold and largely suprathreshold KCl stimuli. Inhibiting AMPARs with NBQX was without effect on the CSD threshold and velocity. Analysis of the CSD subthreshold and threshold neuronal depolarizations in control conditions and in the presence of MK-801 or Ni^{2+} revealed that the mechanism underlying ignition of CSD by a threshold stimulus (and not by a just subthreshold stimulus) is the Ca_V-dependent activation of a threshold level of NMDARs (and/or of channels whose opening depends on the latter). The delay of several seconds with which this occurs underlies the delay of CSD initiation relative to the rapid neuronal depolarization produced by KCl. These data show that both NMDARs and Ca_V channels are necessary for CSD initiation, which is not

determined by the extracellular K^+ or neuronal depolarization levels per se, but requires the Cav-dependent activation of a threshold level of NMDARs. Our data give insights into potential mechanisms of CSD initiation in migraine

Disclosures: **M. Vitale:** None. **A. Tottene:** None. **M. Zarin Zadeh:** None. **K. Brennan:** None. **D. Pietrobon:** None.

Poster

PSTR522. Calcium Channels

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.16/D7

Topic: B.03. Ion Channels
3R01MH131719-01S1
1R01MH131719-01

Title: Novel Cav3.3 potentiators rescue rodent Cav3.3 hypofunction mediated sleep spindle and cognitive deficits relevant for the unmet needs of patients with schizophrenia

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Abstract: Schizophrenia is a debilitating disorder that lacks effective treatments for the negative symptoms (e.g., social withdrawal) and cognitive disruptions (e.g., working memory deficits) in patients. Recently, large scale human genetics studies including genome wide association studies (GWAS) and exome sequencing studies have identified many replicable genomic loci for schizophrenia risk. CACNA1I was implicated in schizophrenia risk by GWAS and rare variations, and it encodes the functional core, α1 subunit, of Cav3.3 voltage-gated calcium channels. Cav3.3 channel expression is enriched in a subset of neurons including GABAergic neurons of the thalamic reticular nucleus (TRN) where they regulate neuronal excitability. The TRN has emerged as a crucial brain nucleus in the generation of sleep spindles, sleep dependent memory, focused attention, and cognitive flexibility, all of which are impaired in patients with schizophrenia. Our group and others have demonstrated that loss of Cav3.3 dramatically impairs TRN neuronal firing and reduces sleep spindles occurrences in mice, and reduction of sleep spindles is a highly reproducible trait of schizophrenia patients. Based on these genetic and biological evidence, we hypothesize that Cav3.3 potentiators could benefit patients with schizophrenia by rescuing sleep spindle deficits and improving sleep dependent cognitive function. Utilizing high throughput molecular pharmacology, automated patch clamp electrophysiology, ex vivo and in vivo electrophysiology, and animal behavior, we identified a

set of potent Cav3.3 potentiators that selectively enhance Cav3.3 function. Furthermore, our Cav3.3 potentiators increase sleep spindles in healthy adult mice and selectively rescue sleep spindle deficits and cognitive deficits in genetic models of Cav3.3 hypofunction. Given the wealth of literature demonstrating a critical role of Cav3.3 function in generating sleep spindles, our demonstration of the first selective Cav3.3 potentiator may represent an exciting step towards the development of a novel class of potential therapeutics for the treatment of sleep disturbances in patients with schizophrenia.

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Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.17/D8

Topic: B.03. Ion Channels

Support: R01 MH115045-01
R01MH118298
U54 NS108874

Title: Functional characterization of missense variants of CACNA1A from patients with neurodevelopmental disorders

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Abstract: *CACNA1A* encodes the Cav2.1 P/Q-type voltage-dependent calcium channels that express throughout regions of brain. Cav2.1 channels play an important role in calcium influx mechanisms that underlies neuronal excitability and presynaptic neurotransmitter release. Coding variants of *CACNA1A* have been implicated in epileptic encephalopathies (EE), familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6), and neurodevelopmental disorders. However, the molecular phenotypes of *CACNA1A* underlying the pathophysiology of these disorders are still elusive. Here, we analyzed an allelic series of 40+ de novo missense changes of *CACNA1A* identified in a large cohort of 31,058 parent-offspring trios of individuals with developmental disorders. In addition, we included 6 novel de novo or likely de novo variants of *CACNA1A* from patients at Boston

Children's and Children's Hospital of Philadelphia (CHOP) in our analyses to characterize the functional properties of *CACNA1A* variants implicated in the *CACNA1A* disorder. We performed functional evaluation of *CACNA1A* missense variants using automated patch-clamp (SyncroPatch384) and compared the functional properties of Cav2.1 channels encoded by de novo variants with those of channels harboring coding changes identified in the Genome Aggregation Database (gnomAD) of normal population. We analyzed four biophysical properties, including the whole cell current density, the voltage-dependent activation/inactivation and the kinetics of inactivation and deactivation, of the missense variants from the cohort and gnomAD. Majority missense variants from the neurodevelopmental cohort encode channels with significantly reduced current densities compared to those identified in gnomAD. Interestingly, several missense variants from neurodevelopmental cohorts showed leftward shift in voltage-dependent activation, while majority of missense variants exhibited rightward shift in voltage-dependent inactivation. We also obtained clinical information from a subset of variants and aim to gain insights on the correlation between molecular phenotypes and clinical manifestations. Taken together, the results of our functional analysis of an allelic series of *CACNA1A* variants from cohort and control groups may provide important insights on the role of *CACNA1A* in neurodevelopmental disorders.

Disclosures: E. Kurganov: None. **N. Budnik:** None. **S. Sooyeon Jo:** None. **A. Poduri:** None. **I. Helbig:** None. **J. Pan:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.18/D9

Topic: B.03. Ion Channels

Support: CIHR F21-04038

Title: Cav3.2 T-type calcium channels modulate dentate gyrus granule cell excitability in a maturational stage dependent manner

Authors: *A.-S. SACK[1,2,3], R. GOPAUL[2,3], E. GARCIA[2,3], T. P. SNUTCH[2,3];
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Abstract: Epileptic seizures are thought to occur as a result of hyperexcitable neurons driving aberrant synchronous activity in the brain. Owing to their properties, low voltage activated T-type calcium channels are well suited to mediate this type of activity and have been implicated in several types of epilepsy. The Cav3.2 T-type channel subtype is highly expressed in the dentate gyrus (DG), a region suggested to be involved in the development of acquired epilepsies. While previous work has implicated Cav3.2 in DG mediated behavior, our understanding of the contribution of Cav3.2 to DG granule cell (GC) excitability remains incomplete. As new neurons

are continuously added via neurogenesis, GCs are a heterogenous population of cells with varying degrees of maturation. To understand how Cav3.2 shapes DG excitability, a comparison of the role of Cav3.2 in GCs at distinct maturational stages is required. Here, we used whole-cell patch clamp recordings in acute brain slices from young (3-4-week-old) male and female C57BL/6 and Cav3.2 knockout (KO) mice to examine the contributions of Cav3.2 to GC excitability at three maturational stages: immature, intermediate and mature. GCs were assigned to a stage based on their intrinsic electrophysiological properties which follow a well-defined pattern during maturation. Our results indicate that Cav3.2 KO slowed the decay of the instantaneous firing frequency during suprathreshold stimulation in intermediate and mature GCs. This led to increased GC excitability as evident during 4-8 Hz stimulation where Cav3.2 KO GCs fired at higher frequencies. Similar results were found with acute pharmacological blockade of T-type calcium channels in WT using the T-type antagonist Z944. In immature GCs, Z944 but not genetic KO inhibited the low threshold calcium spikes characteristic of this maturational stage. Further, Cav3.2 KO did not modify the proportion of cells with immature firing patterns. At the circuit level, field recordings showed that Cav3.2 KO mice were impaired in both mature and immature GC dependent forms of medial perforant path long-term potentiation. Overall, we have identified a maturational stage dependent role for Cav3.2 in GC excitability, where the functional contributions of Cav3.2 increases with GC maturation and loss of Cav3.2 enhances excitability in intermediate and mature GCs. Our results point to a role for Cav3.2 in the filtering of afferent excitation in the DG, where Cav3.2 promotes the low intrinsic excitability of GCs. These findings might shed light on the development of acquired epilepsies such as epilepsy that develops after traumatic brain injury where this filtering function appears reduced.

Disclosures: **A. Sack:** None. **R. Gopaul:** None. **E. Garcia:** None. **T.P. Snutch:** None.

Poster

PSTR522. Calcium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR522.19/D10

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF: IOS-2224262

Title: Ror2 activation regulates phospholipids metabolism through plc and gq protein activation.

Authors: *R. RIQUELME[1], A. BARRIA[2];
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Abstract: The neuronal Wnt signaling pathway regulates the trafficking of NMDARs toward synapses and lowers the threshold for synaptic plasticity. Different Wnt signaling cascades also play crucial roles in embryonic development, tissue homeostasis, and cell differentiation by regulating cellular processes such as cell proliferation, cell fate determination, and cell

migration. One Wnt receptor, the Receptor tyrosine kinase-like orphan receptor 2 (RoR2), is a cell surface receptor that belongs to a conserved family of tyrosine kinase receptors. RoR2 binds to Wnt5a and exerts its function regulating development, neuronal branching, and synapses formation. In our laboratory we have identified that RoR2 is involved in neuronal function by activating PKC and JNK, increasing dendritic calcium (Ca2+) levels, increasing trafficking of GluN2B-containing NMDARs, and depolarizing neurons through PLC activation. PLC is an enzyme involved in various signaling pathways, and exerts its functions by cleaving a specific phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP2), into two secondary messengers: inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 serves as a second messenger that induces the release of Ca2+ from intracellular stores. The increased concentration of Ca2+ can then initiate various downstream signaling events, including the activation of protein kinase C (PKC), which is involved in diverse cellular processes. Considering the previous evidence, we hypothesize that RoR2 may play a role in regulating phospholipid metabolism. To test this hypothesis, we utilized fluorescence probes for PiP2, IP3 and DAG, as well as inhibitors for PLC and Gq protein. Our results show that RoR2 can activate PLC, leading to the generation of IP3 and DAG, which further mediate cellular responses in the context of neuronal Wnt signaling.

Disclosures: R. Riquelme: None. **A. Barria:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.01/D11

Topic: B.04. Synaptic Transmission

Support: UK Ministry of Defence (MOD)

Title: In vitro and ex vivo investigations into the activity of alternative broad-spectrum treatments of nerve agent poisoning

Authors: J. GUNN, M. E. PRICE, J. CONNAH, J. E. H. TATTERSALL, *C. WHITMORE, A. C. GREEN;
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Abstract: Nerve agents inhibit acetylcholinesterase (AChE), the enzyme responsible for degradation of acetylcholine (ACh) to terminate neurotransmission at cholinergic synapses, including the neuromuscular junction. The accumulation of ACh at muscarinic (mAChR) and nicotinic (nAChR) receptors leads to the effects of nerve agent poisoning including miosis, increased secretions, seizures, and respiratory failure. Current nerve agent treatments contain a muscarinic antagonist to reduce the effect of excess ACh at mAChR, an oxime to restore AChE activity and a benzodiazepine to reduce or prevent seizures. In this combination, the oxime is the only component targeting overstimulation of the nAChR, albeit indirectly. The reliance on oxime enzyme reactivators is a fundamental weakness in this approach, since no single oxime

demonstrates adequate reactivating activity against all the known nerve agents. It has been demonstrated that addition of MB327, a compound shown to reduce muscle-type nAChR activity by open channel block, offers therapeutic benefit. MB327 has a narrow therapeutic window and therefore there is an on-going search for alternative treatments to improve function at nerve agent-exposed cholinergic synapses. Our screening pipeline begins *in vitro* using plate-based assays and automated patch-clamp electrophysiology. This is followed by *ex vivo* studies utilising a guinea pig phrenic nerve/hemidiaphragm preparation to assess functional recovery. Dstl are currently investigating several classes of compounds as possible alternative treatments. We are exploring the potential ability of bispyridinium compounds to act as allosteric modulators, and/or increase desensitisation of the human muscle-type nAChR. We are also investigating the activity of 'multi-target' ligands. These compounds are primarily anti-muscarinic but also show anti-nicotinic activity and may have a role in reducing polypharmacy within nerve agent treatments. Finally, a number of partial agonists or allosteric modulators of neuronal nAChR have been explored for additional activity at the muscle-type nAChR that may make them suitable candidate medical countermeasures. Here we present the data we have obtained to date on these classes of compounds which have reached various points in the screening pipeline.© Crown copyright 2023, Dstl

Disclosures: **J. Gunn:** None. **M.E. Price:** None. **J. Connah:** None. **J.E.H. Tattersall:** None. **C. Whitmore:** None. **A.C. Green:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.02/D12

Topic: B.04. Synaptic Transmission

Support: R01 AA027023

Title: Opioids differentially regulate transmission from subclasses of prefrontal GABAergic interneurons

Authors: *R. P. D. ALEXANDER, K. J. BENDER;
Neurol., UCSF, San Francisco, CA

Abstract: Opioid signaling is strongly associated with motivation and reward, and as such has a high liability for abuse and addiction. Multiple opioid receptor subtypes are expressed throughout limbic and cortical structures, including prefrontal cortex (PFC), often with overlapping distribution patterns. In the PFC, delta opioid receptors (DORs) are expressed primarily by GABAergic interneurons where they inhibit synaptic release; however, whether the mechanism of release suppression is conserved between inhibitory subclasses is not known. Here we describe differential DOR modulation of GABAergic inputs in PFC. Application of the DOR-selective agonist DPDPE strongly suppressed electrically-evoked inhibitory currents on layer 5

pyramidal neurons, but had variable effects on short-term plasticity (STP), as assessed by paired-pulse ratio (PPR). Optogenetic targeting of select GABAergic subpopulations in PFC revealed that DPDPE suppressed release from both parvalbumin- (PV+) and somatostatin-expressing (SOM+) interneurons, but with notable differences. All PV+ neurons exhibited canonical presynaptic depression accompanied by increased PPR, while we observed two distinct forms of presynaptic regulation of SOM+ responses. In most SOM+ terminals, GABA release was suppressed without corresponding PPR changes, while others exhibited canonical increases. 2-photon calcium imaging demonstrated that DPDPE reduced action potential-evoked transients in both subtypes, and we are currently investigating the possibility of differential calcium channel regulation. These results demonstrate that the same opioid receptor can regulate inhibitory synapses via multiple mechanisms, even within the same cortical microcircuit. Since inhibitory neurons gate the flow of information through PFC circuitry, subtype-specific release modulation has significant implications for opioid research.

Disclosures: R.P.D. Alexander: None. **K.J. Bender:** 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.; BioMarin Pharmaceutical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regal Therapeutics.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.03/D13

Topic: B.04. Synaptic Transmission

Support: JSPS KAKENHI Grant Number 23H02633

Title: Phospholipid flippases ATP8A1 and ATP8A2 regulate the functional expression of synaptic proteins in hippocampal neurons

Authors: *M. KAWASE[1], T. MATSUDA[1], Y. UMEMURA[1], H. OISHI[2], T. SAKURAI[3], M. HATTORI[4];
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Abstract: Phospholipids are asymmetrically distributed between lipid bilayers in the plasma membrane. This asymmetry is partly regulated by flippases, which transport phosphatidylserine (PS) and phosphatidylethanolamine from the extracellular or lumenal side to the cytoplasmic side. PS asymmetry is important for the prevention of phagocytosis of normal cells and for the formation and fusion of transport vesicles. Among mammalian flippases, ATP8A2 is mainly

expressed in the nervous system, and its dysfunction causes severe motor deficits and higher brain dysfunction. ATP8A1, which has high structural homology with ATP8A2, is located in synaptic vesicles, and mice lacking ATP8A1 exhibit spatial memory deficits. However, their expression patterns in neurons and molecular mechanisms by which they contribute to neural functions are largely unknown. In this study, we aim to clarify the significance of ATP8A1 and ATP8A2 in the nervous system.We generated ATP8A1-deficient (ATP8A1 KO) and ATP8A2-deficient (ATP8A2 KO) mice using the CRISPR/Cas9 system. We then crossed them to generate ATP8A1/ATP8A2 double-deficient (DKO) mice. The amount of glycosylated TMEM30a, the common auxiliary subunit of flippases, was markedly reduced in the DKO mice, suggesting that ATP8A1 and ATP8A2 are major flippases in the brain. We stained primary cultured neurons derived from these mice with PS-binding probes and found that the exposure of PS under unstimulated conditions was observed only in the neurites of DKO neurons. We also found that ATP8A2 is abundantly localized in neurites, especially at inhibitory synapses, suggesting that ATP8A2 may be involved in the regulation of inhibitory synapses. Thus, we performed surface biotinylation experiments using primary cultured neurons and found that the surface expression level of membrane proteins of the inhibitory synapses changed in the neurons of ATP8A1 KO or ATP8A2 KO mice. Our results suggested that both ATP8A1 and ATP8A2 contribute to neural functions by regulating inhibitory synaptic transmission. We are currently exploring the mechanisms by which flippases affect the localization of membrane proteins at inhibitory synapses.

Disclosures: **M. Kawase:** None. **T. Matsuda:** None. **Y. Umemura:** None. **H. Oishi:** None. **T. Sakurai:** None. **M. Hattori:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.04/D14

Topic: B.04. Synaptic Transmission

Support: Swedish research council: 2020-00559

Title: Physical activity may restore neurotransmission in emotional circuits during nicotine abstinence

Authors: *E. LUCENTE, A. DOMI, D. CADEDDU, M. ERICSON, L. ADERMARK; Univ. of Gothenburg, Göteborg, Sweden

Abstract: ***Background****:* Nicotine addiction is a major contributor to the global burden of disease and finding novel interventions to facilitate nicotine cessation is warranted. Emotional dysregulation during nicotine abstinence appears to be associated with impaired amygdala functioning, and normalizing neurotransmission in the amygdala may thus represent a neurobiological target for novel cessation aids. Importantly, later research suggests that learning

new motor skills might be a way to improve neuroplasticity after extended nicotine exposure, and physical activity might thus provide new means to restore brain function. ***Aim****:* The aim of this study was to define the effect by repeated nicotine exposure on neuronal function in the basolateral amygdala (BLA) of female rats, and to determine if learning a new motor skill could act beneficial to restore neurotransmission. ***Methods****:* Female Wistar rats received either nicotine (0.36 mg/kg free-base, s.c.) or vehicle (0.9% NaCl) in a discontinuous manner over three weeks (15 injections), and behavioral sensitization to the locomotor stimulatory properties was monitored in locomotor activity boxes. During nicotine abstinence, animals were allowed to stay in their home cage, or to receive training on the Rotarod. Rotarod training was conducted for four trials a day over five consecutive days. Following Rotarod training, neurotransmission in the BLA was defined by whole cell electrophysiological recordings conducted *ex vivo*. ***Results****:* Repeated nicotine exposure produced a robust behavioral sensitization toward the locomotor stimulatory properties of nicotine. Furthermore, there was a trend towards increased time spent on the rod when monitoring Rotarod performance in rats receiving repeated nicotine exposure. Whole cell recordings demonstrated a significant reduction in the frequency and amplitude of spontaneous excitatory post synaptic currents (sEPSCs), and action potential firing was robustly suppressed in BLA neurons from animals receiving repeated nicotine injections. Nicotine-treated rats trained for five days on the rotarod, however, demonstrated no sustained changes in any neurophysiological parameter when compared to vehicle treated rats receiving rotarod training. ***Conclusions****:* Our results show that repeated nicotine exposure leads to a sustained depression of neuronal excitability in the BLA, but that a brief period of physical activity that involves motor skill learning is sufficient to restore neurotransmission. Learning new motor skills might thus be a way to reduce emotional dysregulation during nicotine abstinence, and putatively facilitate nicotine cessation.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.05/D15

Topic: B.04. Synaptic Transmission

Support: FONDECYT 1200908
FONDECYT 1211082
Millennium Nucleus for the Study of Pain NCN19_038
ANID Doctorado 21201176

Title: Qualitative analysis and actions on neuronal function of a Drimys winteri seed extract

Authors: *M. E. MEZA[1,2], O. FLORES[1], J. GAVILÁN[1], O. RAMÍREZ-MOLINA[1], C. CASTILLO[1], F. FIGUEROA[2], C. PÉREZ[2], J. BECERRA[2], G. E. YÉVENES[1], J.

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Abstract: *Drimys winteri* (*Dw*) seeds are an alimentary product that is currently marketed as "cinnamon pepper" due to its organoleptic characteristics: strong pungency, slightly sweet taste, and balsamic aftertaste with menthol aroma. Despite having unique organoleptic characteristics, the neuroactive compounds responsible for these features have not been characterized. Furthermore, the biological properties or the potential toxicity of these seeds are unknown. However, previous studies have shown that the *Dw* leaves and bark possesses several sesquiterpenes that modulate ion channels, including nicotinic acetylcholine receptors, TRPV1 channels, and voltage-activated Ca^{2+} channels. Therefore, the aim of this study was to define the chemical nature of *Dw* seeds, to subsequently characterize the biological activity of its compounds with focus on the synaptic function. Using gas chromatography coupled to mass spectrometry, we determined that a typical *Dw* seed extract is composed by a 76% sesquiterpenes, 6% monoterpenes, 6% lignans, 6% phytosterols, and 6% of other compounds. The effects of these extracts (1,10 and 100µg/ml) on cell viability were evaluated in PC12 cells, using the 3-(4,5dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Acute treatments (3 hours) ad no cytotoxic effects. However, chronic treatment (24 hours) with 100 µg/ml significantly decreased the cell viability the compared to the control (59.44% ± 3.092, $p<0.001$). We then studied whether these preparations affect intracellular Ca^{2+} dynamics on cultured mouse cortical neurons using Ca^{2+} microfluorometry. Brief treatments (1 hour) with *Dw* seed extracts significantly decreases the amplitude (1 µg/ml, 64.55% ± 1.435, $p<0,001$; 10 µg/ml, 29.50% ± 0.5707, $p<0,001$) and the slope (1 µg/ml, 46.77% ± 1.252, $p<0.001$; 10 µg/ml, 24.81% ± 0.6728, $p<0.001$) of the intracellular Ca^{2+} transients. On the other hand, the frequency of intracellular Ca^{2+} signals was not significantly affected (1 µg/ml, 91.19% ± 8.206; 10 µg/ml, 100.1% ± 7.753). Our studies provide novel information of the chemical composition of *Dw* seeds and explore its potential effects in cell viability and neuronal function. Due to their abundance, we suggest that the modulation of the intracellular Ca^{2+} transients and of cell viability is potentially related to sesquiterpenes. Further characterization of these molecules may identify novel neuroactive compounds useful for basic research or biomedical purposes. Ongoing electrophysiological assays will determine the synaptic targets underlying these effects.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.06/D16

Topic: B.04. Synaptic Transmission

Support: NIH Grant NS120628

Title: Input-specific regulation of discrete populations of lateral habenula neurons by kappa opioid receptors

Authors: S. S. SIMMONS[1], W. J. FLERLAGE[2], B. M. COX[3], F. S. LISCHKA[1], J. S. SMYTH[4], ***F. S. NUGENT**[2];
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Abstract: The lateral habenula (LHb) is an epithalamic brain region associated with value-based decision making and stress evasion through its modulation of dopamine (DA)-mediated reward circuitry. Increased activity of the LHb is associated with drug addiction and stress-related mood disorders. Additionally, dynorphin (DYN)/Kappa opioid receptor (KOR) signaling is an endogenous mediator of stress response in reward circuitry. Previously, we have shown a novel functional role of KOR signaling in LHb of rats which is altered by severe early life stress, 24h maternal deprivation. In non-stressed rats, KOR activation has bidirectional effect on LHb neuronal excitability, which is altered in maternal deprived rats. Here we used several methods to elucidate the neural circuitry and synaptic integration of DYN/KOR signaling in LHb in both rats and mice. First, we used the unbiased high-throughput approach of in-vitro GCAMP calcium signaling in the LHb of mice to identify effects of KOR agonist (U50,488) on LHb spontaneous neuronal activity. To identify KOR-expressing or DYN-expressing projections to the LHb we used viral retrograde tracing in KOR-Cre and DYN-Cre mice and identified entopenduncular nucleus (EP) as a major KOR-expressing input. EP has been implicated in stress-induced mood disorders and may contribute to aberrant LHb excitability in depressive-like phenotypes. KOR activation significantly reduced LHb action potential generation in a subset of LHb neurons in response to optical stimulation of EP inputs. This suggests input and cell-type specific KOR regulation of LHb neurons. In the future we will explore the effects of early life stress on KOR modulation of LHb activity in projection and input-specific manner.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

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Program #/Poster #: PSTR523.07/D17

Topic: B.04. Synaptic Transmission

Support: DA R33041876
Department of Pharmacology and Toxicology
Stark Neurosciences Research Institute
IUSM Strategic Research Initiative

Title: Spinophilin and Neurabin: Biochemical and Functional Divergence in Homologous Synaptic Scaffolding Proteins

Authors: *N. SHAH[1], W. COREY[3], A. J. BAUCUM II[2];
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Abstract: Spinophilin and neurabin are homologous, striatum-enriched scaffolding proteins that regulate dendritic spine morphology, target Protein Phosphatase 1 (PP1), and regulate G protein signaling. Despite considerable structural and functional overlap, knockout studies demonstrate that in the striatum mediated rotarod learning task, neurabin ablation enhances performance, whereas spinophilin ablation impairs performance. However, how these structurally similar proteins bidirectionally impact rotarod performance is unclear. To begin to identify differences between these proteins, a series of RNAScope experiments were conducted in an 8-week-old adult male and female mouse to establish basal distribution of spinophilin and neurabin transcripts within the mouse brain. Ten-micron sagittal sections were obtained in correspondence with plate numbers in the Mouse Brain Atlas. Anatomically similar sections were first stained separately with either a spinophilin probe or neurabin probe, and subsequently, simultaneously with both probes to ensure staining fidelity. Qualitative analysis demonstrated that neurabin and spinophilin displayed largely overlapping distribution within the cortex, striatum, cerebellum, and olfactory bulb, whereas neurabin transcripts predominated in brain stem, and spinophilin transcripts predominated in the thalamus. High resolution confocal studies demonstrated that within regions of transcript overlap, spinophilin mRNA appear enriched in puncta within the neuropil, consistent with local, dendritic spine translation, whereas neurabin mRNA are more nuclear localized, consistent with more classical cell-body mRNA translation. Subsequently, a different subset of mice was subject to standard training (3 trials/day for 5 days, n=3) or intensive training (10 trials/day for 8 days, n=3) on a 5-minute accelerating rotarod paradigm (4-40 RPM). Immunoblot analysis of whole striatal lysates demonstrates rotarod training increases striatal neurabin but not spinophilin protein expression and increases levels of spinophilin and neurabin interacting proteins such as GluA2, as compared to untrained controls (n=3). Collectively, these exploratory studies further delineate unique biochemical properties for spinophilin and neurabin and these, along with ongoing studies, suggest that unique regulation or actions of these proteins may underlie mechanisms by which they bidirectionally impact striatal motor learning.

Disclosures: **N. Shah:** None. **W. Corey:** None. **A.J. Baucum II:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.08/D18

Topic: B.04. Synaptic Transmission

Support: Swedish research council: 2020-00559

Title: Association between neurophysiological properties in prelimbic prefrontal cortex and sub-dimensions of alcohol use in rodents

Authors: *D. CADEDDU[1], A. DOMI[1], E. LUCENTE[1], M. ERICSON[2], B. SÖDERPALM[2], L. ADERMARK[1];
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Abstract: Alcohol abuse is one of the main causes of death worldwide. Alcohol use disorder is diagnosed through the Diagnostic and Statistical Manual of mental disorders (DSM-5), and only a small percentage of alcohol users develops alcohol addiction. To further outline the neurophysiological underpinnings of alcohol use disorder we used an animal model aimed to characterise the inter-individual variability to develop alcohol addictive like-behaviour in rats followed by ex-vivo electrophysiological recordings. Rats were trained to self-administer alcohol in operant boxes for more than 60 days and where then assessed using three different criteria that define AUD-like behaviours based on the DSM-5. Specifically, we measured: i) persistence in responding during a period where alcohol was unavailable and promptly signalled, ii) motivation for alcohol in a progressive ratio (PR) schedule of reinforcement and iii) resistance to punishment when alcohol intake was anticipated by a mild foot shock. Rats that scored more than 66th of the total distribution were considered positive to a criterion. Based on the number of criteria met, rats were distributed in four groups: 0crit,1crit, 2crit and 3crit. To define if neurophysiological properties could be linked to the number of criteria met, whole cell and field potential recordings were performed in the prelimbic prefrontal cortex (PL-PFC) of brain slices from alcohol exposed rats and water drinking control rats. Whole cell recordings conducted in voltage-clamp mode demonstrated a decline in the frequency of excitatory inputs that correlated with the number of criteria met. Current-clamp recordings further showed increased excitability of PL-PFC neurons in the 3crit rats as compared to the other groups. Finally, field potential recordings performed in the PL-PFC demonstrated an increased GABAergic tone in all alcohol drinking groups, but a blunted response to mGluR 2/3 agonist selectively in brain slices from 3crit rats. In conclusion, the data presented here highlights the inter-individual variability in AUD-like behaviours among outbred Wistar rats and suggests that the PL-PFC may play a role in driving alcohol seeking behaviours.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.09/D19

Topic: B.04. Synaptic Transmission

Support: NIH Grant MH66198
NIH Grant AG055577

Title: Single synapse evaluation of spontaneous calcium transients in hippocampal dendritic processes

Authors: *C. I. MCCARTHY[1], L. M. MONTEGGIA[3], E. T. KAVALALI[2];
[1]Pharmacol., [2]Vanderbilt Univ., Vanderbilt Univ., Nashville, TN; [3]Vanderbilt Brain Inst., Vanderbilt Brain Inst., Nashville, TN

Abstract: Dendritic calcium signals elicited by spontaneous release events are critical for the local biochemical signaling and protein synthesis leading to synaptic plasticity. In this work, we use optical imaging of the Ca^{2+} sensor GCamp8s to dissect pharmacologically the origin of postsynaptic spontaneous calcium transients in rat hippocampal cultured neurons. We performed live imaging recordings of neurons sparsely transfected with GCamp8s, focusing on individual dendritic spines to isolate postsynaptic spontaneous calcium transients in the presence of activity blockers and under physiological Ca^{2+} and Mg^{2+} conditions. To locate individual active synapses, we perfused a 90 mM KCl solution at the end of each recording and used these responses to identify regions of interest (ROIs) around the local fluorescence maxima in dendritic spines. In the presence of activity blockers including sodium channels, AMPA, NMDA and GABAergic receptors blockers, we were able to detect rapid spontaneous calcium transients with absolute values that were at least 3 standard deviations above the mean of the preceding baseline period (3 s). These events occurred at a mean frequency of 0.049 ± 0.006 min^{-1} per ROI. We found that acute perfusion of TBOA, a blocker of glutamate transporters, significantly increased soluble GCamp8s spontaneous calcium transient frequency (0.154 ± 0.029 min^{-1} per ROI), without altering their amplitude or kinetic parameters. Interestingly, co-application of the mGluR5 antagonist, MPEP, prevented the effect of TBOA by returning the spontaneous calcium transient frequency to control levels. In further experiments, we expressed a fusion construct of GCamp8s with the postsynaptic scaffolding protein PSD95 (GCamp8s-PSD95) in order to target the calcium sensor specifically to the postsynaptic density. We found that TBOA once more produced an increase in the spontaneous calcium transient frequency, with no changes in the amplitude or kinetic parameters. In summary, we were able to isolate activity-independent calcium transients at the single synapse level in cultured hippocampal neurons. Our results suggest that these postsynaptic calcium signals are dependent on mGluR5, a G-protein coupled receptor that is known to modulate spine Ca^{2+} levels and contribute to fine-tuning of synaptic efficacy at postsynaptic sites. Further experiments are needed to understand the link between these Ca^{2+} transients and spontaneous glutamate release.

Disclosures: C.I. McCarthy: None. **L.M. Monteggia:** None. **E.T. Kavalali:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.10/D20

Topic: B.04. Synaptic Transmission

Title: Acetylcholine Modulation of Associative Interactions of two inputs in Hippocampal Granule Cells

Authors: *T. MONDEN[1], S. KIBA[1], N. NAKAJIMA[2], T. KAMIJO[3], T. AIHARA[1]; [1]Tamagawa Univ., Tokyo, Japan; [2]Kyushu Inst. of Information Sci., Kyushu, Japan; [3]Univ. of the Ryukyus, okinawa, Japan

Abstract: To investigate the associative mechanism among inputs to granule cells (GC) in the hippocampus, we measured the timing-dependent interaction between two inputs induced by stimulating the Lateral Perforant Path (LPP) with non-spatial information and the Medial Perforant Path (MPP) with spatial information. We also observed the influence of acetylcholine, an input from intrinsic neural circuits, on the interaction between the two inputs. The experiments were performed using hippocampal slices from Wistar rats. We conducted paired electrical stimulation with timings ranging from -40 msec to 40 msec, relative to the MPP input as a reference to the LPP input and recorded excitatory postsynaptic potentials (EPSPs) from the GC somatic region using extracellular recordings. We conducted the same experiments while applying the GABA(A) receptor inhibitor picrotoxin, the NMDA receptor inhibitor D-AP5, or the cholinergic agonist carbachol. Arithmetic waveforms of EPSPs were calculated using the two EPSP time courses obtained by a single stimulation of the LPP or MPP. Measured waveforms of EPSPs were obtained by using paired stimulation of the LPP and MPP. The ratio of the measured EPSP to the arithmetic EPSP was compared to observe the linearity of the response to the two inputs. As a result, when the LPP and MPP were stimulated simultaneously, the peak amplitude of the response from the two inputs was linearly summed, and there was no difference between the measured and arithmetic results. The result suggests that the combined response in GC detects the coincidence of the timing for the two inputs from LPP and MPP. It was also shown that NMDA receptors enhance linear summation, while GABA(A) receptors facilitate non-linear summation, applying different timings. Additionally, it was suggested that the cholinergic effect regulates the linearity in the two-input interaction by affecting NMDA receptors.

Disclosures: **T. Monden:** None. **S. Kiba:** None. **N. Nakajima:** None. **T. Kamijo:** None. **T. Aihara:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.11/D21

Topic: B.04. Synaptic Transmission

Support: ANID Fellowship 21202235
FONDECYT 1201816

Title: Maternal high-fat diet consumption during pregnancy and lactation impairs gabaergic efficacy in hippocampal pyramidal neurons of mouse offspring.

Authors: *C. CERNA[1], O. SANTANDER[1], N. VIDAL[2], G. CRUZ[1], M. FUENZALIDA[1]; [1]Fisiología, Univ. De Valparaíso, Valparaíso, Chile; [2]Inst. de Fisiología, Univ. de Valparaíso, Valparaíso, Chile

Abstract: Over the past two decades, maternal obesity (MO) during pregnancy has emerged as a significant global health concern, with projections indicating its continued rise in the future. Extensive evidence suggests that maternal obesity during pregnancy can have profound and long-lasting effects on the health and development of offspring. While the metabolic and cardiovascular consequences of MO have been extensively investigated, its impact on the central nervous system, particularly cognition, is an emerging area of research. The hippocampus, a brain region involved in learning and memory processes, is particularly vulnerable to insults during fetal development, including nutritional changes. Previous studies have demonstrated that maternal high-fat diet (mHFD) leads to cognitive deficits in offspring, accompanied by alterations in the neurogenesis and morphology of hippocampal neurons. However, it is the effect on synaptic transmission has not been examined. Considering the essential role of adequate balance between excitation and inhibition (E/I balance) for its cognitive functions, we hypothesize that the mHFD during the perinatal period would have consequences on synaptic transmission in the CA1 region. To investigate this hypothesis, we employed a maternal obesity model induced by HFD which was administered to female mice one month before pregnancy until the lactation period. We employed the novel object recognition (NOR) and object location memory (OLM) tests to assess memory-associated cognitive function. Our findings revealed impaired cognitive performance in the offspring exposed to mHFD. To gain insights into the underlying synaptic changes, we conducted electrophysiological recordings in CA1 pyramidal neurons in offspring mice. Notably, we observed that mHFD increased inhibitory synaptic transmission without any significant alterations in excitatory transmission. Taken together, these findings suggest that MO can enhance inhibitory synaptic efficacy within the hippocampus, which leads to E/I imbalance. Such modifications may have profound effects on hippocampal-dependent cognitive function in the offspring mice, providing further insights into the potential mechanisms underlying cognitive impairments associated with MO.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

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Topic: B.04. Synaptic Transmission

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Title: Impact of intrinsic neurotransmitter release properties on sensory representations in cerebellar cortex

Authors: *F. BENDER[1], B. SERMET[1], M. COSTREIE[1], A. BARRI[1], G. DIANA[1], S. BORDA BOSSANA[1], F. RUCKERL[1], A. HANTMAN[2], D. A. DIGREGORIO[1];
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Abstract: Neural activity flows through the circuits of the brain, encountering particular synaptic properties at every node. Here, we ask how these intrinsic synaptic properties can increase the signal processing capacity of networks. In the cerebellar cortex, the granule cell (GC) layer is thought to facilitate the discrimination of activity patterns arising from mossy fibers (MFs) in order to learn context-dependent, temporally precise actions. Our previous theoretical study proposed that short-term plasticity at the MF-GC synapse enhances decorrelations between input activity patterns and generates diverse activity basis sets within the population of GCs for temporal learning. We demonstrate, in awake mice, that air-puff stimulation of whiskers generates a prolonged activity response in mossy fibers, which is transformed into diverse, brief GC activity transients whose peak activity responses tile the duration of the stimulus. In acute brain slices, MF-GC synapses are diverse and generate precise temporal patterning of GC activity. Using genetically encoded glutamate indicators *in vivo* we monitored neurotransmitter release in response to air puffs and showed that their dynamics are more similar to GC dynamics than MF activity. Furthermore, GC temporal dynamics across cerebellar lobules mimicked differences in their synaptic dynamics. Together these results support the conclusion that the heterogeneity of short-term plasticity of MF-GC synapses sharpens GC responses and increases temporal sparsity. This synaptic transformation is likely to facilitate temporal associations needed to acquire the precise cerebellar cortical output dynamics required to fine-tune motor and cognitive actions.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.13/D23

Topic: B.04. Synaptic Transmission

Support: R01NS111749

Title: Group II mGluR Liberated Gβγ Inhibits AMPA Receptor Insertion in the Postsynaptic Membrane

Authors: S. HUYNH[1], C. DELBOVE[1], Z. ZURAWSKI[2], A. CABALLERO[3], K.-Y. TSENG[3], ***S. ALFORD**[1];
[1]Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL; [3]Anat. and Cell Biol., [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Long-term potentiation (LTP) is a form of synaptic plasticity that represents a critical step in learning and memory. Following LTP induction, the density of AMPA receptors (AMPARs), which mediate synaptic transmission and are located postsynaptically in synapses, increases, resulting in an increased excitatory postsynaptic current (EPSC). The two main types of AMPARs are calcium-permeable (CP), which are inwardly rectifying, and calcium-impermeable (CI) AMPARs. The insertion of AMPARs is necessary for maintaining LTP, and recent research suggests an especially important role of CP-AMPAR insertion in LTP. AMPAR insertion is mediated through the fusion of trafficking vesicles with the postsynaptic plasma membrane, a type of exocytosis. The exocytosis of AMPARs is facilitated by SNARE protein complexes consisting of proteins such as SNAP-25. Gβγ, as liberated by Gi/o-coupled G-protein coupled receptors (GPCRs), such as Group II metabotropic glutamate receptors (mGluRs), can inhibit exocytosis through binding to SNAREs such as SNAP-25, competing with fusogenic calcium sensors. These interactions have only been studied presynaptically. The effect of Gβγ on the exocytosis and insertion of AMPARs, especially CP-AMPARs, into the postsynaptic membrane is unknown. By comparing the effects of LTP on wild-type (WT) mice vs SNAP-25Δ3 mice, whose mutant SNAP-25 protein is unable to bind Gβγ, we can test whether Gβγ can regulate the exocytosis of AMPAR bearing vesicles through binding to the SNAP-25 t-SNARE complex. In extracellular field recordings, a Group II mGluR agonist inhibits LTP in WT mice but not SNAP-25Δ3 mice. Using iGluSnFR imaging, we show that the Group II mGluR agonist does not affect presynaptic glutamate release. However, from whole cell recordings, we show that the Group II mGluR agonist increases the ratio of CI-AMPARs to inwardly rectifying CP-AMPARs in WT mice but not SNAP-25Δ3 mice. Using Lattice Light-Sheet microscopy (LLSM) imaging of dendritic spines in acute slices, we show that the Group II mGluR agonist inhibits calcium fluorescence transients mediated by AMPARs in WT but not in SNAP25Δ3 mice. Additionally, LLSM imaging of HALO-tagged GluA1 AMPA subunits shows the Group II mGluR agonist changes the ratio of surface AMPARs to intracellular AMPARs. Taken together, we show that Gβγ from Group II mGluRs can inhibit LTP through inhibiting the insertion of AMPA receptor-bearing vesicles with the postsynaptic plasma membrane by binding to the SNAP-25 t-SNARE complex. Additionally, this inhibitory mechanism might preferentially inhibit the insertion of CP-AMPARs.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.14/D24

Topic: B.04. Synaptic Transmission

Support: NIH Grant MH114872

Title: Novel chemokine-like TAFA2 modulates GABAergic synaptic transmission

Authors: *C. P. NUNEZ[1], G. KEARNEY[1], T. KELLY[1], K. HEASTER[1], D. GRAU[1], Q.-S. LIU[2], S. H. LEE[2];
[1]Pharmacol. and Toxicology, [2]Pharmacol. and Toxicology, Neurosci. Res. Ctr., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The dysfunction and loss of inhibitory synapses are associated with multiple neurological and psychiatric diseases, making it important to uncover the molecular mechanisms that underlie these processes and how they affect neuronal function and behavior. TAFA2 (also called Fam19a2 and Sam2) is a chemokine-like, secreted protein highly expressed in the brain. Previous studies showed that global knockout of *TAFA2* increases anxiety and fear in mice and zebrafish, but the mechanisms that manifest this aberrant behavior remain uncertain. We used cultured rat hippocampal neurons and immunocytochemical markers of GABAergic synapses (vesicular GABA transporter and the γ2 subunit of $GABA_A$ receptor) to estimate the changes in the number of GABAergic synapses following overexpression of TAFA2. We found that TAFA2 overexpression or the application of purified TAFA2 protein causes a drastic loss of GABAergic synapses. Furthermore, incubation with TAFA2 protein caused a reduction in the amplitude and frequency of mIPSCs in rat hippocampal slices, indicating that TAFA2 modulates GABAergic synaptic transmission. Consistently, immunohistochemistry of *TAFA2* knockout mice showed greatly increased number of GABAergic synapses in the hippocampus. Calcineurin inhibitor cyclosporin A blocked TAFA2-induced GABAergic synapse loss and TAFA2 decreased the phosphorylation of S327 residue of $GABA_A$ receptor γ2 subunit by protein kinase C, suggesting that TAFA2 promotes dispersal of synaptic $GABA_A$ receptors. Further studies are ongoing in the lab to tease out the TAFA2 signaling pathway leading to GABAergic synapse loss. Taken together, our studies suggest that TAFA2 functions as a novel neuromodulator controlling GABAergic synaptic transmission and provide evidence that TAFA2-mediated disinhibition is involved in the control of emotional behaviors.

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Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.15/D25

Topic: B.04. Synaptic Transmission

Support: KAKENHI Grant 22K17169

Title: Muscarinic regulation of GABAergic synaptic transmission by ChAT-VIP interneurons in the rat cerebral cortex

Authors: *K. KANEKO[1], M. KOBAYASHI[2];
[1]Dept. of Anesthesiol., [2]Dept. of Pharmacol., Nihon Univ. Sch. of Dent., Chiyoda-ku, Japan

Abstract: Choline acetyltransferase (ChAT)-positive interneurons are immunopositive for vasoactive intestinal peptide (VIP) in layers II/III of the cerebral cortex. ChAT-VIP interneurons are activated via nicotinic receptors by cholinergic projections from the basal forebrain (BF). Several studies have reported that ChAT-positive interneurons in the neocortex do not directly exert inhibitory postsynaptic responses in neighboring pyramidal neurons (PyNs), but indirectly increase the frequency of spontaneous EPSCs in adjacent PyNs. It has also been reported that VIP-positive interneurons are activated by cholinergic inputs from BF, then they suppress the activities of other subtypes of interneuron particularly parvalbumin- and somatostatin-positive interneurons, resulting in the disinhibition of PyNs in the neocortex. However, little information is available regarding the cellular mechanisms of neuromodulation by ChAT-VIP double-positive interneurons. Therefore, we performed multiple whole-cell patch-clamp recordings from ChAT-VIP interneurons and adjacent GABAergic interneurons in the rat insular cortex (IC). The acute brain slices were obtained from ChAT-tdTomato and VGAT-Venus double-positive rats. To record the unitary synaptic transmission from ChAT-VIP interneurons to GABAergic interneurons, we targeted both ChAT-VGAT double-positive interneurons and VGAT single-positive interneurons using a multiple patch-clamp technique. We observed the chemical synapses from ChAT-VIP interneurons connecting to low threshold spike interneurons (LTS-INs) and fast-spiking interneurons (FS-INs). Unitary inhibitory postsynaptic currents (uIPSCs) in LTS-INs and FS-INs induced by ChAT-VIP interneurons were attenuated with bath application of atropine. These uIPSCs were blocked by picrotoxin and nicotinic responses were not observed in the postsynaptic GABAergic interneurons in IC. However, bath application of pilocarpine increased the amplitude of uIPSCs. Further, we coincidentally observed that ChAT-VIP interneurons had mutual connections with other ChAT-VIP interneurons via electrical gap-junctions, which resulted in their synchronized spike firings. These results suggest that cholinergic projections from BF activate ChAT-VIP interneurons via nicotinic receptors, and ChAT-VIP interneurons generate action potentials coordinately via electrical gap-junctions. And the co-released acetylcholine from ChAT-VIP interneuron may potentiate the GABAergic inhibitory synaptic strength via postsynaptic muscarinic receptors.

Disclosures: K. Kaneko: None. **M. Kobayashi:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.16/D26

Topic: B.04. Synaptic Transmission

Support: College of Arts and Sciences and Department of Neuroscience summer fellowship and Beckman Scholarship (K.E.B.).

Title: The bacterial endotoxin lipopolysaccharide (LPS) recruits synaptic vesicles for evoked transmission at glutamatergic synapses.

Authors: *R. MCINTOSH, K. BROCK, R. COOPER;
Univ. of Kentucky, Lexington, KY

Abstract: The direct action of the Gram-negative bacteria toxin, (i.e., lipopolysaccharides-LPS), affects synaptic transmission independent of systemic secondary immune responses. High concentration of LPS (500 μg/mL) from Serratia marcescens increased synaptic efficacy at glutamatergic synapse at the crawfish (Procambarus clarkii) neuromuscular junction (NMJ) ($N=6$; $P<0.05$). LPS appears to promote vesicles in the reserve pool to the readily releasable pool. The action of LPS at the glutamatergic synapses of the crayfish neuromuscular junction is unique in promoting synaptic transmission as compared to other glutamatergic synapses in Drosophila and mammals, where synaptic transmission is depressed. Through quantal analysis of evoked and spontaneous quantal events, we can also address if all the effect is presynaptic in recruiting vesicles only for evoked responses or randomly. By analysis in the shape of the quantal events the postsynaptic receptor sensitivity to glutamate is being examined. This content is being addressed with intracellular recording of the muscle at NMJ, as well as focal macropatch recordings over defined synaptic varicosities. To date, it appears evoked responses increase quantal content $N=6$ ($P<0.05$) without significant effects of the occurrences of spontaneous quantal events. This will help to address the direct effect of LPS on synaptic transmission.

Disclosures: R. McIntosh: None. **K. Brock:** None. **R. Cooper:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.17/D27

Topic: B.04. Synaptic Transmission

Title: Exploring synaptic vesicle protein function in human neurons using CRISPR/Cas9 gene editing

Authors: *A. HOUCEK[1], B. UZAY[2], E. T. KAVALALI[2];
[1]Vanderbilt Brain Inst., Nashville, TN; [2]Vanderbilt Univ., Vanderbilt Univ., Nashville, TN

Abstract: The advent of generating induced human neurons (iNs) from embryonic stem cells represents a milestone in the translation relevance of *in vitro* model systems. We have recently employed CRISPR gene editing in this iN model system to explore the impact of deleting two presynaptic proteins: the Ca2+ sensor for neurotransmitter release Synaptotagmin-1 (Syt1), and synaptic vesicle glycoprotein 2A (SV2A). Mutations in Syt1 and SV2A have been associated with neurodevelopmental diseases that include epileptic seizures and hyperkinetic movement disorders. SV2A is also a target of the anti-epileptic drug levetiracetam (Keppra) although it has a poorly understood function. Our glutamatergic iNs generated from Syt1 or SV2A KO stem cells show predicted phenotypes previously associated with loss of Syt1 or SV2A, including desynchronization of neurotransmitter release with respect to action potentials and increased short-term synaptic facilitation, respectively. This model system in combination with targeted gene editing may serve as a valuable platform for uncovering enigmatic protein function and mechanisms of therapeutic action in human synapses.

Disclosures: A. Houcek: None. **B. Uzay:** None. **E.T. Kavalali:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.18/D28

Topic: B.04. Synaptic Transmission

Support: NIH Grant MH124934

Title: Serotonergic regulation of excitatory synaptic transmission in the mouse prefrontal cortex

Authors: *E. LIU[1], A. T. GULLEDGE[2];
[1]Mol. & Systems Biol., Dartmouth Col., Hanover, NH; [2]Mol. & Systems Biol., Geisel Sch. of Med. At Dartmouth Col., Hanover, NH

Abstract: The prefrontal cortex (PFC) contributes to executive functions such as decision making, working memory, and emotional regulation. In the mouse medial PFC, serotonin (5-HT) modulates the intrinsic excitability of layer 5 pyramidal neurons according to their long-distance axonal projections. Less is known about how 5-HT regulates the excitatory synaptic transmission that drives projection neurons. To test the impact of 5-HT on excitatory synaptic transmission, we recorded spontaneous excitatory postsynaptic potentials (sEPSPs) in layer 5 pyramidal neurons in slices of prelimbic cortex from 6- to 12-week-old female and male C57Bl/6 mice. Layer 5 pyramidal neurons were classified as either intratelencephalic (IT) or pyramidal tract (PT) neurons based on their physiological characteristics, and sEPSPs were recorded in current-clamp with a slow bias ("dynamic holding") current to keep neurons near their initial resting membrane potential. In 14 IT neurons, bath application of 40 µM 5-HT for 7 minutes had no

effect on sEPSP amplitudes or frequencies but did cause a small outward hyperpolarizing current of 22 ± 6 pA ($p < 0.001$). In 30 PT neurons, 5-HT generated a much larger outward current (84 + 11 pA; $p < 0.001$; $p < 0.001$ vs IT neurons) and transiently increased sEPSP amplitudes and frequencies by 25 ± 8% ($p = 0.003$) and 20 ± 8% ($p = 0.013$), respectively. Unlike the long-lasting effects of 5-HT on postsynaptic holding current, increases in sEPSP amplitudes and frequencies reversed well before the end of 5-HT exposure (peaking at ~3 minutes after 5-HT application). This result is consistent with prior reports of 5-HT increasing spontaneous excitatory transmission in layer 5 neurons (e.g., Lambe et al., Cerebral Cortex 10:974-980, 2000), and further demonstrates that enhanced excitatory input occurs selectively in PT-type projection neurons.
To examine the impact of 5-HT on evoked excitatory synaptic transmission, we measured optically evoked EPSPs in 7 IT and 5 PT neurons in mice expressing channelrhodopsin-2 in one cerebral hemisphere (delivered by AAV injection). At a concentration of 10 µM, 5-HT suppressed light-triggered EPSPs by 52 ± 19% ($n = 12$; Student's t-test for paired data, $p = 0.023$), while at 40 and 100 µM, 5-HT suppressed EPSPs by 70 ± 5% and 71 ± 5%, respectively ($p < 0.001$ for each). The magnitude of serotonergic suppression of glutamate release from callosal afferents did not depend on the subtype of target neuron. These data demonstrate that 5-HT enhances network excitatory transmission selectively in PT neurons, but also suppresses glutamate release from some excitatory afferents targeting IT and PT neurons.

Disclosures: E. Liu: None. **A.T. Gulledge:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.19/D29

Topic: B.04. Synaptic Transmission

Title: Characterization of CaMKIIbeta in synaptic transmission and homeostatic plasticity

Authors: *A. WEIT[1], E. KAVALALI[1,2], L. MONTEGGIA[1,2];
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Abstract: Changes in synaptic strength (i.e. plasticity) are fundamental processes that allow us to adapt in response to environmental influences. Hebbian plasticity, caused by long-term potentiation (LTP) and depression (LTD), is an important form of plasticity that may underlie learning and memory formation in the hippocampus. Many studies indicate that the α isoform of calcium/calmodulin-dependent protein kinase II (CaMKIIα) is a critical sensor of activity-driven synaptic transmission and regulator of LTP. While the function of CaMKIIα in synaptic transmission and plasticity is well-described, our understanding of CaMKIIβ, the other major isoform in the hippocampus, remains underdeveloped. Previous studies suggest that CaMKIIβ could be involved in a different form of plasticity, homeostatic plasticity, which works differently than LTP by counteracting long-term global changes in activity to maintain a

homeostatic environment. Furthermore, studies have found CaMKIIβ protein expression is enriched in low activity environments driven by tetrodotoxin. Collectively, these data have led us to hypothesize that CaMKIIβ is a key regulator in multiple mechanisms of spontaneous transmission-mediated homeostatic upscaling. Here, we use whole cell patch clamp electrophysiology and microscopy in rodent hippocampal cultures to evaluate how manipulating CaMKIIβ expression and enzymatic activity influences upscaling in postsynaptic homeostatic mechanisms. In addition, we are also using microscopy to investigate the understudied basal function of CaMKIIβ in the presynapse. The completion of this study examining CaMKIIβ dynamics and function will provide novel insight into the role of CaMKIIβ in synaptic transmission and advance our understanding of fundamental and clinically relevant mechanisms of synaptic homeostasis.

Disclosures: **A. Weit:** None. **E. Kavalali:** None. **L. Monteggia:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.20/D30

Topic: B.04. Synaptic Transmission

Support: Office of the Principal Scientific Adviser (PSA) to the Government of India vide sanction number: Prn.SA/Epilep/2017(G).

Title: Molecular and cellular mechanisms of altered synaptic transmission in peritumoral cortex of low-grade gliomas.

Authors: ***J. BANERJEE**[1], K. KUMAR[2], V. DUBEY[5], A. DIXIT[6], M. TRIPATHI[7], R. DODDAMANI[3], M. C. SHARMA[4], P. CHANDRA[3];
[1]Biophysics, All India Inst. of Med. Sci. (AIIMS), New Delhi, India, New Delhi Delhi, India; [3]Neurosurg., [4]Neuropathology, [2]All India Inst. of Med. Sci. (AIIMS), New Delhi, New Delhi, India; [5]All India Inst. of Med. Sci. (AIIMS), New Delhi, India; [6]ACBR, Univ. of Delhi, Delhi, India; [7]Neurol., All India Inst. of Med. Sci., New Delhi, India

Abstract: Several patients with low-grade gliomas (LGG), like oligodendrogliomas and astrocytomas, present with a history of seizures where tumor-induced changes in the peritumoral neocortex is known to contribute to hyperexcitability. In this study we investigated the molecular and cellular players potentially involved in abnormal synaptic activity in peritumoral cortex of LGGs. Intraoperative peritumoral brain tissues resected from LGG patients with seizures (GRS) or without seizures (GNS) were used in this study. We correlated differentially expressed genes (DEGs) in GRS compared to GNS with the electrophysiological properties of pyramidal neurons in these samples. Histology was performed to assess the infiltration of proliferating cells at the peritumoral tissues. RNA sequencing (RNA-seq) followed by comparative transcriptomics was used to identify (DEGs) in GRS compared to GNS. GSEA was performed to identify

significantly enriched KEGG pathways and Gene Ontology terms. Expression of key genes was validated at the transcript and protein levels in the peritumoral region using qPCR and immunohistochemistry, respectively. Whole-cell patch clamp technique was used to measure the spontaneous glutamatergic and GABAergic synaptic activity onto pyramidal neurons in the peritumoral samples. We found that DEGs in GRS were highly enriched in the "Glutamatergic Synapse" pathway. We observed increased mRNA expression of glutamate receptor subunits GRIN2A (NR2A), GRIN2B (NR2B), GRIA1 (GLUR1), GRIA3 (GLUR3) and increased immunoreactivity for NR2A, NR2B, and GLUR1 proteins in the peritumoral tissues of GRS. We observed enhanced glutamatergic activity onto pyramidal neurons of the peritumoral samples of GRS. Although we observed higher frequency of spontaneous GABAergic synaptic transmission onto pyramidal neurons of the peritumoral samples of GRS, but there was no alteration in the expression of $GABA_A$ receptor subunits. It may be possible that excitatory GABAergic response renders peritumoral cortex hyperexcitable and susceptible to seizures in LGG. Despite similar histopathological features, the pyramidal neurons in the peritumoral samples of GRS and GNS patients showed differences in spontaneous excitatory and inhibitory synaptic neurotransmission. These findings suggest that altered glutamatergic transmission could be a driving factor in glioma-related seizures. This explorative study suggests a tight association between altered glutamatergic synaptic transmission and differential expression of glutamate receptor subunits in the peritumoral samples of patients with LGG presenting with seizures.

Disclosures: **J. Banerjee:** None. **K. Kumar:** None. **V. Dubey:** None. **A. Dixit:** None. **M. Tripathi:** None. **R. Doddamani:** None. **M.C. Sharma:** None. **P. Chandra:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.21/D31

Topic: B.04. Synaptic Transmission

Support: NIH/NINDS R15NS099850

Title: Quantal size affects synaptic fidelity in Huntington's disease mouse neuromuscular junctions

Authors: *K. M. BARNES[1], K. A. TRITTSCHUH[1], A. A. VOSS[1], A. SINGH[2];
[1]Biol. Sci., Wright State Univ., Dayton, OH; [2]Electrical & Computer Engin., Univ. of Delaware, Newark, DE

Abstract: Huntington's disease (HD) is a neurodegenerative disorder caused by an expanded trinucleotide repeat in exon 1 of the *huntingtin* (*htt*) gene. Symptoms include progressive cognitive decline and muscle defects, including bradykinesia, chorea, and muscle weakness. These muscle defects were previously attributed to neurodegeneration, but more recent work has shown that the ubiquitous expression of mutant *htt* also causes primary peripheral pathologies.

Previously, we reported muscle hyperexcitability due to a reduction in chloride currents in the late stage of the R6/2 mouse model of HD. This hyperexcitability amplifies changes in the membrane potential in R6/2 muscle. We also showed depressed neuromuscular transmission in R6/2 neuromuscular junctions (NMJs) during high-frequency stimulation, suggesting a decrease in vesicle mobilization. In this study, we sought to determine the factors contributing to this synaptic depression in 12-week R6/2 mice and age-matched control littermates. We designed a 3-part protocol consisting of 2 seconds of 50 Hz stimulation followed by 6 seconds of 2 Hz stimulation, repeated 3 times. Voltage clamp recordings of this 3-part protocol showed that the amount of charge passing through the R6/2 acetylcholine receptors decreased 67% compared to control, but the total number of synaptic vesicles released was only 1.5% lower in R6/2 than in control. Plasticity changes in control but not R6/2 NMJs contributed to the differences. The area of the control miniature endplate currents (mEPCs) increased 12% following the first 50 Hz stimulation and remained elevated during the protocol, whereas it only increased 0.4% in R6/2. The lack of an increase in quantal size appears to be responsible for the decreased charge movement in R6/2 muscle. In addition, computational modeling reveals a decrease in the refilling rate of the readily releasable pool in R6/2 NMJs. Current-clamp recordings of the same 3-part protocol showed no significant difference between control and R6/2 membrane potentials, indicating that the muscle hyperexcitability we previously reported is compensating for the reduced charge movement
caused by a constant quantal size and by the decreased vesicle mobilization. This work provides a mechanism to help explain central synaptic dysfunction in HD and helps us better understand the motor defects experienced in HD, both of which may lead to the development of future treatments. In addition, our discovery of increasing mEPC area in control muscle leads to a better understanding of exercise and fatigue in healthy animals.

Disclosures: **K.M. Barnes:** None. **K.A. Trittschuh:** None. **A.A. Voss:** None. **A. Singh:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.22/D32

Topic: B.04. Synaptic Transmission

Support: DP1EY033975

Title: Functionally diverse populations of inhibitory synapses innervate somatostatin-expressing interneurons in the visual cortex interneurons

Authors: *J. GUPTA[1], M. J. HIGLEY[2];
[1]Neurosci., Yale Med. Sch., New Haven, CT; [2]Neurobio., Yale Sch. of Med., New Haven, CT

Abstract: Somatostatin-expressing GABAergic interneurons (SST-INs) play a critical role in the normal function of neocortical circuits. These cells canonically innervate pyramidal cell

dendrites and influence synaptic integration and dendritic spiking. In addition to being excited by local glutamatergic circuits, SST-INs are also inhibited by GABAergic inputs from neighboring parvalbumin (PV) and vasoactive intestinal peptide (VIP)-expressing interneurons. Indeed, inhibition of SST-INs and subsequent PN disinhibition may be central to behavioral state-dependent modulation of cortical activity. However, the functional properties and potential differences of these two populations of GABAergic synapses on SST-INs are largely unknown. Here, we generate mice in which a subgroup of SST-INs are labeled with GFP (GIN line) and Cre recombinase is expressed transgenically in either PV- or VIP-INs. Adult double transgenic mice (PV-Cre;GIN or VIP-Cre;GIN) were injected with an adenoassociated viral (AAV) vector to express the excitatory opsin ChroME in PV- or VIP-INs of the primary visual cortex. We obtained whole-cell voltage clamp recordings from labeled SST-INs and optogenetically stimulated the presynaptic interneurons. Both pathways evoked robust inhibitory postsynaptic currents (IPSCs) mediated by A-type GABA receptors. However, analysis of IPSC kinetics and voltage-jump experiments suggest that VIP-INs and PV-INs form synapses distally and proximally, respectively, on SST-IN dendrites. In addition, paired stimulation indicated that PV-IN synapses exhibit higher release probability and greater short-term depression versus those formed by VIP-INs. Pharmacological studies demonstrated that GABA receptors comprising an α5 subunit contribute to VIP- but not PV-IN synapses. Finally, we examined the role of acetylcholine in shaping these inhibitory circuits. We found that muscarinic receptor activation strongly suppresses PV-IN inputs to SST-INs but does not alter inputs from VIP-INs. Overall, our results provide evidence that different populations of interneurons make distinct patterns of innervation along SST-IN dendrites and exhibit marked differences in their functional synaptic properties and response to modulatory influence. These findings will further our understanding of inhibitory microcircuit organization in the neocortex.

Disclosures: J. Gupta: None. **M.J. Higley:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.23/D33

Topic: B.04. Synaptic Transmission

Support: Beckman Scholar's Program

Title: The similarities and differences in the effects of bacterial endotoxin lipopolysaccharide (LPS) on synaptic transmission at glutamatergic synapses

Authors: *K. BROCK, R. MCINTOSH, R. COOPER;
Univ. of Kentucky, Lexington, KY

Abstract: The endotoxin lipopolysaccharides (LPS), secreted from Gram-negative bacteria, has direct effects on synaptic transmission independent of systemic secondary cytokine responses.

High concentrations of LPS (500 μg/mL) from *Serratia marcescens* increased synaptic efficacy at glutamatergic synapse at the crayfish neuromuscular junction (NMJ) but depressed synaptic efficacy at glutamatergic synapse at the larval *Drosophila* NMJ (N=12 for each; P<0.05). Both preparations resulted in transient hyperpolarization (N=6 for each; P<0.05). At the *Drosophila* NMJ, quantal responses and evoked excitatory junction potentials (EJPs) decreased in amplitude, and it appeared that postsynaptic glutamate receptors were blocked. However, this is not the case at the crawfish NMJ, despite the receptor subtypes at both NMJs being classified as quisqualate receptors. The hyperpolarization appears to be due to transiently activating K2p potassium channels at the *Drosophila* NMJ, since doxapram blocks the LPS response (N=6 for each; P<0.05). Doxapram is a therapeutic compound used clinically to improve the respiratory drive of carotid bodies by blocking the pH sensitive K2p channels (TASK subtypes). Doxapram (10 mM) depolarizes the larval *Drosophila* muscle in all preparations (N=6; P>0.5), but not as substantially for crayfish muscle (1 to 10 mV) for all preparations (N=6). Doxapram at the crayfish NMJ does not cause the NMJ to spontaneously fire action potentials as it does at the larval *Drosophila* NMJ. Doxapram does not block the LPS response on glutamate receptors in Drosophila but does block the hyperpolarization induced by LPS. Investigations at the crayfish NMJ is still underway. Perhaps doxapram will be able to be used to block the LPS direct responses during Gram-negative bacterial septicemia.

Disclosures: K. Brock: None. **R. McIntosh:** None. **R. Cooper:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.24/D34

Topic: B.04. Synaptic Transmission

Support: R37MH080046
R01MH119826

Title: A Dual Fluorescence Reporter Strategy for Imaging Synaptic Transmission

Authors: *S. T. BARLOW[1], T. A. BLANPIED[2];
[1]Physiol., Univ. of Maryland Baltimore, Baltimore, MD; [2]Dept. of Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Dysfunction of NMDA receptor (NMDAR)-mediated synaptic transmission is implicated in pathogenesis and progression of numerous diseases, and defining fundamental mechanisms of NMDAR activation in synapses is likely a precondition for understanding disruption in disease-associated states. An attractive approach for this is via direct optical dissection of synaptic transmission. We reasoned that GluSnFR3 measurements of glutamate release deployed in tandem with Ca^{2+} sensors targeted to the postsynaptic compartment could disentangle pre- and postsynaptic function at individual synapses. This would open new lines of

inquiry and enable discovery of, for instance, how spatiotemporal properties of glutamate release influence Ca^{2+} flux associated with NMDAR opening. To accomplish this, we established a dual-reporter approach using GluSnFR3 and a new solution to postsynaptic Ca^{2+} sensing based on HaloTag and the far-red Ca^{2+}-sensitive dye, JF646-HaloTagLigand-BAPTA-AM. We fused HaloTag to a Utrophin-binding domain, which selectively enriched the reporter in actin-rich compartments including dendritic spines. By co-expressing GluSnFR3 with this "spine-HaloTag" in dissociated rat hippocampal neurons, we achieved a direct optical readout of NMDAR-mediated Ca^{2+} flux following release of single presynaptic vesicles. To deploy this approach as an end-to-end assay in an easily scalable format, we integrated automated microscopy and data analysis routines to efficiently extract functional properties of hundreds of individual synapses imaged simultaneously. We validated the automated feature extraction with a number of manipulations, first incorporating automated fluid exchange for pharmacological and ionic titrations. We regulated presynaptic release probability and found that synaptic transmission efficacy, i.e. the consistency with which a Ca^{2+} transient followed a GluSnFR3 transient, was as expected proportional to the concentration of Ca^{2+} or inversely proportional to the concentration of Mg^{2+} in the bath. Postsynaptically, we observed that transmission efficacy was decreased significantly by the NMDAR antagonist APV. Together, this set of tools will facilitate dissection of how synaptic function is influenced by disease-relevant conditions and acute perturbations.

Disclosures: S.T. Barlow: None. **T.A. Blanpied:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.25/D35

Topic: B.04. Synaptic Transmission

Support: 5R01DA007418-28

Title: Amphetamine-mediated changes in midbrain neurons firing underline changes in striatal dopamine release

Authors: *E. MAKOWICZ;
Columbia Univ., Astoria, NY

Abstract: Amphetamine (AMPH) and its derivatives are highly addictive substances that elicit their response by increasing striatal extracellular dopamine (DA) levels. Mechanisms that have been suggested to account for this include depletion of DA vesicular stores and blockade and reversal of dopamine uptake transporters (DAT) thus promoting non-exocytotic DA efflux from striatal DA terminals. Additionally, in vivo studies suggest that AMPH augments action potential- mediated presynaptic DA release (Ramsson et al., 2011). Here, we studied the relationship between the firing activity of SNpc dopaminergic neurons and DA release in the

dorsal striatum evoked by midbrain electrical stimulation in vivo in anesthetized animals. We found a ~5-fod increase in evoked striatal DA release accompanied by a ~50% decrease in DA neurons spontaneous firing following 10 mg/kg i.p. AMPH injection. The effect of AMPH on striatal DA release appears to be calcium-independent. Furthermore, deficiency of alpha-synuclein - a protein implicated in Parkinson's Disease - diminishes AMPH-mediated increase in striatal evoked DA release. Although still preliminary, these results suggest that both inhibition of DAT and a decrease in tonic neuronal firing mediate the effect of the psychostimulant on striatal DA release. Further evaluation of the relationship between DA neuron tonic activity and DA release from their striatal terminals will help to better understand the mechanisms of AMPH and alpha-synuclein involvement in these processes.

Disclosures: E. Makowicz: None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.26/D37

Topic: B.04. Synaptic Transmission

Support: MH002386

Title: Pituitary adenylate cyclase-activating polypeptide (PACAP)-dependent neurotransmitter function at hypothalamic and central amygdalar synapses mediating stress endocrine and behavioral stress responses

Authors: *S. JIANG[1], H.-Y. ZHANG[2], W. BRANCALEONE[2], L. EIDEN[2];
[1]NIMH/NIH, Bethesda, MD; [2]NIMH, Bethesda, MD

Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a master regulator of central and peripheral stress responses. Peripherally, PACAP acts as a transmitter at splanchnicoadrenomedullary synapses to mediate catecholamine release following systemic or psychogenic stress. The synapses at which PACAP acts to regulate psychogenic stress responses in the brain are still being characterized. We used anatomically specific viral injection in PACAP-Cre and PACAP floxed mice to identify the PACAP-containing circuits that mediate behavioral responses, and hypothalamo-pituitary adrenal (HPA) axis activation, following acute psychogenic (restraint) stress (ARS). Surprisingly, endocrine (hypothalamic) responses to ARS, ablated by constitutive PACAP knockout in mice, were unaffected by hypothalamus-wide PACAP gene deletion in Sim1-Cre::PACAP$^{fl/fl}$ mice. Rather, ablation of PACAP expression in a newly-discovered projection from prefrontal cortex directly to the paraventricular nucleus of the hypothalamus impaired both c-fos activation and corticotropin-releasing hormone messenger RNA elevation in the paraventricular nucleus after 2 hours of restraint, without affecting ARS-induced hypophagia, or c-fos elevation in nonhypothalamic brain. ARS-induced hypophagia (anorexia), on the other hand, was eliminated in mice in which PACAP expression in projections

from lateral parabrachial nucleus to capsular centrolateral amygdala (CeCL) and oval bed nucleus of the stria terminal (ovBNST), the sole PACAPergic inputs to these two extended amygdalar nuclei, was eliminated. This occurred without affecting ARS-induced CRH messenger RNA elevation or fos elevation in the paraventricular nucleus. PACAP projections to CeCL and ovBNST terminate at protein kinase C delta type (PKCδ) neurons in both nuclei. Silencing PKCδ neurons in the central amygdala, but not in the ovBNST, attenuates ARS-induced hypophagia without affecting endocrine stress responses. Defining two separate limbs of the acute stress response provides broader insight into the specific brain circuitry engaged by the psychogenic stress response, and provides a potential alternative view to the hypercortisolemia hypothesis for explaining clinical stress-induced behavioral responses potentially underlying melancholic depression.

Disclosures: **S. Jiang:** A. Employment/Salary (full or part-time):; National Institute of Mental Health, NIH. **H. Zhang:** A. Employment/Salary (full or part-time):; National Institute of Mental Health. **W. Brancaleone:** A. Employment/Salary (full or part-time):; National Institute of Mental Health. **L. Eiden:** A. Employment/Salary (full or part-time):; National Institute of Mental Health.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.27/D38

Topic: B.04. Synaptic Transmission

Support: MUSC Institutional Research Acceleration Award

Title: Elevated O-GlcNAcylation depresses inhibitory synaptic neurotransmission onto GABAergic interneurons in CA1 stratum radiatum.

Authors: *M. BAGLEY, L. L. MCMAHON;
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: O-GlcNAcylation, a post translational modification resulting from the addition of O-GlcNAc moieties onto serine or threonine residues on target proteins plays a critical role in regulation of cellular processes throughout the body, including the brain. Addition of O-GlcNAc occurs via the enzyme, O-GlcNAc transferase (OGT), and it is removed by O-GlcNAcase (OGA), both of which are highly expressed in hippocampus. We previously reported that pharmacologically increasing O-GlcNAcylation induces long-term depression (LTD) of both evoked excitatory transmission (eEPSC) and inhibitory transmission (eIPSC) onto CA1 pyramidal cells and dentate gyrus granule cells. GABAergic interneurons play an important role in the excitation-inhibition balance in hippocampus, therefore, understanding whether their output is regulated by O-GlcNAcylation is key to hippocampal function. In this study, we investigated whether excitatory and inhibitory transmission onto GABAergic interneurons are

similarly depressed following an acute, pharmacologically induced increase in O-GlcNAcylation. We performed whole-cell voltage-clamp recordings from visually identified interneurons in CA1 stratum radiatum in acute slices from 5-12-week-old male and female rats. O-GlcNAcylation was increased by bath applying glucosamine (5mM) and the OGA inhibitor thiamet-G (1μM). Inhibitory (N=16, n=12), and excitatory transmission (N=12, n=6) transmission were pharmacologically isolated using AMPAR and NMDAR antagonists or $GABA_AR$ antagonists, respectively. As in CA1 pyramidal cells, we find that within minutes, increasing O-GlcNAcylation induced a long-term depression of eIPSCs onto interneurons (53% of baseline). However, the effect of increasing O-GlcNAcylation on eEPSC transmission displays a mixed effect with one-third of the population exhibiting a long-term depression and two-thirds exhibiting an acute potentiation followed by a prolonged depression. To gain further understanding of the variable effect of increasing O-GlcNAcylation on excitatory transmission onto interneurons, future experiments will explore subtypes of interneurons that contain AMPARs with specific subunit combinations.

Disclosures: M. Bagley: None. **L.L. McMahon:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.28/D39

Topic: B.04. Synaptic Transmission

Support: NIH Grant 1 F99 NS134163-01

Title: Forskolin reverses the O-GlcNAcylation dependent decrease in $GABA_AR$ current amplitude at hippocampal synapses possibly through action at the neurosteroid site on $GABA_ARs$.

Authors: *S. PHILLIPS[1], L. L. MCMAHON[2], J. C. CHATHAM[3];
[1]Univ. of Alabama at Birmingham, North Charleston, SC; [2]Med. Univ. of South Carolina, Charleston, SC; [3]Dept. of Pathology, Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Recent work from our lab and others have shown that increasing protein O-GlcNAcylation depresses GABAergic transmission in hippocampus and dentate gyrus. Serine phosphorylation and O-GlcNAcylation are fundamental modulators of $GABA_ARs$, yet no study has examined whether these modifications interact to control $GABA_AR$ function. For decades, forskolin has been used to activate adenylyl cyclase to drive protein kinase A (PKA) dependent phosphorylation of AMPARs at excitatory synapses in hippocampus, leading to potentiation. Therefore, using forskolin, we sought to determine whether PKA dependent serine phosphorylation before and after pharmacologically increasing O-GlcNAcylation inhibits or amplifies the effect on $GABA_AR$ function. Using whole-cell recordings of evoked IPSCs (eIPSCS) from CA1 pyramidal cells and dentate granule cells in acute slices from 3-5 week old

male and female rats, we bath applied forskolin (50μM), either before (*CA1*:N=9,n=11; *Dentate:*N=6,n=6) or after (*CA1:*N=10,n=11; *Dentate:*N=9,n=10) bath application of glucosamine (5mM) and the O-GlcNAcase inhibitor, thiamet-G (1mM) to increase O-GlcNAcylation. In CA1 pyramidal cells, but not in dentate granule cells, forskolin induces a small but significant depression of baseline eIPSC amplitude, (10.4%), and had no effect on the magnitude of the O-GlcNAc dependent depression of eIPSC amplitude compared to control. However, in both CA1 and dentate, a prior increase in O-GlcNAcylation elicits a forskolin-dependent increase in IPSC amplitude (CA1:19.7%, Dentate:13.1%), thereby reversing the O-GlcNAc-induced synaptic depression. To confirm forskolin was working via PKA dependent phosphorylation, we used the PKA inhibitor, KT 5720 (N=6,n=8) and the adenylyl cyclase inhibitor SQ22536 (N=7,n=8), separately. Surprisingly, neither inhibitor prevented the forskolin dependent increase in $GABA_AR$ current amplitude following a prior increase in O-GlcNAcylation, indicating that this potentiation occurs through another mechanism. Interestingly, the inactive forskolin analog, 1,9-dideoxy Forskolin (N=6,n=8), used as a negative control also elicited a significant potentiation of eIPSC amplitude, also consistent with a non-PKA dependent mechanism. A previous study in carp amacrine-like cells (*Li& Yang, 2021)* found that forskolin can act directly at the neurosteroid site on $GABA_ARs$. Currently, we are testing the hypothesis that following a prior increase in O-GlcNAcylation, 5α-pregnane-3α,21-diol-20-one (THDOC;100 nM) application will mimic forskolin and increase the eIPSC, indicating that O-GlcNAcylation enhances access to the neurosteroid site on $GABA_ARs$.

Disclosures: S. Phillips: None. **L.L. McMahon:** None. **J.C. Chatham:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.29/D40

Topic: B.04. Synaptic Transmission

Support: R01AG066489

Title: Effects of long-term increases in O-GlcNAcylation on astrocytes and microglia

Authors: *M. GARCIA[1,2], N. JACKSON[2], L. L. MCMAHON[2];
[1]Dept. of Cellular, Developmental, and Integrative Biol., Univ. of Alabama, Birmingham, Birmingham, AL; [2]Neurosci. Dept., Med. Univ. of South Carolina, Charleston, SC

Abstract: The addition of a single O- linked N-acetylglucosamine moiety (O-GlcNAc) to serine or threonine residues on cytosolic or nuclear proteins creates the post-translational modification O-GlcNAcylation. The O-GlcNAc modification is tightly regulated and ubiquitous, and is controlled by two enzymes O-GlcNAc Transferase (OGT) and O-GlcNAcase (OGA) that are highly expressed in hippocampus. O-GlcNAc can modify a plethora of proteins and cellular processes, either by itself or through interplay with phosphorylation. Chronic dysregulation of O-

GlcNAcylation has been implicated in the etiology of several diseases including cancer, diabetes, as well as neurodegenerative diseases and aging. We have previously reported that acute increases in O-GlcNAcylation induces a long-term depression of both excitatory and inhibitory transmission onto CA1 pyramidal cells and dentate granule cells, and limits the magnitude of LTP. Increased O-GlcNAc can depress hyperexcitability in epilepsy models in vitro and in vivo, but also negatively impact some forms of learning and memory during the period of increased O-GlcNAcylation. Much less is known regarding how increasing O-GlcNAc impacts morphology and function of glial cells. In this study, we first wanted to establish the time course of the elevated O-GlcNAc levels following in vivo administration of the OGA inhibitor thiamet-G (TMG), using a 3 times/per week protocol. By 48-72 hrs after the last injection, O-GlcNAc levels to return to baseline, quantified through western blots. Next, we wanted to determine how chronic increase (3 months) in O-GlcNAcylation modulates the morphology of astrocytes and microglia in the CA1 region and the dentate gyrus of adult male rats. Preliminary analysis shows a region-specific increase in GFAP and Iba1 in TMG injected animals. Immunohistochemistry targeting GFAP and Iba-1 and confocal imaging is being used to assess morphological characteristics to understand how increased O-GlcNAc is affecting astrocyte and microglia morphology, and if this is also region specific. Preliminary analysis suggests a likely significant difference in astrocytic morphology, which will likely be linked to a change in function. This information will help guide future studies focused on understanding how pathophysiological increases in O-GlcNAc such as in diabetes and cancer impact the function of glial cells

Disclosures: M. Garcia: None. **N. Jackson:** None. **L.L. McMahon:** None.

Poster

PSTR523. Pre- and Postsynaptic Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR523.30/D41

Topic: B.04. Synaptic Transmission

Support: UW Bridge Fund
UW Tacoma Mary Cline Award

Title: Fast-spiking and regular-spiking inhibitory perisomatic spinule-bearing boutons are the largest perisomatic boutons and envelop distinct spinule subtypes within CA1 hippocampus

Authors: E. J. WELLS, X. TREGUBOV, E. TURNER, ***M. NAHMANI**;
Div. of Sci. & Mathematics, Univ. of Washington – Tacoma, Tacoma, WA

Abstract: Fast-spiking (FS) and regular-spiking (RS) inhibitory perisomatic synapses in CA1 hippocampus play crucial and distinct roles in regulating network timing and behavior, whereas disruptions in their development or activity can result in disease states. Thus, a complete understanding of FS and RS perisomatic synaptic function is critical to elucidating their roles in regulating behavioral output and neurological disorders. Recent work investigating excitatory

synapse ultrastructure in CA1 found that 75% of these synapses contain mysterious structures called synaptic spinules. Synaptic spinules are thin, finger-like projections from one neuron that are embedded within the presynaptic or postsynaptic compartments of another neuron. While the function of synaptic spinules remains enigmatic, recent data suggests they may act as novel forms of neuronal communication and/or increase the strength and stability of spinule-bearing synapses. Yet, the prevalence and types of synaptic spinules within FS and RS inhibitory perisomatic boutons remains unclear. Here, we performed a 3D analysis of FS and RS perisomatic presynaptic boutons within a focused-ion beam scanning electron microscopy image volume from CA1 hippocampus of an adult male mouse. We sought to determine the prevalence, types, and sizes of spinules within these two distinct subsets of perisomatic inhibitory boutons. Using conservative criteria to analyze hundreds of perisomatic synapses, we found that approximately 50% of all inhibitory perisomatic boutons contained spinules, and that the majority of these spinules emanated from their postsynaptic pyramidal soma partners. In addition, we found that RS and FS spinule-bearing boutons (SBBs) were significantly larger than their non-spinule bearing bouton counterparts, comprising the largest perisomatic boutons in CA1. Yet, while a majority of both FS and RS SBBs contained spinules from their postsynaptic soma(s), they also contained distinct percentages of spinules from other neurites. Together, these findings suggest that the presence of a spinule within FS and RS boutons is a marker for synaptic maturity and stability, and that FS and RS boutons may participate in distinct forms of spinule-driven neuronal communication and circuit remodeling.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.01/D42

Topic: B.05. Synaptic Plasticity

Support: Research funded by the Polish National Science Center (grant 2019/34/E/NZ4/00387).

Title: Protein S-palmitoylation in hippocampal synaptic plasticity

Authors: *T. WOJTOWICZ[1], A. PYTYś[1], A. BUSZKA[1], M. ROSZKOWSKA[2], E. KERSTEIN[3], R. DZAKPASU[3], K. KALITA-BYKOWSKA[2], J. WŁODARCZYK[1];
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Abstract: Understanding the mechanisms of synaptic plasticity and neuronal network code lies at the center of contemporary neurobiology. Recently, post-translational protein modification S-palmitoylation which involves covalent addition of palmitic acid has been proposed to play a crucial role in synaptic plasticity and learning. However, it is largely unknown how this

modification affects synaptic function. To determine to what extent S-PALM is required for neuronal plasticity, we recorded neuronal network spiking from primary neurons (DIV14) cultured on micro-electrode arrays (MEAs). We found that a long-term increase in neuronal spiking after associative stimulation of network nodes was abolished in cultures treated with depalmitoylating agent NtBuHA. In addition, in control cultures, the Fano factor, defined as the ratio of the variance-to-mean firing rate, within an observation time window decreased with time in control cultures, unlike those treated with NtBuHA. In addition, we recorded extracellular field potentials in hippocampal slices of adolescent rats incubated with NtBuHA. We found that in the CA1 region, pharmacological treatment impaired both short and long-term synaptic plasticity in both stratum oriens and stratum radiatum. Next, we studied S-PALM profiles following the induction of chemical long-term potentiation (cLTP) with acyl-biotinyl exchange assay (ABE) method and bioorthogonal labeling of S-palmitoylated proteins with ω-alkynyl palmitic acid with a subsequent azide-based click reaction. cLTP did not result in any global shift in the palmitoylation of neuronal proteins. However, we identified individual synaptic proteins (i.e. PSD-95, synaptophysin) which were specifically up- or down-regulated following cLTP, and those for which S-PALM state remained (i.e. SNAP25, VAMP2). Altogether, we show that protein S-PALM is crucial for synaptic plasticity in hippocampal excitatory synapses and the temporal structure of neuronal spiking following enhanced neuronal network activity. Changes to protein S-PALM levels can occur rapidly - in a matter of minutes - and are protein-specific rather than proteome-wide. Our findings further support the role of dynamic lipid modifications of proteins in functional neuronal plasticity and further emphasize S-PALM's contribution to learning and memory.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.02/D43

Topic: B.05. Synaptic Plasticity

Support: NIH grant MH112151
NIH grant NS036715
NIH grant T32MH015330
SynGAP Research Fund
SFARI Pilot Award 731581

Title: Syngap regulates synaptic plasticity and cognition independent of its catalytic activity

Authors: *Y. ARAKI[1,2], K. E. RAJKOVICH[2], T. R. GAMACHE[2], E. E. GERBER[2], R. C. JOHNSON[2], I. HONG[2], H. TRAN[2], B. LIU[2], A. KIRKWOOD[2], R. L. HUGANIR[2];
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Abstract: SynGAP is an abundant synaptic GTPase-activating protein (GAP) critical for synaptic plasticity, learning, memory, and cognition. Deleterious mutations in *SYNGAP1* in humans result in intellectual disability, autistic-like behaviors, and epilepsy. Heterozygous *Syngap1* knockout mice display deficits in synaptic plasticity, learning and memory. It is unclear whether the unusual abundance of SynGAP at synapses imparts unique synaptic structural properties independent of its GAP activity. Here, we report that specific inactivating mutations within the SynGAP GAP domain in mice do not inhibit synaptic plasticity or cause behavioral deficits in both heterozygous and homozygous mutant mice. In cultured neurons, the c-terminal PDZ ligand motif of SynGAP, together with the coiled-coil domain required for liquid-liquid phase separation (LLPS), and not GAP activity, dominate the ability of SynGAP to rescue chemical LTP deficits after *Syngap1* knockdown. These data reveal that SynGAP regulates synaptic plasticity and cognition due to its unique structural role, mediated through its PDZ-ligand sequence and LLPS formation, independently of its GAP catalytic activity. Our results inform therapeutic strategies for treating SYNGAP1-related neurodevelopmental disorders in the future.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.03/D44

Topic: B.05. Synaptic Plasticity

Support: NIH Grant MH112151
NIH Grant NS036715
Simons Foundation Pilot Award 731581
SynGAP Research Fund (2020-2021)

Title: Mouse models of SYNGAP1-related intellectual disability

Authors: Y. ARAKI[1], ***E. GERBER**[2], K. RAJKOVICH[1], I. HONG[1], R. JOHNSON[1], H.-K. LEE[1], A. KIRKWOOD[1], R. HUGANIR[1];
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Abstract: SYNGAP1 is a Ras-GTPase activating protein highly enriched at excitatory synapses in the brain. *De novo* loss-of-function mutations in *SYNGAP1* are a major cause of genetically defined neurodevelopmental disorders (NDD). These mutations are highly penetrant and cause

SYNGAP1-related intellectual disability (SRID), a NDD characterized by cognitive impairment, social deficits, early-onset seizures, and sleep disturbance. Studies in rodent neurons have shown that Syngap1 regulates developing excitatory synapse structure and function, and heterozygous *Syngap1* knockout mice have deficits in synaptic plasticity, learning and memory, and have seizures. However, how specific *SYNGAP1* mutations found in humans lead to disease has not been investigated in vivo. To explore this, we utilized the CRISPR-Cas9 system to generate knock-in mouse models with two distinct known causal variants of SRID: one with a frameshift mutation leading to a premature stop codon, *SYNGAP1; L813RfsX22,* and a second with a single-nucleotide mutation in an intron that creates a cryptic splice acceptor site leading to premature stop codon, *SYNGAP1; c.3583-9G>A*. While reduction in *Syngap1* mRNA varies from 30-50% depending on the specific mutation, both models show ~50% reduction in Syngap1 protein, have deficits in synaptic plasticity, and recapitulate key features of SRID including hyperactivity and impaired working memory. These data suggest that half the amount of SYNGAP1 protein is key to the pathogenesis of SRID. These results provide a resource to study SRID and establish a framework for the development of therapeutic strategies for this disorder.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.04/D45

Topic: B.05. Synaptic Plasticity

Support: NIH Grant MH112151
NIH Grant NS036715
SynGAP Research Fund(2020-2021)
SFARI Pilot Award 731581

Title: Non-canonical splicing of SYNGAP1

Authors: *I. HONG[1,2], Y. HAN[1,2], Y. ARAKI[1,2], J. LING[3], R. C. JOHNSON[1,2], B. LANGMEAD[4], C. WILKS[4], R. L. HUGANIR[1,2];
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Abstract: Human *SYNGAP1* haploinsufficiency is a leading cause of intellectual disability, epilepsy, and autism. SYNGAP1 is the third most abundant protein in the post-synaptic density (PSD) and is critical for NMDA receptor/CaMKII-dependent synaptic plasticity. A PDZ ligand unique to the α1 SYNGAP1 c-terminal isoform allows for interaction with PSD-95 at the PSD and is crucial for its role in synaptic plasticity. Here we find that the SYNGAP1 α1 isoform arises from a GG-AG non-canonical splicing event unique to the brain and conserved across

many mammalian species. The non-canonical splice donor site is a single base upstream of the α2 canonical GT splice donor and introduces a frameshift in the resulting c-terminal protein sequence of SYNGAP1, which is critical for the interaction with PSD-95. A trichromatic *SYNGAP1* minigene splice reporter allowed base-level interrogation of this splice junction and provided means to screen antisense-oligonucleotides that modulate alternative splicing. Systematic deletions within introns of the splice reporter revealed that *SYNGAP1* non-canonical splicing requires a well-conserved region spanning the first 40 bases of the intron following the splice donor. Further single nucleotide deletions and mutations established a critical role of the splice donor region (-4 to +3) and revealed critical bases in the intron necessary for α1 splicing. Antisense oligonucleotide (ASO) screening on the minigene reporter led to several top candidate splice-switching ASOs that increase (or decrease) α1 splicing. As a novel therapeutic strategy for haploinsufficient individuals, α1-increasing ASO treatment has the potential to increase this key SYNGAP1 isoform while avoiding overt overexpression. Finally, in a genome-wide survey of splice junctions, we find that non-canonical splice junctions that deviate a single base from the canonical ones are extremely rare, with the only other gene displaying more than 10% of such alternative non-canonical splicing being *LDB1*. The c-terminus of LDB1 is also frameshifted by the non-canonical splicing, leading to a functionally distinct c-terminus. These findings illuminate a rare but highly conserved, brain-specific splicing mechanism that governs synaptic plasticity. Additionally, they pave the way for a potentially promising therapeutic approach for neurodevelopmental disorders, achieved through modulation of non-canonical splicing.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR524.05/D46

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS084111
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NIH Grant NS119512

Title: Scribble regulates Rac1 activity during LTD

Authors: *T. A. KHAN[1], M. SHELLY[2];
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Abstract: The RhoGTPase Rac1 is a major regulator of the actin cytoskeleton in dendritic spines. Spines serve as the functional receivers of synaptic input where the reorganization of spine actin cytoskeleton underlies the functional outcomes of synaptic plasticity such as AMPAR trafficking and spine remodeling. Intriguingly, recent evidence suggests that Rac1 is a critical

regulator of both forms of synaptic plasticity, LTP and LTD. LTP is triggered by a strong but brief stimulus, resulting in the addition of AMPARs to the synaptic surface and an increase in spine size. In contrast, LTD, has been shown to be initiated by a weak and prolonged stimulus, reducing the number of GluA2 containing AMPARs and shrinking the spine size. However, what determines Rac1 activity towards one form of plasticity over the other remains largely unknown. Rac1 requires activation by guanine nucleotide exchange factors (GEFs) that catalyze the GDP to GTP exchange of Rac1-bound GDP to Rac1-bound GTP. Many Rac1 GEFs are intimately associated with LTP but few with LTD. Here we reveal that Rac1 is a significant regulator of LTD and that its function is facilitated by the scaffold protein Scribble by forming a signaling complex with a Rac1 GEF. Scribble is a multidomain signaling organizer that can interact with a plethora of proteins to conduct distinct signaling pathways. We hypothesize that Scribble regulates Rac1 activity during LTD via a Rac1 GEF and is critical for GluA2 containing AMPAR endocytosis and actin remodeling.

Disclosures: **T.A. Khan:** None. **M. Shelly:** None.

Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

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Topic: B.05. Synaptic Plasticity

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Title: Copine-6 is a Ca^{2+}sensor for activity-induced AMPA receptor exocytosis

Authors: *A. BATALLAS BORJA[1], J. TAN[1], S. JANG[1], N. BHEMBRE[1], M. CHANDRA[2], L. ZHANG[1], H. GUO[1], M. RINGUET[1], J. WIDAGDO[1], B. COLLINS[2], V. ANGGONO[1];
[1]The Univ. of Queensland Queensland Brain Inst., St Lucia, Australia; [2]The Univ. of Queensland Inst. for Mol. Biosci., St Lucia, Australia

Abstract: The recruitment of synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors underlies the strengthening of neuronal connectivity during learning and memory. This process is triggered by NMDA (N-methyl-D-aspartate) receptor-dependent postsynaptic Ca^{2+} influx. Synaptotagmin (Syt)-1 and -7 have been proposed as Ca^{2+}-sensors for AMPA receptor exocytosis but are functionally redundant. Here we identify a cytosolic C2

domain-containing Ca^{2+}-binding protein Copine-6 that forms a complex with AMPA receptors. Loss of Copine-6 expression impairs activity-induced exocytosis of AMPA receptors in primary hippocampal neurons, which is rescued by wild-type Copine-6, but not Ca^{2+}-binding mutants. In contrast, Copine-6 loss-of-function has no effects on steady-state expression or tetrodotoxin-induced synaptic upscaling of surface AMPA receptors. Loss of Syt-1/-7 significantly reduces Copine-6 protein expression. Interestingly, overexpression of wild-type Copine-6, but not the Ca^{2+}-binding mutant, restores activity-dependent exocytosis of AMPA receptors in Syt-1/-7 double-knockdown neurons. We conclude that Copine-6 is a postsynaptic Ca^{2+}-sensor that mediates AMPA receptor exocytosis during synaptic potentiation.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.07/D48

Topic: B.05. Synaptic Plasticity

Support: 2 R01 MH071739

Title: Uncovering how activity regulates cell type-specific alternative splicing of ion channels

Authors: *Y.-C. SUN[1], Y. QIN[2], B. LI[2], X. CHEN[3], R. W. TSIEN[1];
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Abstract: Neurons can regulate their firing properties through two processes: positive feedback plasticity, which amplifies response to activity, and negative feedback plasticity, which maintains functional stability. These processes allow incorporation of new information through Hebbian mechanisms while stabilizing excitability through homeostatic mechanisms. Balancing these two forms of plasticity involves changes in ion channels that determine firing properties. One way ion channels can be altered is by regulating alternative splicing, but how splicing changes contribute to the different forms of plasticity is unclear. To investigate the activity-dependence of ion channel splicing, we analyzed RNAseq and collected PCR confirmatory results of alternative splicing in different ion channels. We found that splicing is regulated in distinct ways. For example, the inclusion of an NTD of Cavb4 increases under chronic inactivity and decreases under chronic depolarization. Expression of this splice variant has been shown to enhance current density of calcium channels, and its splicing changes suggest homeostatic regulation of neuronal excitability. On the other hand, Li et al. (2020) found that exclusion of an exon in BK channel is favored under both low and high activity, a non-monotonic pattern echoed by Nav1.2. For BK, exon exclusion widens the action potential, leading to homeostatic

regulation under inactivity and Hebbian plasticity under depolarization. Thus, our results show exemplars of both monotonic and non-monotonic patterns of activity-dependent alternative splicing.
We next asked how the splicing changes we found might influence overall circuit function, a task that requires interrogation of individual cell types. Cell types play distinct roles in neural circuits, so for example, splicing of an ion channel that increases excitability will have opposite effects on the circuit depending on whether it occurs in an excitatory or inhibitory cell type. Detecting cell type-specific splice variants requires distinguishing diverse neuronal types in the brain and probing splice variants in those same neurons simultaneously. To do this, we capitalized on BARseq, a high-throughput in situ sequencing method that can resolve neuronal types in brain slices (Sun et al. 2021). Adapting BARseq for exon detection allowed us to characterize differential expression of splice variants across brain regions and cell types. This will enable us to assay activity-dependent splicing in intact circuits and investigate the cell type-specific molecular basis of different forms of plasticity.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.08/D49

Topic: B.05. Synaptic Plasticity

Support: Canadian Institutes of Health Research FRN 162179
Natural Sciences and Engineering Research Council of Canada RGPIN-2016-05538

Title: Pharmacological isolation of non-ionotropic NMDA receptor signaling reverses long-term potentiation in the spinal cord dorsal horn and attenuates pain hypersensitivity in vivo

Authors: *D. RODRIGUEZ[1], H. ZHANG[1], A. D'SOUZA[1], M. ZAIN[1], S. FUNG[1], L. BENNETT[1], D. HE[2], R. P. BONIN[1,3,4];
[1]Pharmaceut. Sci., [2]Anesthesiol. and Pain Med., [3]Cell and Systems Biol., Univ. of Toronto, Toronto, ON, Canada; [4]Univ. of Toronto Ctr. for the Study of Pain, Toronto, ON, Canada

Abstract: Pathological pain is associated with changes to the strength of synaptic connections within ascending nociceptive pathways. In particular, the long-term potentiation (LTP) of synapses between primary sensory neurons and spinal dorsal horn neurons has been closely linked to the development of pain hypersensitivity (i.e. hyperalgesia). At the cellular/molecular level, synaptic plasticity is highly dependent on NMDA receptor (NMDAR) function; activation of this ligand-gated ion channel leads to the influx of calcium ions (Ca^{2+}) which, in turn, activates intracellular signaling cascades that drive LTP induction and promote the sensitization of pain pathways. Interestingly, recent investigations in the brain have described a novel, non-

ionotropic NMDAR (NI-NMDAR) signaling mechanism that does not require channel opening and, instead, favours long-term depression (LTD) and synaptic spine shrinkage. In this study, we test the hypothesis that NI-NMDAR activity can be used to depotentiate previously-sensitized nociceptive pathways in the spinal cord, and promote the reversal of hyperalgesia in animal models of pathological pain. For ex vivo experiments, C fiber-evoked postsynaptic field potentials (fPSPs) were recorded in acute spinal cord explants isolated from adult mice. Dorsal roots containing primary sensory afferents were stimulated using a bipolar electrode, and a recording electrode was inserted into the superficial dorsal horn of the spinal cord to measure postsynaptic responses. For behavioural experiments, hyperalgesia was induced via intraplantar injections of capsaicin or complete freund's adjuvant (CFA); Von Frey filaments were then used to measure mechanical sensitivity in the injected paw. NI-NMDAR signaling was pharmacologically isolated using NMDAR glycine-site antagonists or open-channel blockers. We found that NI-NMDAR activity significantly decreased the overall magnitude of dorsal horn LTP in spinal cord explants. Importantly, this reversal of hyperexcitability translated into significant decreases in mechanical sensitivity following capsaicin- or CFA-induced hyperalgesia. Our results identify NI-NMDAR signaling as a novel target for the treatment of chronic pain. Furthermore, the ability to reverse LTP in an activity-dependent manner may have important implications beyond nociceptive processing, for example in the modulation of hippocampal LTP which is thought to be crucial for memory formation and learning.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.09/D50

Topic: B.05. Synaptic Plasticity

Support: Lundbeck Foundation

Title: Inhibition of astrocytic glycogen metabolism impairs synaptic plasticity, high-frequency activity, and post-train recovery -rescue by L-glutamine

Authors: J. K. ODDERSHEDE[1], P. F. MARKER[1], S. R. ANDREASEN[1], I. S. TVILLING[1], E. JAKOBSEN[2,3], L. K. BAK[2,3], M. S. JENSEN[1], ***M. M. HOLM**[1];
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Abstract: Astrocytes serve as the main glycogen stores of the brain. Glycogen can be metabolized to glutamine (L-Gln) which serves as the glutamate precursor in neurons. This study aimed to perform a detailed mapping of synaptic mechanisms which are dependent on astrocytic

glycogen metabolism. We took advantage of the glycogen phosphorylase inhibitor, 1,4-Dideoxy-1,4-imino-D-arabinitol hydrochloride (DAB). We prepared 400 µM thick acute coronal brain slices from deeply anesthetized two to three months old male C57BL/6J mice. We used *in vitro* extracellular field recordings to analyze CA1 synapses while stimulating Schaffer collaterals. Recordings were performed in standard artificial cerebrospinal fluid (ACSF). First, we evaluated the impact of DAB on long-term potentiation (LTP) triggered by 2 x 100 Hz stimulations. In ACSF, the field excitatory postsynaptic potential (fEPSP) slopes increased to 151.34 ± 15.72% (n = 5) compared to only 100.56 ± 11.16% (n = 5) in 30 µM DAB ($P < 0.05$). These data support findings *in vivo* (Suzuki et al., 2011). The impaired LTP could be rescued by 500 µM L-Gln; 150.74 ± 11.92%, n = 17. We expanded our studies by testing sustained high-frequency activity. We applied 4000 stimulations at 20 Hz and observed a pronounced facilitation followed by a depression that flattened out at around 30% of baseline. 100 µM DAB caused a downward shift of the fEPSP slopes (n = 8, p = 0.037). The application of 3 mM L-Gln + 100 µM DAB significantly reversed this downward shift.
Afterward, we challenged the synapses with more complex activity patterns triggered by four trains of 1000 stimulations at 20 Hz, with inter-train recovery phases of 200 sec with 40 stimulations at 0.2 Hz (4 x 1000) (Tani et al., 2014). 100 µM DAB caused significant reductions of all trains ($P_1 = 0.0055$, $P_2 = 0.0101$, $P_3 = 0.0052$ and $P_4 = 0.0043$, n = 8). 3 mM L-Gln could rescue these impairments.
Finally, we demonstrated that post-train recovery strongly depends on glycogen metabolism. 10 min after 4 x 1000 stimulations in ACSF, the fEPSP slopes were increased to 255.68 ± 21.68% (n = 11) normalized to baseline. 30 µM DAB reduced this potentiation to 193.14 ± 18.49% (n = 12, P = 0.0361) while in 100 µM DAB it was 154.88 ± 14.51% (n = 9, P = 0.0051). 3 mM L-Gln rescued the recovery to 211.69 ± 17.89% (n = 15, P = 0.0236).
In conclusion, our analyses document that hippocampal astrocytic glycogen stores are critical to sustaining LTP and maintaining performance during repetitive synaptic activity, post-train recovery, and more complex activity. Exogenously applied L-Gln rescues the impaired activity, strongly suggesting that astrocytically delivered L-Gln is critical for normal synaptic functionality.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

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Program #/Poster #: PSTR524.10/D51

Topic: B.05. Synaptic Plasticity

Support: NRF-2019M3C7A1031742
NRF-2020R1A2C2005868

Title: Sustained inhibitory long-term depression on the paraventricular nucleus of the hypothalamus in the absence of FKBP5

Authors: *S. ZHANG[1], C. CHUNG[2];
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Abstract: The hypothalamic-pituitary-adrenal (HPA) axis governs stress-induced physiological responses. Stress exposure triggers the release of glucocorticoids, which then provide feedback signals to the brain to prevent excessive hormonal responses. The physiological model of this negative feedback is known as activity-dependent long-term depression in inhibitory synapses (iLTD) of the paraventricular nucleus (PVN) of the hypothalamus. FKBP5 is a well-known negative modulator of glucocorticoid receptors (GR), which plays a role in modulating stress-induced behaviors. Mice overexpressing FKBP5 have been reported to be susceptible to stress, exhibiting slow stress-coping behaviors and anxiogenic behaviors. On the other hand, FKBP5-deficient mice show resilience to stress and exhibit anxiolytic behaviors. Given that PVN-specific deletion of FKBP5 led to increased GR activity, we investigated the impact of FKBP5 deficiency on iLTD in the PVN. Interestingly, PVN neurons lacking FKBP5 exhibited iLTD even in the absence of GR activation, suggesting continuous negative feedback in the hypothalamus. The mechanisms underlying iLTD in the FKBP5-deficient PVN were found to involve the same signaling pathways as previously reported, including metabotropic glutamate receptor 5 and opioid receptors. Additionally, we discovered that the BDNF-TrkB signaling pathway also mediates iLTD in the PVN. Overall, these findings highlight the role of FKBP5 and the associated signaling pathways in regulating stress-induced behaviors and the negative feedback mechanism in the hypothalamus to maintain physiological balance during stress.

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Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

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Topic: B.05. Synaptic Plasticity

Support: NIH NS019895
NIH NS102490

Title: Differences in the long term dynamics of kinase activation after distinct serotonin protocols that induce long term synaptic facilitation in aplysia

Authors: *Y. ZHANG, R.-Y. LIU, P. SMOLEN, J. BYRNE;
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Abstract: Long-term synaptic facilitation (LTF) of the Aplysia sensorimotor synapse is a well-established model system for studying the molecular mechanisms of long-term memory.

Empirical studies have identified three serotonin (5-HT) protocols that induce LTF: the Standard protocol of five, 5-min pulses of 5-HT with regular interstimulus intervals (ISIs) of 20 min; the Enhanced protocol of 5 pulses of 5-HT with computationally designed irregular ISIs; and the Two-pulse protocol of two 5-min pulses of 5-HT with an ISI of 45 min. The protocols result in substantial differences in the strength and duration of LTF. The mechanisms underlying the differential efficacy of protocols remain unclear. 5-HT-induced LTF depends on the activation of the transcription activator CREB1 and inactivation of the transcription repressor CREB2 (Bartsch et al. 1995,1998). In this study, we examined the dynamics of four kinase cascades that are critical for activation of CREB1 and CREB2: mitogen-activated protein (MAP) kinase isoforms ERK and p38 MAPK, protein kinase A (PKA), and p90 ribosomal S6 kinase (RSK) (Guan et al. 2002, 2003; Zhang et al. 2017; Liu et al. 2020). We treated isolated Aplysia sensory neurons with the above LTF-inducing protocols, and compared the dynamics of activation of these four kinases, for up to 24 h after 5-HT treatment.
Compared to vehicle control examined at the same time points, each kinase showed complex dynamics of activation after all three protocols. In general, two waves of increase in kinase activities were observed for all kinases over 24 h. The first wave of increase occurred shortly after treatment and returned to basal levels within 5 h. The second wave of increase was evident at 18 h. Most kinase activities returned to basal level at 24 h. However, ERK activity remained elevated 24 h after the Enhanced protocol, but not after the other two protocols. This prolonged elevation may explain, in part, why the Enhanced protocol is superior to the Standard protocol in prolonging LTF (Zhang et al. 2012). The complex dynamics and interaction of these kinase pathways will, in turn, govern the dynamics of CREB1 and CREB2 activation after 5-HT protocols, which are key in determining the efficacy of 5-HT protocols to form LTF. New empirical results will be incorporated into the computational model of molecular network of LTF. The updated model will be used to predict optimal intervals for single-block or multiple-block, training protocols to induce persistent LTF and LTM.

Disclosures: **Y. Zhang:** None. **R. Liu:** None. **P. Smolen:** None. **J. Byrne:** None.

Poster

PSTR524. LTP and LTD: Signaling Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR524.12/D53

Topic: B.05. Synaptic Plasticity

Support: NIH grant NS019895

Title: Mecp2 represses the induction and maintenance of long-term synaptic plasticity

Authors: *R.-Y. LIU, Y. ZHANG, P. SMOLEN, J. H. BYRNE;
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Abstract: Mechanisms of memory deficits associated with Rett syndrome are poorly understood, at least in part because mutations of methyl-CpG binding protein 2 (MECP2) have confounding effects on nervous system development and basal synaptic transmission. To mitigate such empirical uncertainties, we exploited the technical advantages of the *Aplysia* sensorimotor synapse, a well-established model system that has provided insights into mechanisms of synaptic plasticity. We examined the physiological role of MeCP2 in long-term synaptic facilitation (LTF) as well as short-term facilitation (STF). Four lines of evidence demonstrate the roles of MeCP2 in LTF. First, MeCP2 levels 2 h after 5-HT treatment are significantly reduced by three temporally distinct stimulus protocols. Three 5-HT protocols can be used to induce LTF: 1) the Standard protocol, consisting of five, 5-min pulses of 50 μM 5-HT, with interstimulus interval (ISI) of 20 min, 2) the Enhanced protocol (5 pulses of 5-HT with computationally designed irregular ISIs of 10-10-5-30 min), which induces stronger and more persistent LTF, and 3) the two-pulse protocol, with an ISI of 45 min, which induces LTF comparable to the Standard protocol. Comparing these protocols, the extent of MeCP2 reduction correlated with the effectiveness of each protocol in inducing LTF. This reduction suggests MeCP2 may act as a transcription repressor, with relief of repression necessary for LTF formation. Second, one 5-min pulse of 5-HT, a protocol that usually only induces STF lasting ~10 min, induced significant LTF 24 h after 5-HT application in a group in which anti-MeCP2 antibody was injected 1 h before 5-HT treatment, compared to an IgG control group, suggesting repression of MeCP2 activity facilitates induction of LTF. Third, LTF is prolonged from persisting for 1 day to persisting for at least 5 days by removing the inhibitory effects of MeCP2, using MeCP2 antibody injected into sensory neurons 1 h before the Standard 5-HT treatment. Finally, LTF is repressed by boosting MeCP2 levels with injection of recombinant MeCP2 protein into sensory neurons in sensorimotor co-cultures 1 h before the Standard 5-HT treatment, further supporting that MeCP2 is a repressor for LTF. Neither boosting nor inhibiting MeCP2 function affected STF. The results indicate MeCP2 may act as an inhibitory constraint for formation, and also maintenance, of long-term synaptic plasticity.

Disclosures: **R. Liu:** None. **Y. Zhang:** None. **P. Smolen:** None. **J.H. Byrne:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.01/D54

Topic: B.08. Epilepsy

Support: NIH

Title: The effect of neuron-to-neuron forces on neuronal activity and viability

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Abstract: Cells are attracted to each other by mechanical forces mediated by cell adhesion molecules and cytoskeletal tension. We have previously showed that these forces are present in rat cortical neurons and neurons derived from human induced pluripotent stem cells. Neuron-to-neuron attraction causes tissue contraction in dissociated neuronal aggregates. Neurons at the edge of aggregates which were experiencing disbalanced contractile forces also had more baseline and dynamic Ca^{2+} activity. We hypothesized that an imbalance in cell attraction forces in the brain tissue after trauma may result in tissue contraction and hyperexcitability and may contribute to post-traumatic epileptogenesis. Here, we used organotypic hippocampal culture model to determine whether contractile forces exist in ex vivo tissue, and whether they influence epileptogenesis. First, organotypic hippocampal slices were embedded in fluorescent-bead containing Matrigel. After 48 hours, significant movement of beads toward the slice occurred, confirming that cells in the slice exerted contractile forces. Then, we cultured slices on substrate printed with patterns of cell-substrate adhesion molecule poly-D-lysine (PDL). We found that tissue contraction was accelerated in slice sub-regions located over PDL-free regions. Effect of contraction varied depending on the force direction (along or perpendicular to apical-basal axis). Contraction along apical-basal axis led to loss of neuronal viability and decreased Ca^{2+} dynamics. On the other hand, neurons in hippocampal sub-regions subjected to contraction in transverse direction were viable and had increased Ca^{2+} activity. Overall, our research aims to explore the relationship between trauma-induced imbalance in contractile forces and neuronal activity. Understanding the underlying mechanisms of this phenomenon can help identify strategies to mechanically relax the tissue and prevent the onset of epilepsy.

Disclosures: L. Dalir: None. **Y. Berdichevsky:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.02/D55

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: BBSRC Lilly CASE studentship

Title: The effects of Glucagon-like peptide 1 receptor agonist Exendin-4 on neuronal activity in the lateral septum

Authors: *B. GURUNG[1], A. RANDALL[2], J. BROWN[1];
[1]Univ. of Exeter, Exeter, United Kingdom; [2]Jazz Pharmaceuticals, Cambridge, United Kingdom

Abstract: Glucagon-like peptide-1 receptor (GLP-1R) is an important G-protein coupled receptor in the periphery, which when activated stimulates the glucose-dependent release of insulin by the pancreatic β-cells to lower blood glucose. As such drugs that are agonists of this receptor are an approved class of drugs used for Type 2 diabetes mellitus (T2DM). T2DM and the neurodegenerative disease Alzheimer's disease (AD) share common pathophysiological characteristics such as insulin resistance and decreased glucose metabolism. The GLP-1R agonist liraglutide completed a Phase IIb clinical trial (NCT01843075) in patients with mild AD dementia to examine the drug's effect on the hallmarks of the progression of the disease with promising results. Other agonists of this receptor have also shown improved spatial learning and memory in animal models of AD. However, to date the details of the effects this receptor system has at a neural circuit level in the brain is not well known. GLP-1R is expressed in many brain structures but it is most highly expressed in the lateral septum. Here, we first explore the effects of GLP-1R activation on neuronal activity in the lateral septum. We have used multi-electrode array and whole cell patch clamp methods to show the diverse effects of GLP-1R agonist Exendin-4 on neuronal activity within the mouse lateral septum. Consequently, our work will provide a basis for future works to study whether the effects of GLP-1R activation on neuronal activity is a result of direct or modulatory mechanism, and also how the regulation of neural circuits by this receptor system guides behaviour.

Disclosures: B. Gurung: None. **A. Randall:** None. **J. Brown:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.03/D56

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Evelyn F. McKnight Brain Research Foundation R01NS123424

Title: Modulation of hippocampal theta power and phase during a bimanual string-pulling behavior

Authors: *G. HOLGUIN, A. K. TAPIA, K. JORGENSEN, G. JORDAN, A. VISHWANATH, S. A. MIRON, E. C. VIGIL, A. L. WEBSTER, S. L. COWEN;
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Abstract: The hippocampus plays an integral role in navigation, learning, and episodic memory. These processes are supported by hippocampal involvement in processing sensorimotor information. This is evident in the strong relationship between running speed and the frequency and power of the hippocampal theta oscillation (5-12 Hz) observed in rodents. Most research on theta has been performed while animals run on tracks or open fields. Consequently, little is known about relationships between theta and the motion of individual limbs or when vestibular

or visual self-motion cues are absent. String-pulling tasks have been used for centuries to study learning and behavior, and these tasks require complex bimanual forelimb coordination. Surprisingly, these behaviors have rarely been used to investigate neural correlates of fine-motor behavior and have never been used in hippocampal electrophysiology. In this study, we investigated relationships between theta and forelimb paw speed and acceleration as well as the phase of the reach/pull motion. Methods: Local-field potentials were recorded from CA1 in Sprague Dawley rats (*N*=6) during string pulling and circular track traversal. Results: While there was clear theta-band activity during string pulling, the relationship between acceleration (average acceleration of both paws during string pulling vs. running acceleration on the track) and theta frequency did not differ between the two behaviors. In contrast, while increased deceleration (larger negative values) was associated with higher theta power during track running, this relationship was reversed during string pulling. Furthermore, fine-grained analysis of the lift, advance, grasp, push phases of string-pulling revealed that theta power and frequency were modulated by movement phase, and theta phase reset near the start of the pull phase. These observations provide further support for the notion that dorsal hippocampal theta is not only modulated by movement through space, but that modulation extends to fine limb movements in a task where the animal is stationary and does not receive visual or vestibular information. Even so, these data indicate that relationships between theta and paw movement differ in significant ways from running-associated theta.

Disclosures: **G. Holguin:** None. **A.K. Tapia:** None. **K. Jorgensen:** None. **G. Jordan:** None. **A. Vishwanath:** None. **S.A. Miron:** None. **E.C. Vigil:** None. **A.L. Webster:** None. **S.L. Cowen:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.04/D57

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01NS093866
NIH Grant R35NS127219

Title: Incidence and interval of multiple spikes in dentate granule cells altered by novel experience.

Authors: *W.-C. SHU[1,2], M. B. JACKSON[1];
[1]Dept. of Neurosci., UW-Madison, Madison, WI; [2]Biophysics Program, Univ. of Wisconsin-Madison, Madison, WI

Abstract: The dentate gyrus (DG) within the hippocampus processes perforant path inputs from the entorhinal cortex as part of the execution of functions such as learning, episodic memory, and

spatial navigation. The DG exhibits sparse coding, which has been proposed to play a role in minimizing the overlap of responses to similar stimuli (pattern separation). GCs can generate bursts of activity during distinct physiological behaviors, and these bursts can enhance transmission to their targets through synaptic facilitation. Our previous research used voltage imaging with a hybrid voltage sensor (hVoS 1.5) to study GC double spikes (doublets), the simplest form of bursting. Under control conditions, most of mature GCs in slices can generate doublets with interspike intervals of ~ 3-7.5 msec. GCs are homogeneous in this regard and generate doublets at a low incidence of ~ 5%. Generation of doublets is regulated by the interplay of GC intrinsic excitability, recurrent inhibition, and recurrent excitation. Since GC bursts have been proposed to encode information during distinct behaviors, we investigated doublets in GCs that are active when a mouse is placed in a novel environment (Novelty-GCs). Using a genetic strategy of targeted recombination in active populations (TRAP) to drive probe expression with Cre recombinase under temporal control of the *c-fos* gene, we targeted voltage sensor to behaviorally-activated cells. We then employed voltage imaging in slices from these animals to record and compare the spiking behavior of Novelty-GCs and home-cage controls (Ctrl-GCs). In contrast to mature GCs (labeled postnatally with the Prox1-Cre driver), we see heterogeneity within TRAP-labeled Novelty-GCs, with some generating doublets more frequently. The blockade of GABAergic transmission has a stronger effect on Novelty-GCs, increasing doublet incidence to 24% versus only 17% in Ctrl-GCs. With GABA receptors blocked TRAP labeled Novelty-GCs also displayed a mean doublet interval of 9.06 msec, which was 2.8 msec longer than TRAP labeled Ctrl-GCs under the same conditions. In the presence of a GABA receptor blocker, TRAP-labeled Ctrl-GCs and mature GCs were similar in their incidence and interval of doublets. Doublets with prolonged intervals (>6 msec) occurred in half of the Novelty-GCs, which was twice as frequent as Ctrl-GCs. This suggests that doublets may serve a role in encoding experience. In summary, our results show that GCs activated by novelty are modified by that experience. The modifications in GC doublets have the potential to alter DG processing.

Disclosures: **W. Shu:** None. **M.B. Jackson:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.05/D58

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NINDS R01NS115233
NIMH R01MH117964

Title: Submillisecond influence of action potential provides evidence for neuron-to-neuron ephapses in the rodent brain

Authors: *A. D. STANKOV[1], N. R. KINSKY[2], U. KAYA[2], B. GIRI[2], A. AMARASINGHAM[3], K. DIBA[2];
[1]Psychology (Neuroscience), The Grad. Center, CUNY, New York, NY; [2]Anesthesiol., Univ. of Michigan, Ann Arbor, MI; [3]Mathematics, City Col. of New York, New York, NY

Abstract: Submillisecond synchrony has been observed and characterized between interneurons in the rat hippocampus (Diba et al., 2014). Our recent analysis, using new internal and publicly available external data, reveals that this synchrony has an intricate temporal structure featuring rapid periods of both precise excitation and precise inhibition. One possibility suggested by studies in other brain regions is that such pairwise features, especially submillisecond inhibition, may be due to ephapses. With this possibility in mind, we examine high-density recordings in rodent hippocampal CA1 and mouse cortical, subcortical, and cerebellar regions from various sources, including publicly-available data sets (Dimitriadis et al., 2020; Meyer, 2023; Steinmetz & Ye, 2022). We are examining the cross-correlograms of spike times to quantify the relative likelihood with which a spike in a reference neuron is followed by spikes in a target neuron with various spike timing relationships. When we align the resulting cross-correlograms with the action potential waveforms of the reference units, we find a remarkable alignment between these features. This effect can be observed both locally and at distances up to 500 microns, and can be observed both uni- and bidirectionally. In some instances, the pairwise interactions also show rapid modulation between different types of neurons (i.e. interneurons and pyramidal cells). We have observed these results in more than 60 animals, spanning a wide range of experimental in-vivo preparations. Prior electrophysiology and review studies show these attributes are not characteristic of electrical synapses and are likely to instead support an ephaptic coupling hypothesis. Our observations also reveal that extracellular conduction of action potentials are detectable much further away than previously believed to be, suggestive of distal neuron arborization of interneurons and pyramidal cells. This is evident in the cross-correlograms of reference neurons with many target neurons and/or with the grouping of rejected spikes regarded as noise. To better scrutinize the evidence of ephaptic coupling we are automating the search for neuronal pairs that exhibit these phenomena and whose component spike times are unlikely to be contaminated by spike sorting error. In conclusion, we argue that extracellular conduction of action potentials reveal putative neuron-to-neuron ephapses and have further-than-expected detectable activity.

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Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.06/D59

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant T32-GM142521
NIH Grant R01-MH111604

Title: Circuit-specific androgen receptor regulation of hippocampal neuronal excitability

Authors: *C. SUGIMOTO, A. L. EAGLE, A. J. ROBISON;
Physiol., Michigan State Univ., East Lansing, MI

Abstract: Major depressive disorder (MDD) is a highly prevalent psychiatric disorder that has a complex multifactorial etiology, with stress vulnerability emerging as a critical risk factor. MDD disproportionately affects women, with female prevalence almost double that of men, but the biological basis of this sex difference is not understood. Additionally, roughly half of MDD patients do not respond to existing treatments, and therefore there is an urgent need to understand MDD's biological etiology and the molecular mechanisms underlying sex differences to develop new therapeutic strategies. MDD is associated with abnormalities of the brain's reward circuitry, and studies have implicated the ventral hippocampal (vHPC) projections to the nucleus accumbens (NAc; vHPC-NAc) in stress-induced susceptibility to anhedonia in male mice. However, despite the higher prevalence rates of MDD in females, studies that include both male and female subjects are lacking. Our lab used subchronic variable stress (SCVS), which shows an anhedonia phenotype in female mice but not males, to investigate the sex differences in stress-induced anhedonia. We found that female mice are susceptible to SCVS-induced anhedonia and have increased basal vHPC-NAc circuit excitability compared to males. Moreover, vHPC-NAc circuit excitability is reduced by adult testosterone but the mechanisms by which androgen receptors (AR) regulate this excitability remain unknown. We used *ex vivo* whole cell slice electrophysiology on WT- and AR^{flox}-L10GFP mice to examine the role of AR in vHPC-NAc excitability in male and female mice. We found circuit excitability to be increased in females compared to males, which was dependent on non-aromatized androgens, and conditionally knocking out the androgen receptor in the vHPC-NAc circuit reversed these effects. We performed action potential waveform analysis, and results suggest that potassium channels may play a key role in this excitability change. Therefore, we used translating affinity ribosomal purification sequencing (TRAP-seq) to examine downstream targets of AR and observed changes in expression of multiple ion channels and signaling pathways which drive sex-specific behaviors and vulnerability to stress. Future experiments will investigate the roles of these channels in circuit excitability and sex-specific stress responses.

Disclosures: C. Sugimoto: None. **A.L. Eagle:** None. **A.J. Robison:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.07/D60

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01NS115508

Title: Regulation of hippocampal CA1 pyramidal neuronal excitability and associative learning by the endoplasmic reticulum Ca^{2+} sensors, STIM1 and STIM2

Authors: *K. S. KORSHUNOV, M. E. MARTIN, M. PRAKRIYA;
Pharmacol., Northwestern Univ., Chicago, IL

Abstract: A ubiquitous Ca^{2+} mechanism that serves as the primary source of Ca^{2+} entry in many non-excitable and excitable cells is store operated Ca^{2+} entry (SOCE). SOCE is initiated by Ca^{2+} release from the endoplasmic reticulum (ER), which triggers the opening of the Ca^{2+}-release activated Ca^{2+} (CRAC) channels formed by the Orai proteins, including Orai1. In the canonical pathway, the ER Ca^{2+} sensors STIM1 and STIM2 sense the depletion of Ca^{2+} stores and physically interact with and gate Orai1 channels to trigger Ca^{2+} entry. SOCE is known to drive a variety of essential effector functions within the immune system, but its physiological roles in the central nervous system are poorly understood. Our lab's previous work has shown that mice with a knockout of the Orai1 in hippocampal neurons show attenuated glutamate-evoked dendritic spine Ca^{2+} signals and long-term potentiation (LTP) at the CA3-CA1 synapse, and defects in a variety of learning and memory tasks. Thus, while Orai1 is now known to regulate neuronal and cognitive functions, the roles of its canonical ligands, STIM1 and STIM2, are much less understood. Because the STIM proteins regulate cellular Ca^{2+} homeostasis, we hypothesized that conditional deletion of these proteins in excitatory neurons of the brain may also dysregulate Ca^{2+}-dependent neuronal activity and behavior. In this work, we used a combination of electrophysiological and behavioral approaches in mice with conditional deletion of STIM1 or STIM2 in excitatory neurons to test this hypothesis. Our current results indicate that loss of STIM1 or STIM2 does not affect the passive membrane properties of CA1 pyramidal neurons but alters their firing properties. Interestingly, like Orai1 cKO mice, STIM cKO mice did not show changes in baseline synaptic transmission. We are currently analyzing the potential implications of these excitability changes for behavioral learning using tests of working and associate memories. We anticipate that these studies will expand on the growing knowledge of the role of SOCE proteins in regulating excitability of central nervous system neurons and implications for cognitive functions.

Disclosures: K.S. Korshunov: None. **M.E. Martin:** None. **M. Prakriya:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.08/D61

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF GRFP DGE 1752814
NSF GRFP DGE 2146752

Title: Transsynaptic coupling of synaptic and intrinsic excitability through neurexin signaling

Authors: *A. ALEJANDRO-GARCÍA;
UC Berkeley, Berkeley, CA

Abstract: Neurons are highly compartmentalized cells, which can be observed at the level of axons, dendrites, and dendritic spines. Having discrete compartments with unique electrical and biochemical features can increase the number of distinct 'processing states' that a neuron can have. What are the molecular rules that instruct this organizational arquitecture? The tight balancing of synaptic and intrinsic activities is thought to define input-output relationships of excitatory neurons, and although the molecular mechanisms that determine the set-points for these relationships are unclear, they are thought to involve the spatial and temporal binding of inputs to enhance excitation. Concurrently, neurons possess homeostatic mechanisms that balance excitation across their inputs together with their spiking activity. Within the hippocampal trisynaptic circuit, we previously identified Slm2 as an RNA-binding protein that changes alternative splicing of neurexin mRNAs, leading to increased synaptic strength in the postsynaptic cell. We sought to determine if this increase in synaptic strength cooperates with the cell's neuronal firing properties. We performed whole-cell electrophysiology on acute slices of mouse hippocampus and recorded from hippocampal CA1 pyramidal neurons while selectively deleting Slm2 from presynaptic CA3 pyramidal neurons. We explored whether an increase in synaptic strength is matched with a change in the cell's intrinsic excitability due to an increase in hyperpolarization-activated currents (Ih). We report an increase in expression of Ih currents due to an upregulation of HCN channel activity. Our results may be evidence of local homeostatic coupling of intrinsic activity with synaptic strength.

Disclosures: A. Alejandro-García: None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.09/D62

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: ANR-18-CE16-0006
Fondation pour la Recherche Medicale EQU202003010457
ANR-18-CE370020-01

Title: Environmental enrichment leads to a rescue of social memory in a 22q11.2DS mouse model via sPLA2G5

Authors: C.-L. ZHANG[1], M. NOGUERES[2], M. LOISY[1], M. AHMADI[1], L. THERREAU[1], M. CHATEAU[1], A. FAFOURI[1], J. COGNET[1], V. CHEVALEYRE[1], J. CHABRY[2], ***R. PISKOROWSKI**[1];
[1]Inserm, Univ. Paris Cite, Paris, France; [2]CNRS IPMC, VALBONNE, France

Abstract: Environmental factors play a complex and interdependent role with genetic and epigenetic factors in numerous psychiatric diseases. Of particular importance is social cognition, as social impairment is among the earliest features of schizophrenia, correlating highly with poor functional outcome. While it has been clearly demonstrated that environmental factors can either preclude or precipitate pathogenesis in psychiatric diseases, the physiological and cellular mechanisms underlying these phenomena are poorly understood. In human postmortem studies for numerous disorders, hippocampal area CA2 has been shown to be particularly affected. Using a mouse model of the 22q11.2 deletion syndrome (22q11.2DS), we have shown that both parvalbumin-expressing (PV+) interneurons and pyramidal neurons (PNs) in this region undergo alterations that result in reduced activity in this region. We have found that three weeks in an enriched environment (EE) results in a depolarization of the resting membrane potential of CA2 pyramidal neurons is more depolarized for deletion mice and littermate controls. Furthermore, we show that this depolarization is due to changes in TREK-1 potassium channel conductance. Regulation of the TREK-1 conductance is linked to increased levels and activity of sPLA2G5 in EE, a secreted lipase enriched in hippocampal area CA2. The increased amount of sPLA2G5 leads to major changes in the lipid composition of CA2 Pyramidal neurons, and decreased conductance of TREK1. The consequence of this environmentally induced change in intrinsic properties is that an endocannabinoid-mediated synaptic plasticity of inhibitory transmission is rescued in these animals. Furthermore, blocking sPLA2G5 prevents endocannabinoid plasticity in animals reared in an enriched environment. 22q11.2DS mice also have rescued social recognition memory after three weeks in an enriched environment, indicating that hippocampal area CA2 is highly sensitive to intrinsic states of the animal. We are optimistic these results may shed light on the mechanisms underlying effective non-pharmacological treatment for psychiatric disorders.

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Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

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Program #/Poster #: PSTR525.10/D63

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01MH118258

Title: Activation of the ghrelin hormone secretagogue receptors excites the dentate gyrus granule cells by depressing GIRK channels

Authors: *S. LEI, C. ORAEGBUNA@UND.EDU;
Univ. of North Dakota, Grand Forks, ND

Abstract: Ghrelin is an orexigenic hormone involved in a variety of bodily functions including stimulating growth hormone release, suppressing insulin release, maintaining energy homeostasis and it is also associated with chronic stress conditions. The ghrelin hormone secretagogue receptors (GHSRs) are G protein coupled receptors that are widely expressed in different parts of the brain including the dentate gyrus of the hippocampus. Although the GHSRs are robustly expressed within the dentate gyrus, the signaling and ionic mechanisms through which ghrelin affects the neurons of the dentate gyrus remain undetermined. Using whole-cell patch clamp recording, we demonstrated that application of ghrelin produced significant depolarization and increased action potential firing in the dentate gyrus granule cells (GCs). Ghrelin-mediated depolarization was dependent on the activation of adenylate cyclase and cyclic AMP, whereas inhibition of phospholipase C beta did not affect ghrelin-mediated excitation of dentate gyrus neurons. The I-V curve of the net currents generated by ghrelin showed inward rectification with a reversal potential close to the K^+ reversal potential, suggesting that activation of GHSRs excites GCs by depressing an inwardly rectifying K^+ channel. Further experiments demonstrated that tertiapin-Q and SCH23390 blocked ghrelin-induced depolarization, suggesting that GIRK channels are involved.

Disclosures: S. Lei: None. **C. Oraegbuna@und.edu:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

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Program #/Poster #: PSTR525.11/D64

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF grant IOS 2002863 (HGR)
NSF grant IOS 2002971 (XH)

Title: A CA1 pyramidal cell model explains the theta and gamma rhythmic coding properties through complex and single spike modes

Authors: R. PENA[1], U. CHIALVA[2], E. LOWET[3], X. HAN[4], *H. ROTSTEIN[5,6];
[1]New Jersey Inst. of Technol., Jersey City, NJ; [2]Dept. de Matemática, Univ. Nacional del Surand CONICET, Bahía Blanca, Argentina; [3]McGovern Inst. for Brain Res., MIT, Cambridge, MA; [4]Boston Univ., Boston, MA; [5]NJIT, Hoboken, NJ; [6]Federated Dept. of Biol. Sci., New Jersey Inst. of Technol. & Rutgers Univ., Newark, NJ

Abstract: Hippocampal networks display theta (~5-10Hz) and gamma (~30-100Hz) rhythmic activity, which play functional roles in learning, memory and navigation among other cognitive processes. CA1 pyramidal cells (PYR) exhibit two spike modes: complex (CS) and single (SS) spikes. Recent experimental results (Lowet et al., bioRxiv 2022) show that these spike modes preferentially phase-lock to gamma (SS) and theta (CS) network oscillations. While a number of mechanisms for the generation of SSs and CSs have been proposed, the ones underlying their phase-locking properties remain unknown. In particular, it is unclear whether and how they depend on the theta-resonance generating ionic currents in PYR: dendritic hyperpolarization-activated mixed Na^+/K^+ current (I_h), active at hyperpolarized levels, and somatic persistent Na^+ (I_{Nap}) and muscarinic sensitive K^+ (I_M) active at depolarized levels. We address these issues by using a two-compartment biophysical model of Hodgkin-Huxley type including ionic currents known to be present in CA1 PYR, particularly I_h, I_{Nap} and I_M, white (Gaussian) noise and mixed theta/gamma input currents differentially distributed in the somatic and dendritic compartments mimicking the effects of network activity. We used the sequential neural posterior estimation (SNPE) method (Tejero-Cantero et al., J Open Source Softw 2020) to adjust parameters from *in vivo* observations (Lowet et al.). This Bayesian artificial neural network approach minimizes the loss function from the observed and model signals by considering several attributes that characterize these signals (e.g., membrane potential and spike train statistics, phase-locking values with respect to the theta and gamma rhythms). We identify the biophysical parameter ranges for which the model captures the observed SS-gamma and CS-theta preferential phase-locking. Additionally, our model shows that the theta input must be stronger than the gamma one in the dendritic compartment, while the gamma input must be stronger than the theta one in the soma, consistent with the fact that OLM and PV^+ interneurons project to the distal and proximal dendrites, respectively. Finally, we tested the NMDA receptor hypothesis (Grienberger et al., Neuron 2014), finding an increase in CSs with NMDA, with no impact on gamma phase-locking, suggesting NMDA alone is not enough to explain the phenomenon. Our results shed light on the mechanisms responsible for the generation of SSs and CSs and their relationship with the ongoing theta and gamma rhythms. Importantly, our results show that the individual PYR biophysical properties are enough to explain this phenomenon in the presence of theta and gamma rhythms.

Disclosures: **R. Pena:** None. **U. Chialva:** None. **E. Lowet:** None. **X. Han:** None. **H. Rotstein:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.12/D65

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: the National Natural Science Foundation of China (32071040 to BL, 82071241 and 81871048 to LH)

Guangdong Basic and Applied Basic Research Foundation (2023B1515040019 to BL)
Guangdong Project (2017GC010590 to BL)

Title: Precision control of intrinsic excitability homeostasis by subcellular L-type calcium channels

Authors: *Z. WEI, C. WEI, Z. LUO, L. CHEN, X. ZHANG, L. HUANG, B. LI;
Sun Yat-sen Univ., Guangzhou, China

Abstract: The stability of neuronal circuitry depends on the homeostasis of neural firing properties, but how such plasticity is precisely regulated remains to be elucidated. Here we report that neocortical pyramidal neurons increase their firing rate (FR) and action potential duration (APD) in order to adapt to a chronic suppression of activity. During chronic inactivity, somatic Ca_V1 channels in the soma are primarily closed, whereas dendritic Ca_V1 channels tend to be open. In the soma, the closed Ca_V1 channels recruit SAMD3 to the plasma membrane via protein-protein interaction, thereby reducing SMAD3 levels in the nucleus and downregulating the SMAD3-dependent transcription of *Kcnq3*, which encodes the $K_V7.3$ potassium channel. This reduction in $K_V7.3$ channels eventually leads to a homeostatic increase in the neuron's firing rate. Together with our previous finding that activation of Ca_V1 channels in dendritic spines engages the CaMKK-CaMKIV-Nova-2 pathway to regulate the alternative splicing of BK channels, which play a critical role in APD homeostasis, our findings reveal how Ca_V1 channels can regulate the homeostasis of both FR and APD simultaneously in a state-dependent, subcellular localization-dependent, and mechanism-dependent manner. This effect is physiologically relevant, as we found that homeostatic adaptation to chronic neuronal inactivity in the visual cortex led to a stronger innate defensive response to visual stimuli in freely moving mice. Our results provide a framework for understanding how the same protein ($Ca_V1.2$) residing in different states (closed versus open) and subcellular locations (the soma versus dendritic spines) can coordinately regulate critical cellular functions at the molecular, cellular, and behavioral levels. These findings have broad implications with respect to understanding the changes in these homeostatic mechanisms that underlie neurological disorders and for guiding the development of new therapeutic strategies designed to target these mechanisms.

Disclosures: Z. Wei: None. **C. Wei:** None. **Z. Luo:** None. **L. Chen:** None. **X. Zhang:** None. **L. Huang:** None. **B. Li:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.13/D66

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NS100016
NS120720

Title: The organization and properties of cortical gamma rhythms driven by L6-corticothalamic neurons

Authors: *F. S. SUSI, B. CONNORS;
Neurosci., Brown Univ., Providence, RI

Abstract: Corticothalamic (CT) neurons in primary sensory cortex provide massive projections to the thalamus which allow the cortex to modulate its own inputs. Most CT feedback arises from neurons in layer 6 (L6). L6-CT cells also influence the cortex itself via local collaterals in deep layers and ascending connections to middle and upper layers. Recent work has shown that L6-CT cells can also drive a subset of L6 fast-spiking (FS) inhibitory interneurons exerting inhibitory effects on cortical input layers. This circuit configuration suggests that L6-CT cells are well-poised to influence sensory processing at two early and sequential steps: the sensory thalamus and cortical L4, the major input layer. Our laboratory previously found that stimulating L6-CT cells can drive fast, synchronous, gamma-like rhythms in cortex and thalamic relay cells. FS cells are critical for the generation of cortical gamma rhythms generally, so L6-FS cells may play a role in L6-CT-imposed rhythms in superficial layers.
Here we investigate the scope and mechanisms by which L6-CT neurons drive fast, synchronous rhythms in overlying cortical circuits. To do this we used an *Ntsr1*-Cre- mouse line to target L6-CT cells for optogenetic stimulation in S1 cortex *in vitro*. A 16-channel electrode array was aligned to the cortical column to record LFPs and single units while whole-cell recordings were obtained from FS and RS neurons. Ramps of light evoked robust gamma oscillations in L6 and layers above. We asked to what spatial extent gamma rhythms were imposed superficially in the cortex, and measured differences in the power and phase across layers of the column. We also aimed to determine whether different cell types and layers preferentially participated in these rhythms, and studied coherence and spike-field coupling between neurons and LFPs over multiple layers. We further characterized intrinsic and network properties across neuronal subtypes, characterized their heterogeneity, and asked whether specific cell types in particular layers preferentially participated in fast rhythms.

Disclosures: F.S. Susi: None. **B. Connors:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.14/D67

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: 2021ZD0202500
2019-01- 07-00-07-E00062
2020CXJQ01
No.2018SHZDZX01

Title: Correlations of molecularly defined cortical interneuron populations with morpho-electric properties

Authors: *Y.-C. YU;
Fudan Univ., Shanghai, China

Abstract: Cortical interneurons can be categorized into distinct subtypes based on multiple modalities; however, it remains unclear to what extent these modalities are correlated. Here, we utilized patch-clamp single-cell RT-PCR (Patch-PCR) to investigate the correlations between molecular marker expressions and morpho-electric properties of over 600 layer 5 (L5) interneurons in the mouse somatosensory cortex (S1). Based on extracted morpho-electric features and differential expressions of neurochemical markers and transcriptomic signatures, we identified 11 morpho-electric subtypes (M/E types), 9 neurochemical cell groups (NC groups) and 20 transcriptomic cell groups (TC groups). We found that cells in NC groups and TC groups typically comprised several M/E types, yet combinatorial expression of certain neurochemical markers and expression levels of specific transcriptomic signatures were statistically correlated to given M/E types. Moreover, we found that, at the subclass level (Pvalb, Sst, Vip and Non-Vip), most interneurons exhibited distinct morpho-electric properties, and this distinction was relatively weak between individual TC groups. Similar results were also obtained in the primary visual cortex (V1) and motor cortex (M1) using recently published Patch-seq data. Interestingly, a significantly stronger correlation between morpho-electric properties and TC groups in V1 compared to S1 and M1 was observed. Systematic comparison of TC groups between these brain areas suggested that, compared to V1, S1 interneurons were morpho-electrically more similar to M1. Together, this study revealed a complex multimodal correlation landscape across different cortical areas.

Disclosures: Y. Yu: None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.15/D68

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant K08 NS118114
NIH Grant R01 NS100016

Title: Activity-dependent ectopic action potentials in regular-spiking neurons of the mouse neocortex

Authors: *S. SAPANTZI[1], Y. ZHANG[4], A. LIN[4], B. W. CONNORS[2], B. B. THEYEL[3]; [2]Neurosci., [3]Psychiatry and Human Behavior, [1]Brown Univ., Providence, RI; [4]Brown Univ. Neurosci. Grad. Program, Providence, RI

Abstract: Action potentials typically originate at axon initial segments and propagate toward axon terminals. Some neurons can also fire "ectopic action potentials" (EAPs) that originate in distal axons or terminals and travel antidromically. EAPs have been detected in various types of neurons under both pathological and physiological conditions. The goals of this study were to test whether regular-spiking (RS) cells of the neocortex can fire EAPs in physiological conditions and, if they can, to determine what stimulus patterns most readily evoke EAPs. We made whole-cell recordings from somata of 60 RS cells (pyramidal neurons) in layers 2/3 of mouse orbitofrontal cortex in vitro. Cells were stimulated with either 600 ms, incrementally increasing (by 5 pA), current steps presented every 2 sec, or brief-pulse trains at 30, 60, or 100 Hz. We tested three groups of cells (20 in each) with different patterns: 1) fixed pulse train of 1 sec duration presented every 2 sec with a 5 min rest between protocols (3 sec with rest), 2) fixed number of pulses (180) presented once every 10 sec with a 5 min rest between protocols (10 sec with rest), and 3) fixed number of pulses (180) presented every 10 sec (10 sec without rest). Among 60 RS neurons, 34 (56.6%) fired EAPs. The step protocol was least effective (EAPs evoked in 16.7% of 60 cells). The frequency-based protocol in the '10 sec with rest' group was most effective (EAPs in 75% of 20 cells). The '3 sec with rest' group (EAPs in 50% of 20 cells) and the '10 sec without rest' (EAPs in 45% of 20 cells) were moderately effective. Among the 34 cells that fired EAPs, one fired EAPs in response to all four protocols (steps, 30, 60, and 100 Hz), 13 cells only responded to one protocol, and the rest (20) were responsive to two or three protocols. At least half of neocortical RS neurons in nonpathological mouse orbitofrontal cortex were capable of firing EAPs after sufficient somatic excitation. Among the three groups, the '10 sec without rest' approach was the most effective. Some cells fired EAPs only after stimulation at a single frequency, while others responded to more than one frequency. Future work will explore the ability of RS cell subtypes to fire EAPs, and the mechanisms underlying EAPs in RS cells.

Disclosures: S. Sapantzi: None. **Y. Zhang:** None. **A. Lin:** None. **B.W. Connors:** None. **B.B. Theyel:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.16/D69

Topic: B.08. Epilepsy

Support: NIH Grant NS130042

Title: Heterozygous expression of a Kcnt1 gain-of-function variant has differential effects on SST- and PV-expressing cortical GABAergic neurons.

Authors: A. SHORE[1], W. N. FRANKEL[2], ***M. WESTON**[3];
[1]Fralin Biomed. Res. Inst., Roanoke, VA; [2]Columbia Univ. Med. Ctr., New York, NY; [3]Fralin Biomed. at Virginia Tech., Roanoke, VA

Abstract: More than twenty recurrent missense gain-of-function (GOF) mutations have been identified in the sodium-activated potassium (K_{Na}) channel gene *KCNT1* in patients with severe developmental and epileptic encephalopathies (DEEs), most of which are resistant to current therapies. A consistent finding in mouse models of KCNT1-related epilepsy is reduced GABAergic interneuron excitability, however, the molecular identities of the most affected subtypes are unknown. Here, we assessed the effects of heterozygous expression of a *Kcnt1* GOF variant (Y777H) on K_{Na} currents and neuronal physiology among cortical GABAergic neuron subtypes, including those expressing vasoactive intestinal polypeptide (VIP), somatostatin (SST), and parvalbumin (PV), in mice to identify and model the pathogenic mechanisms of autosomal dominant *KCNT1* GOF variants in DEEs. Although the *Kcnt1*-Y777H variant had no effect on VIP neurons, it increased subthreshold K_{Na} currents in both SST and PV neurons but with opposite effects on neuronal output; SST neurons became hypoexcitable with a higher rheobase current and lower AP firing frequency, whereas PV neurons became hyperexcitable with a lower rheobase current and higher AP firing frequency. Further neurophysiological and computational modeling experiments showed that the differential effects of the Y777H variant on SST and PV neurons are not likely due to inherent differences in these neuron types, but to an increased persistent sodium current in PV, but not SST, neurons. The Y777H variant also increased excitatory input onto, and chemical and electrical synaptic connectivity between, SST neurons. Together, these data suggest differential pathogenic mechanisms, both direct and compensatory, contribute to disease phenotypes, and provide a salient example of how a pathogenic ion channel variant can cause opposite functional effects in closely related neuron subtypes due to interactions with other ionic conductances.

Disclosures: A. Shore: None. **W.N. Frankel:** None. **M. Weston:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.17/D70

Topic: B.08. Epilepsy

Title: GABAergic interneuron subtypes and their GABA-A receptor outputs are differentially tuned and targeted by select neurostimulation paradigms.

Authors: *D. NAYLOR;
Veterans Admin. - UCLA, Los Angeles, CA

Abstract: Interneurons (INs) shape normal and pathological circuit dynamics. Two key types are the parvalbumin- (PV) and somatostatin- (SOM) INs. PV-INs somatically innervate and are highly divergent to 1000 or more excitatory cells and are fast-spiking and provide feedforward inhibition. They drive normal circuit oscillations over multiple frequency domains and also are implicated as early participants with seizures. SOM-INs act on distal dendrites and fire sparsely for local processing and feedback inhibition. The differential activation properties of PV- and SOM-INs were probed to various patterns of electrical stimulation in vitro using calcium imaging of select IN subpopulations. Data-optimized computational models of GABA-AR phasic and tonic currents predicted subsequent post- and extra-synaptic inhibitory responses to IN driving by similar frequency/burst stimulation patterns. SOM-IN dynamic calcium fluorescence effectively tracks individual pulses while PV-IN fluorescent activation is favored by high-frequency trains of stimulation (SOM- or PV-Cre / AAV1-Syn-FLEX-GCaMP6f). After hi-freq hi-intensity activation, the PV-IN fluorescence lasts 2-3x longer than for SOM-INs, often >10s. SOM-INs show much lower activation thresholds than PV-INs, with as robust induced fluorescence at 0.1 as 10 mA stimulus intensity. Spontaneous hypersynchronous activity in SOM-INs also is more easily induced with 0 Mg++ aCSF. With data-optimized computational modeling of evoked GABA-AR responses in dentate gyrus granule cells that includes synaptic 'spillover', above 40 Hz the extrasynaptic δ subunit-containing GABA-ARs cannot track the input and integrate the individual evoked responses. This DC contribution from δ relative to γ2 subunit-containing GABA-ARs at synapses becomes more pronounced with increase to 150 Hz, with significant desensitization with the latter. Between 3-6 Hz, responses are oscillatory and track stimulation while also maintaining synaptic GABA-AR desensitization that presumably maintains circuit hyperactivity. Adding 'bursting' to this lower frequency driving increases the peak phase of inhibition 60%, but accentuates desensitization 250%. In summary, specific patterns of stimulation selectively engage subpopulations of INs as well as the post- and extrasynaptic GABA-ARs that mediate their inhibitory output to excitatory cells. The effects from even brief patterns of stimulation can long outlast the stimulus by 10s of sec or longer. Optimization of complex and/or intermittent stimulation should facilitate specific targeting of cell subtypes, as well as their downstream outputs, for greater control of circuit dynamics.

Disclosures: D. Naylor: None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.18/E1

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF Grant Neuronex 2015276

Title: Input-output transformation properties of parvalbumin interneurons from mouse visual and prefrontal cortices

Authors: *Y. NISHIHATA[1], T. MIYAMAE[2], O. L. KRIMER[2], D. A. LEWIS[2], G. G. BURGOS[2];
[2]Dept. of Psychiatry, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Parvalbumin-positive (PV) cells are a prominent class of interneurons which control pyramidal neuron activity via perisomatic inhibition in all areas of the mammalian neocortex. Interestingly, PV cells represent a larger fraction of all interneurons in sensory relative to association neocortical areas. For instance, PV cells are >40% of all interneurons in primary visual cortex (VC) but <20% in prefrontal cortex (PFC). Despite these differences between areas, little is known vis-à-vis the regional specializations of PV cell properties. Here we compared the electrophysiological properties of PV cells between mouse VC and PFC using patch clamp electrophysiology in acute slices. To compare the capacity to transform excitatory input into spike output, we studied the intrinsic membrane properties using injection of rectangular steps of excitatory current and more natural/variable patterns of input current. In both VC (n=37 cells) and PFC (n=32 cells), PV cells exhibited typical Fast Spiking (FS) properties. However, as reported for the somatosensory cortex, in VC we found two phenotypes of PV cells based on their intrinsic membrane properties. These phenotypes were distinguished by the presence or absence of a delay to fire the first spike at rheobase and termed delayed FS (dFS) and continuous FS (cFS), respectively. Additionally, the dFS and cFS PV cells from VC differed in 6 of the 16 membrane properties measured, with cFS cells showing greater excitability than dFS cells. In VC, dFS neurons were the majority of the PV cells (dFS: 28/37; cFS: 9/37). Importantly, consistent with the idea that the first spike delay naturally defines two phenotypes of PV cells in VC, cluster analysis revealed two main groups of PV cells with dFS and cFS properties. Although PV cells from PFC could also be divided into two groups via the first spike delay at rheobase (dFS: 17/32; cFS: 15/32), the PFC PV cells with or without first spike delay did not differ in any of the other 16 membrane properties assessed. Moreover, cluster analysis generated multiple groups of PV neurons in PFC, which were not clearly related to the presence/absence of first spike delay at rheobase. sEPSCs recorded from PV cells from VC (n=26) and PFC (n=17) did not differ in peak amplitude, suggesting that the strength of excitatory drive onto PV cells is similar in VC and PFC. Although analysis of the response to stimulation with natural patterns of excitatory current is in progress, our current findings support the idea that PV neuron electrophysiology shows regional specializations in association cortical areas relative to primary sensory cortices.

Disclosures: Y. Nishihata: None. **T. Miyamae:** None. **O.L. Krimer:** None. **D.A. Lewis:** None. **G.G. Burgos:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.19/E2

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant 1R01AA028861-01A1

Title: The Role of Short- Term Alcohol Administration on the Intrinsic Excitability of Medial Prefrontal Cortex Neurons.

Authors: *S. R. RODRIGUEZ[1], J. T. O'BRIEN[1], Y. LUO[2], S. MENDEZ[2], J. JAMES[2], Z. KANWAL[2], N. SUJI[3], S. JALILVAND[2], A. KHAN[3], R. NUNA[2], R. JUPELLY[2], S. TATA[3], S. KROENER[4];
[1]Neurosci., Univ. of Texas Dallas, Plano, TX; [2]Neurosci., [3]Univ. of Texas Dallas, Dallas, TX; [4]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas Sch. of Behavioral and Brain Sci., Richardson, TX

Abstract: Alcohol use disorder (AUD) is linked to prefrontal cortex (PFC) impairments that compromise cognitive and executive functions leading to heightened risk of relapse. In rodent models, activation of the prelimbic (PL) or infralimbic (IL) regions of the PFC are thought to promote or extinguish alcohol intake, respectively. However, even within these regions, neurons may show heterogeneous responses to alcohol or other reinforcers. Using a transgenic mouse line (TRAP2/Ai9) which permits fluorescent tagging of active populations, we identified PL and IL neurons that were active during withdrawal in mice that self-administered for alcohol or sucrose. We conducted whole-cell patch-clamp current-clamp recordings in PL and IL neurons that were either active (Ai9+) or inactive (Ai-) following 16-hour withdrawal. Neurons received acute application of alcohol (20mM) ex vivo following initial baseline recordings. Our behavioral experiments indicate that mice self-administering for alcohol exhibit similar patterns of responding and cue-induced reinstatement values over a 3-week period to those self-administering for sucrose, a potent reinforcer. Our electrophysiological recordings from the IL reveal increased action potential firing rates during withdrawal in both Ai9+ and Ai- neurons in alcohol compared to sucrose mice, suggesting a broad neural response of the IL region to alcohol withdrawal. Among alcohol mice, IL Ai9+ neurons exhibited significantly greater firing rates than their PL counterparts, suggesting that alcohol withdrawal effects may be more pronounced in these neurons. Our electrophysiological recordings from the PL reveal increased action potential firing in Ai9+ neurons in sucrose versus alcohol mice, whereas Ai- neurons showed no difference in firing rates between groups, suggesting differential, substance-specific modulation of neuronal excitability in the PL region. Acute alcohol application had no effect on firing rates across all assessed cell types indicating that acute exposure to alcohol does not directly modulate neuronal excitability and may suggest that the observed neuronal hyperactivity during withdrawal may be more likely associated with long-term adaptive changes. Overall, our results highlight the heterogeneity of neuronal responses in the PFC during withdrawal from a history of alcohol exposure and support the existence of a PL/IL dichotomy in the context of reward-seeking. These findings underscore the need for further research to elucidate the specific neurophysiological mechanisms underlying alcohol withdrawal, which may pave the way for more targeted therapeutic interventions for AUD.

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Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.20/E3

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIMH Grant K00MH134674

Title: Selective, use-dependent block of sodium channel isoforms identifies differing roles in excitability of neocortical excitatory and inhibitory neurons.

Authors: *J. D. GARCIA[1], C. WANG[2], D. H. HACKOS[4], K. J. BENDER[3];
[1]Neurol., The Univ. of California, San Francisco, San Francisco, CA; [3]Dept. of Neurol., [2]UCSF, San Francisco, CA; [4]Neurosci., Genentech, South San Francisco, CA

Abstract: The sodium channel isoforms Na$_V$1.1, Na$_V$1.2, and Na$_V$1.6 are expressed throughout the mature central nervous system. In neocortex, Na$_V$1.6 is expressed in all neurons. Functional Na$_V$1.2 and Na$_V$1.1 expression, by contrast, appears to be more segregated, with Na$_V$1.2 found largely on excitatory pyramidal neuron membranes and Na$_V$1.1 found instead on inhibitory parvalbumin and somatostatin neurons. In addition to differential cell type expression, membrane expression within a class can change over development, with isoforms shifting localization and density in somatic, axonal, and dendritic regions dynamically over the first few weeks of mouse postnatal development. Previous work has leveraged constitutive and conditional knockout animals for sodium channel genes (*Scn1a, Scn2a, Scn8a*) to understand their differing roles in neuronal excitability, but interpretation can be limited by known or unknown compensatory changes in the expression of other ion channels. Thus, methods to potently, acutely, and reversibly block specific Na$_V$ isoforms could provide insight into their relative roles. Aryl sulfonamide-based molecules (ASMs) have strong potency at Na$_V$1.2 and Na$_V$1.6, and Na$_V$1.7, but weak potency at all other Na$_V$s. Differing potencies are due to sequence differences in the extracellular part of the fourth voltage-sensing domain, where aryl-sulfonamide Na$_V$ inhibitors bind to inactivated channels. Here, we developed knockin mice in which the aryl-sulfonamide Nav inhibitor binding pocket in Na$_V$1.2 or Na$_V$1.6 is replaced with one from Na$_V$1.1/1.3, reducing binding affinity by over 3 orders of magnitude. With these mice, we found that acute block of Na$_V$1.2 and Na$_V$1.6 have very different effects on neocortical pyramidal cells. Na$_V$1.6 blockade depolarized action potential (AP) threshold markedly, with modest effects on the peak speed of AP depolarization (dV/dt). By contrast, block of Na$_V$1.2 had no effect on AP threshold, but instead reduced peak AP speed and led to an acute increase in AP output per given somatic current stimulus. This latter effect is consistent with previous work identifying paradoxical

hyperexcitability from conditional *Scn2a* knockout, and indicates that such effects are due to selective $Na_V1.2$ loss and not compensatory changes in other ion channels. Furthermore, examination of AP initiation in parvalbumin interneurons revealed no role for $Na_V1.2$ in excitability, but a major role for $Na_V1.6$ in setting AP threshold in mature mice. Overall, these techniques may permit a better understanding of the differing roles of Na_V isoforms across diverse cell classes and developmental periods.

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Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.21/E4

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF Grant 1650113
NIH Grant AA027023
NIH Grant MH112729

Title: Axon initial segment GABA inhibits action potential generation throughout periadolescent development

Authors: *A. LIPKIN[1], K. J. BENDER[2];
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Abstract: Neurons are remarkably polarized structures: dendrites spread and branch to receive synaptic inputs while a single axon extends and transmits action potentials to downstream targets. Neuronal polarity is maintained by the axon initial segment (AIS), a region between the soma and axon proper that is also the site of action potential (AP) generation. This polarization between dendrites and axons extends to inhibitory neurotransmission. In adulthood, the neurotransmitter GABA hyperpolarizes dendrites but instead depolarizes axons. These differences in function collide at the AIS. Multiple studies have shown that GABAergic signaling in this region can share properties of either the mature axon or mature dendrite, and that these properties evolve over a protracted period encompassing periadolescent development. Here, we explored how developmental changes in GABAergic signaling affect AP initiation. We show that GABA at the axon initial segment inhibits action potential initiation in Layer 2/3 pyramidal neurons in prefrontal cortex from mice of either sex across GABA reversal potentials observed in periadolescence. These actions occur largely through current shunts generated by $GABA_A$ receptors and changes in voltage-gated channel properties that affected the number of channels that could be recruited for AP electrogenesis. These results suggest that GABAergic neurons targeting the axon initial segment provide an inhibitory “veto” across the range of

GABA polarity observed in normal adolescent development, regardless of GABAergic synapse reversal potential.

Disclosures: **A. Lipkin:** None. **K.J. Bender:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BioMarin Pharmaceuticals Inc..

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.22/E5

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01 MH124695

Title: Loss of Shank3 decreases excitability of prefrontal parvalbumin-positive interneurons but not pyramidal cells in early development

Authors: *Y.-C. SHIH[1], L. NELSON[2], M. JANECEK[3], R. PEIXOTO[4];
[1]Ctr. For Neurosci., [2]Neurosci., [3]Ctr. for Neurosci., [4]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Loss of Shank3 decreases excitability of prefrontal parvalbumin-positive interneurons during postnatal development
Accelerated maturation of corticostriatal synapses onto striatal spiny projection neurons (SPNs) is associated with the onset of behavior abnormalities in *Shank3B*$^{-/-}$ mice, a model of autism spectrum disorders (ASD). Previous studies have shown that global deletion of Shank3 results in cortical hyperactivity and corticostriatal hyperconnectivity during early postnatal development. Here we provide evidence that corticostriatal hyperconnectivity during early postnatal development is not caused by cell-autonomous changes in *Shank3B*$^{-/-}$ SPNs, supporting that this phenotype is caused by extrinsic changes in network activity. To determine the circuit mechanisms that underlie early cortical hyperactivity, we characterized the development of glutamatergic inputs onto layer II/III pyramidal neurons (Pyr) and parvalbumin-positive interneurons (PV) of anterior cingulate cortex (ACC) of *Shank3B*$^{-/-}$ mice during P14-P15 period and adulthood. We found no difference in mEPSC amplitude and frequency in both cell-types between wild-type and *Shank3B*$^{-/-}$ mice but decreased mEPSC amplitude and frequency appear later in adulthood. We then examined the intrinsic cell properties of both types of neurons in ACC during this period. Interestingly, loss of Shank3 specifically decreases excitability of PV but not Pyr neurons in ACC during early development. These findings provide evidence of abnormal maturation of PV neurons in the prefrontal cortex of Shank3 KO mice, suggesting that this mechanism might contribute to cortical hyperactivity and corticostriatal hyperconnectivity in *Shank3B*$^{-/-}$ mice during early postnatal development.

Disclosures: **Y. Shih:** None. **L. Nelson:** None. **M. Janecek:** None. **R. Peixoto:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.23/E6

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF Grant NSF1456302
NSF Grant NSF1645199
NIH Grant GM120464
NIH Grant DC004274
NIH Grant DC012938
NIH Grant AG055378
NSF Grant NSF2154646

Title: Mechanisms of serotonin effects on spike firing in a songbird motor cortex analog neuron

Authors: *B. M. ZEMEL[1], L. E. S. TAVARES[2], C. V. MELLO[3], H. VON GERSDORFF[4]; [1]Oregon Hlth. and Sci. Univ., Portland, OR; [2]Vollum Institute, OHSU, Portland, OR; [3]Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR; [4]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Serotonin (5-HT) is involved in modulating an array of complex behaviors. In zebra finches, brainstem-projecting vocal-motor neurons of the robust nucleus of the arcopallium (RAPNs) express HTR2 receptors, similar to layer V cortical pyramidal neurons in mammals. Previous studies found that activation of HTR2 receptors led to increased excitation in nucleus RA, increasing spontaneous firing rates as measured by extracellular recordings *in vitro* and *in vivo* (Wood et al., *J Neurosci*, 2011). Additionally, 5-HT was shown to be involved in modulating spectral properties of song, likely via changes in the signal to noise ratio during burst-pause-burst activity in RAPNs (Wood et al., *J Neurosci*, 2013). However, it remains unknown 1) whether the 5-HT mediated effect on firing rates is due to changes in the RAPN active (i.e. spike waveform) and/or passive (i.e. subthreshold) properties and 2) what molecular mechanisms, including second messenger cascades, underlie the changes in RAPN firing in response to 5-HT exposure. We have recently identified RAPNs as a unique class of upper-motor neuron that shares several properties with the large layer V Betz cells found in primates and cats, but not in rodents. Interestingly, previous studies in the cat motor cortex also found a class of Betz cells that increase their firing rates in response to 5-HT exposure. Here we used whole cell patch clamp electrophysiology from brain slices to investigate the effects of 5-HT on the intrinsic excitable properties of RAPNs. Administration of 5-HT led to increases in both the spontaneous and evoked firing rates of RAPNs. This increase in firing rates coincided with an apparent depolarization of the average resting membrane potential. Interestingly, whereas the interspike periods decreased substantially, we saw relatively modest changes in the spike waveform which may result from the depolarized membrane potential. This membrane depolarization could originate from a number of sources including, but not limited to, closure of leak or low threshold

voltage-gated K^+ channels, or potentiation of low threshold voltage gated Ca^{2+} channels, the hyperpolarization activated current or the persistent Na^+ current we identified in a previous study. We are currently investigating which of these conductances may be affected by 5-HT, as well as the second messenger pathways that may be involved. Our current and future findings are aimed at providing insight into the regulation of excitability in specialized cortical circuits that are involved in fine motor control.

Disclosures: B.M. Zemel: None. **L.E.S. Tavares:** None. **C.V. Mello:** None. **H. von Gersdorff:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.24/E7

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: CIHR
Neuronex-Working Memory

Title: Using calcium imaging to assess physiological response diversity of ex vivo marmoset and murine cortical pyramidal neurons during spontaneous activity and electrical stimulation.

Authors: *S. VIJAYRAGHAVAN[1], H. IGARASHI[2], W. INOUE[3], A. PRUSZYNSKI[3], J. C. MARTINEZ-TRUJILLO[4];
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Abstract: A hierarchy of intrinsic timescales of neuronal activation across the different sensory and association cortical areas is a potential mechanism by which cortex encodes and maintains information in working memory for longer periods of time (Murray et al., Nat. Neurosci. (2014), 17 (12), 1661-1663). The timescales of activation of neurons as measured by their autocorrelation function during cognitive tasks shows a gradient along the cortical hierarchy from sensory to prefrontal association areas. Several mechanisms could contribute to the longer timescales in the neuronal autocorrelation functions in association cortices. Ca^{++} imaging with genetically encoded indicators such as GCaMP in cortical slices combined with electrical microstimulation offers an attractive methodology to examine the response profiles of many neurons simultaneously to further address the mechanisms by which activation timescales vary between different areas. Here, we examined calcium dynamics with two photon microscopy while electrically stimulating cortical slices from the marmosets and mice. We virally expressed GCaMP6f in multiple marmoset and murine cortical areas under the control of the CAMKII

promoter. We characterized the time-course, latency of onset, amplitude, and other parameters of Ca^{++} responses of pyramidal neurons to electrical stimulation of prefrontal and sensory cortical slices with and without blockade of ongoing synaptic transmission. Additionally, we examined spontaneous Ca^{++} oscillations in cortical slices in a modified artificial cerebrospinal fluid that is previously shown to induce spontaneous rhythmic activity in ex vivo preparations. We show that there is considerable diversity in the stimulation-induced and spontaneous Ca^{++} activation dynamics of cortical pyramidal neurons. Pyramidal neurons in cortical slices show Ca^{++} oscillations of varying strength and timescales delineating potentially tractable model for studying the diversity in intrinsic physiological properties of pyramidal neurons along the cortical hierarchy.

Disclosures: S. Vijayraghavan: None. **H. Igarashi:** None. **W. Inoue:** None. **A. Pruszynski:** None. **J.C. Martinez-Trujillo:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.25/E8

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH NINDS R01 NS119977
Burroughs Wellcome Fund Career Award

Title: Ndnf interneuron excitability is spared in a mouse model of Dravet Syndrome

Authors: *S. R. LIEBERGALL[1], E. M. GOLDBERG[2];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]The Children's Hosp. of Philadelphia, Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Dravet syndrome (DS) is a neurodevelopmental disorder characterized by epilepsy, developmental delay/intellectual disability, and features of autism spectrum disorder, caused by heterozygous loss-of-function variants in SCN1A encoding the voltage gated sodium channel a subunit Nav1.1. The dominant model of DS pathogenesis is the “interneuron hypothesis,” whereby GABAergic interneurons (INs) express and preferentially rely on Nav1.1-containing sodium channels for action potential generation. This has been shown for three of the major subtypes of cerebral cortex GABAergic INs: those expressing parvalbumin, somatostatin, and vasoactive intestinal peptide. Here, we define the function of the fourth major subtype of INs, those expressing Neuron-derived neurotrophic factor (Ndnf). Ndnf-INs have unique properties and have been linked to a broad range of normal brain functions, such as the gating of long-range thalamocortical feedback loops, gain-modulation of sensory inputs, regulation of associative learning and plasticity, and decoupling pyramidal cells from local synchronous activity. Whole cell patch clamp electrophysiological recordings of Ndnf-IN in acute brain slices from male and

female DS (*Scn1a+/-*) mice and age-matched wild-type controls revealed normal intrinsic membrane properties, properties of action potential generation, and synaptic transmission across development, suggesting that Ndnf-INs are the only interneuron subtype with preserved excitability in DS. We extended these *ex vivo* findings to *in vivo* recordings of Ndnf-IN activity using two-photon calcium imaging, in which Ndnf-INs in Scn1a+/- mice show similar baseline activity and are recruited during arousal similarly to wildtype controls. This discovery of an IN subtype with spared function in a mouse model of DS suggests a refinement of the "interneuron hypothesis." Additionally, the preserved excitability and synaptic function of Ndnf-INs indicates that these cells are candidates for targeted manipulation for the treatment of DS and potentially other epilepsy syndromes.

Disclosures: S.R. Liebergall: None. **E.M. Goldberg:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.26/E9

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Israel Science Foundation (ISF) Grants ISF 946/17and ISF 258/20 (to K.R.).

Title: Intrinsic properties of layer 5 Anterior Insula (aIC) projection neurons represent taste valence encoding.

Authors: *S. KOLATT CHANDRAN[1], A. YIANNAKAS[1,3], H. KAYYAL[1], R. SALALHA[1], F. CRUCIANI[1], M. KHAMAISY[1], K. ROSENBLUM[1,2];
[2]Ctr. for Gene Manipulation in the Brain, [1]Univ. of Haifa, Haifa, Israel; [3]Inst. of Biochem. and Mol. Med., Univ. of Bern, Bühlstrasse 28, 3012, Bern, Switzerland, Switzerland

Abstract: Identifying and avoiding potentially harmful food is crucial for the survival of many organisms. Conditioned taste aversion (CTA) is an associative learning paradigm when a novel appetitive tastant (e.g., saccharin) paired with a malaise-inducing agent such as an intraperitoneal injection of lithium chloride induces aversion toward the same tastant, presented again. Recent studies have highlighted the crucial role of the bidirectional connectivity of the insular cortex (IC) and basolateral amygdala (BLA) in CTA memory acquisition and retrieval. In separate studies using in vivo Ca2+ imaging and chemogenetic tools in BLA-projecting neurons of the mouse IC, we have shown that activity in the projection is correlated, necessary, and sufficient for taste valence representation. However, how taste valance is encoded, predicted, or computed remains unknown. Using retrograde viral tracing and whole-cell patch-clamp electrophysiology, we demonstrated that retrieval of the same taste with different valances changes the intrinsic properties of aIC layer V/VI but not II/III neurons projecting to the BLA and different cortical

regions. These changes were specific to taste valence but not sensory information. Our current results suggest that taste valence is encoded, at least in part, by changes in the intrinsic properties of layer 5 projecting neurons in anterior IC. Our study highlights the importance of the plasticity of neuronal intrinsic properties of IC projection neurons in the confidence of taste valence encoding.

Disclosures: S. Kolatt chandran: None. **A. Yiannakas:** None. **H. Kayyal:** None. **R. Salalha:** None. **F. Cruciani:** None. **M. Khamaisy:** None. **K. Rosenblum:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.27/E10

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: ERA-NET NEURON Program (ANR-20-NEUR-0005 VELOSO)
FLAG-ERA HBP Program (ANR-21-HBPR-0002 VIPattract)

Title: Projection specific subpopulations of anterior thalamic neurons possess distinct anatomical and electrophysiological properties.

Authors: *D. LIM[1], J. JOHN-VYANI-RAJALINGAM[1], M. GRAUPNER[2], D. FRICKER[1]; [1]Univ. Paris Cité, CNRS UMR8002, Paris, France; [2]Saints-Pères Paris Inst. For the Neurosciences, Saints-Pères Paris Inst. for the Neurosciences, Paris Cedex 06, France

Abstract: The Head Direction (HD) system is crucial for spatial orientation. The anterior thalamic nucleus (ATN) is one of its key brain regions. It is composed of two main subdivisions: the anterodorsal (AD) and the anteroventral nucleus (AV). ATN receives HD input originating from the lateral mammillary nucleus (LMN), and it sends HD information to the retrosplenial cortex (RSC) and to the presubiculum (PrS). However, it has been unclear which thalamic projecting neurons target RSC or PrS or both. In this study, anterior thalamic neurons were investigated to elucidate their 1) anatomical output connectivity to these two cortical regions, 2) electrophysiological intrinsic properties and 3) electrophysiological responses to the activation of LMN inputs.
First, we demonstrate a topographical segregation and some overlap of gRSC-projecting and PrS-projecting populations of neurons in the ATN in mice. Retrograde tracers (CTB) conjugated with different fluorescent proteins were injected in gRSC and PrS, and labelled neurons in the ATN were quantified. In AD, gRSC-projecting neurons were ventro-laterally located, while PrS-projecting neurons were dorso-medially located. 38% of labelled AD neurons were doubly labelled. In AV, gRSC-projecting neurons were ventro-medially located, and PrS-projecting neurons were dorso-laterally located. 5% of the labelled AV neurons were doubly labelled.
Second, we found that AD and AV neurons possess distinct electrophysiological intrinsic

properties. A total of 36 neurons were recorded in acute brain slices containing the thalamus, using the whole cell patch clamp technique. Neurons in the two nuclei differed in their mean resting membrane potential (AD: -61.5 mV vs. AV: -71.8 mV), time constant (12.3 ms vs. 18.5 ms), sag ratio (1.42 vs. 1.06), input-output relation (382 Hz/nA vs. 173 Hz/nA), firing patterns (adaptive vs. irregular), and the presence or absence of a post-train hyperpolarization. This result suggests that these two thalamic nuclei differently process information sent to cortex.
Lastly, AAV-Chronos was injected into the LMN. Axons originating from LMN targeted the AD and AV. The projecting fibers were strongly innervating AD and dorsal AV, and to a lesser degree the ventral AV. Initial results found short latency excitatory responses in ATN neurons following the optogentic stimulation of axons from LMN in slices.
Taken together, our data provide new insight in the routing of HD information via the ATN to Presubiculum and the Retrosplenial Cortex.

Disclosures: D. Lim: None. **J. John-Vyani-Rajalingam:** None. **M. Graupner:** None. **D. Fricker:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.28/E11

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: R01NS110079
P01NS074972
NSF 2015276

Title: In search of Genetic Correlates of Neuronal Firing-Rate Adaptation: A Patch-seq Analysis of Transcriptomic Subtypes

Authors: Y. KANG[1], ***J. MENG**[1], S. S. DELLAL[2], I. KRUGLIKOV[3], B. RUDY[4], X.-J. WANG[1];
[1]New York Univ., New York, NY; [2]Neurosci., NYU Langone Sch. of Med., New York, NY; [3]Neurosci. Insitutute, NYU Langone Med. Ctr., New York, NY; [4]Physiol. & Neurosci., NYU Sch. of Med., NEW YORK, NY

Abstract: The firing behavior of a neuron is affected both by its input and recent firing history. One example is the firing-rate adaptation (Adap), which has been observed in pyramidal cells, Somatostatin-expressing (SST) Interneurons (IN) but not Parvalbumin-expressing (PV) INs. The strength of the Adap is believed to be related to different cognitive functions, such as working memory. However, how the genetic differences between these cell types explain the Adap differences is poorly understood.
The recently developed Patchseq technology, which collects genetic, electrophysiological, and

mo morphological data from the same cell, provides a new perspective for studying this problem. In this study, we analyzed two public Patchseq datasets from Allen. We first systematically studied the electrophysiological features of PV and SST cells from mouse V1 data. Surprisingly, we found that the half-width of the action potential is strongly correlated with the Adap. Next, we investigated the mechanism of this correlation by including transcriptomic data. The Adap is believed to be the result of a strong medium after-hyperpolarization current (mAHP), mediated by small-conductance calcium (Ca)-dependent potassium (SK) channels. Interestingly, the SK channel coding genes are not significantly differentially expressed between PV and SST INs, but the upstream voltage-dependent calcium channels (Cav) encoding genes are. This led us to hypothesize that the Cav channels control the flow rate of Ca and the half-width fixes the time window of the Ca flow, and the product of these two controls the magnitude of the Adap. A similar analysis was conducted on a mouse M1 dataset that includes pyramidal cells. Finally, we sought experimental evidence that supports this hypothesis. Previously it had been shown that blocking voltage-gated Kv3 channels abolished the fast-spiking (FS) features of PV INs and Adap. In addition, another experiment on rat somatosensory L5 pyramidal cells showed that blocking Cav channels caused a reduction of mAHP. Our preliminary data show that blocking T-type Ca channels by TTA-P2 decreased the degree of adaptation. Taken together, we argue that the Adap differences across cell types are controlled by upstream Ca influx but not the downstream SK channel, and this mechanism is robust in different brain regions.

Disclosures: **Y. Kang:** None. **J. Meng:** None. **S.S. Dellal:** None. **I. Kruglikov:** None. **B. Rudy:** None. **X. Wang:** None.

Poster

PSTR525. Intrinsic Properties and Modulation of Neuronal Firing: Hippocampus and Cortex

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR525.29/E12

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Application of phenotypic calcium imaging screening to evaluate CNS effects for consumer product-relevant nutraceutical combinations

Authors: *C. READ[1], C. CADDICK[1], A. STINCONE[2], E. TORCHIO[2], A. DAVIES[1], A. PAGLIARO[2], G. CALUSI[3], D. FEDERICO[3], A. SAVA[2], E. BETTINI[2], T. IANNITTI[1]; [1]Scientific Res., BAT Investments Ltd, Southampton, United Kingdom; [2]In Vitro Biol., [3]Pharmacometrics, Aptuit, An Evotec Co., Verona, Italy

Abstract: Dietary supplements, functional foods and nutraceuticals describe food products or supplements consumed to support health and wellbeing. Consumer interest in such products continues to grow with a global market value of $291.33 billion in 2022 and an expected compound annual growth rate of 9.4% by 2030. These products often lack scientific evidence to support their claims, many of which relate to improving cognition, reducing stress, increasing

relaxation, and providing energy, effects that could be mediated through central nervous system (CNS) targets. To address this, 32 compounds and plant extracts were screened using whole-cell Ca^{2+} imaging in *ex vivo* rat cortical cultures. A phenotypic assay was chosen to ensure that different neuronal cell types were included, and multiple neuronal targets captured. Perturbations in spontaneous network firing behaviour were assessed after 14 days *in vitro* with compounds/extracts classified as producing either an increase, decrease, biphasic or no effect. Despite this being at an early stage in the discovery process using a rodent system with applications performed directly on neuronal cells, it was interesting that many of the compounds/extracts tested showed robust responses. In at least some cases, this is the first evidence of direct CNS activity. Consumer products often rely on combinations of compounds and extracts under the assumption that this will lead to enhanced effectiveness. To address such "stacking" effects, 39 combinations were chosen from the previously tested compounds/extracts to identify concentration-dependent combinatorial effects by measuring their effects alone and in combination across a concentration matrix. Using four reference models, drug-drug interactions were quantified under the null hypothesis of no interaction. Overall, 11 synergistic pairings, 16 antagonistic pairings, 11 additive pairings and 1 pairing with mixed effects were identified. This study demonstrates that many wellbeing ingredients can be combined to improve their efficacy, which may allow for biologically relevant effects to be achieved, despite the limited dosages administered in many products. Furthermore, the study highlights that although some combinations might logically be considered for products as they are reported to achieve the same effect, antagonistic mechanisms could make this disadvantageous. A scientific approach to wellbeing product development will provide better outcomes for consumers and, in turn, help to increase trust in their effectiveness.

Disclosures: **C. Read:** A. Employment/Salary (full or part-time):; BAT Investments Ltd. **C. Caddick:** A. Employment/Salary (full or part-time):; BAT Investments Ltd. **A. Stincone:** A. Employment/Salary (full or part-time):; Aptuit, An Evotec Company. **E. Torchio:** A. Employment/Salary (full or part-time):; Aptuit, An Evotec Company. **A. Davies:** A. Employment/Salary (full or part-time):; BAT Investments Ltd. **A. Pagliaro:** A. Employment/Salary (full or part-time):; Aptuit, An Evotec Company. **G. Calusi:** A. Employment/Salary (full or part-time):; Aptuit, An Evotec Company. **D. Federico:** A. Employment/Salary (full or part-time):; Aptuit, An Evotec Company. **A. Sava:** A. Employment/Salary (full or part-time):; Aptuit, An Evotec Company. **E. Bettini:** A. Employment/Salary (full or part-time):; Aptuit, An Evotec Company. **T. Iannitti:** A. Employment/Salary (full or part-time):; BAT Investments Ltd.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.01/E13

Topic: B.08. Epilepsy

Title: Noninvasive brain stimulation for epilepsy reduces seizures and alters low frequency EEG power

Authors: *K. STARNES, K. ISLAM, N. GREGG, B. JOSEPH, G. WORRELL, B. LUNDSTROM;
Mayo Clin., Rochester, MN

Abstract: Introduction:Noninvasive brain stimulation (NIBS) includes transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) and is an emerging modality for influencing brain states and treating epilepsy. Previous data from invasive brain stimulation show power spectral density (PSD) changes near the seizure onset zones (SOZ), especially for frequencies less than 4 Hz, may be linked to neural adaptation feedback mechanisms. Specifically, a decrease in infraslow (less than 1 Hz) activity has been seen near the SOZ.
Methods:Nine patients undergoing NIBS (4 tDCS, 5 TMS) for treatment of focal epilepsy underwent high-density (128-channel, 1000 Hz sampling, DC-couple amplifier) awake EEG before and after 5 days of outpatient therapy. PSDs near the targeted area of stimulation (defined as maximal dipole for TMS and center of cathode of tDCS) were calculated and normalized by total mean power per frequency bin. Pre- and post-therapy PSDs were compared for changers, and infraslow (0.1-1 Hz) activity was compared to higher frequency activity - high delta (2-4 Hz) for tDCS and broadband (4-20 Hz) for TMS - across all channels.
Results:Six patients were responders (50% or greater reduction in seizure frequency); these included all four tDCS patients and 2/5 TMS patients. There was a significant increase of infraslow activity following treatment in three tDCS patients. Increase in infraslow activity was seen in 2/5 TMS patients (1/2 responders and 1/3 nonresponders). Overall, a significant increase of infraslow activity compared to delta activity was seen in 4/6 responders and 1/3 nonresponders.
Conclusion:NIBS can reduce seizures for focal epilepsy patients and may be accompanied by a relative increase of infraslow activity on EEG power spectra. These changes may reflect underlying alteration of adaptive feedback mechanisms that control excitability.

Disclosures: K. Starnes: None. **K. Islam:** None. **N. Gregg:** None. **B. Joseph:** None. **G. Worrell:** None. **B. Lundstrom:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.02/E14

Topic: B.08. Epilepsy

Support: NIH grant NS106957 (RS)
NIH grant 033310 (JE)
Christina Louise George Trust

Title: Neuronal Dynamics in Epileptic Brains: Differences in Gamma-Modulated Firing Rates Inside and Outside the Seizure Onset Zone

Authors: *M. SHAMAS, C. SANTANA-GOMEZ, I. FRIED, J. ENGEL, JR, R. J. STABA; UCLA, Los Angeles, CA

Abstract: Gamma is believed to play an important role in cognition. In the epileptic brain, studies suggest gamma event coupling can localize brain regions where seizures begin and this could be due to alterations in the level and/or pattern of neuronal firing. To understand why some gamma activity is associated with epileptogenic tissue, we quantified single neurons and their rate of firing with respect to local gamma events in the seizure onset zone (SOZ) and non-SOZ. Ten patients with drug-resistant epilepsy who underwent diagnostic intracerebral electrodes implantation were included in this study. A total of 185 channels in hippocampus and entorhinal cortex were analyzed after removing channels containing artifacts. Interictal wide bandwidth recording were 71.5 ± 36.9 minutes in duration and sampled at 27 kHz. Neuronal spikes were detected and sorted into 480 single units. In the analyses of local field potentials, individual gamma events were detected after removing periods containing high amplitude action potentials, and the phase of each event divided into seventy-two 5-degree bins. The relative difference between the preferred phase associated with maximal unit firing and all other phases was quantified for each unit, as was unit firing with and without gamma events in the SOZ and non-SOZ. In the SOZ, 59.5% of single units preferentially fired at 180^{o} corresponding to the trough of the local gamma wave, whereas in outside the SOZ, 72.3% of units discharged during the gamma trough. The preferred phase of unit firing was more than twice the level of firing at all other phases, and this difference was larger for units in the SOZ than non-SOZ ($p = 0.0082$, effect size $d = 0.42$). Unit firing was significantly higher during a gamma event than in the absence of gamma ($p < 0.0001$, $\eta^2 = 0.032$), especially for units in non-SOZ ($p < 0.0001$,$\eta 2 = 0.07$). However, during periods without gamma events, unit firing was higher in the SOZ than in non-SOZ ($p = 0.019$, $\eta 2 = 0.012$). The preferred phase of unit firing near the trough of local gamma events is consistent with a strong modulatory effect of gamma in coordinating unit activity. However, the strength of this modulatory effect changes with respect to the seizure network and seemingly it is weaker in the SOZ. These results could be due to local circuits alterations that are capable of generating seizures and disrupt gamma-mediated temporal coding by units during normal information processing. Ongoing work will classify neurons as excitatory and inhibitory cells and quantify cell-type specific firing with respect to gamma in order to get insights on the mechanisms that makes some neuronal circuits gamma-insensitive.

Disclosures: M. Shamas: None. **C. Santana-Gomez:** None. **I. Fried:** None. **J. Engel, Jr:** None. **R.J. Staba:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.03/E15

Topic: B.08. Epilepsy

Support: NIH/NINDS U01-NS0804143 SUDEP Research Alliance
R01-NS123155
F31-NS110333

Title: Limbic circuit involved in apnea induction in$Scn1a^{R1407X/+}$and $Scn8a^{N1768D/+}$ mice, possible link tosudden unexpected death in epilepsy (SUDEP).

Authors: *E. BRAVO[1], I. L. ANANIA[2], F. A. TERAN-GARZA[1], M. S. CROTTS[1], B. J. DLOUHY[3], G. B. RICHERSON[1];
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Abstract: Rationale: Refractory epilepsy increases risk for SUDEP. Fatal apnea has been shown as the leading cause of death in the MORTEMUS study. The mechanisms involved in seizure-induced apnea remain unknown, but evidence has suggested the amygdala plays a critical role in the pathway by which seizures propagate to the brainstem respiratory network. When the hippocampus (HC) or central amygdala (CeA) is electrically stimulated, prolonged apnea is observed and lesioning the CeA increases survival in mice. Here, we analyzed the role of the CeA in seizure-induced apnea and death using both *Scn1a*$^{R1407X/+}$ mice (DS) and *Scn8a*$^{N1768D/+}$ (Scn8a) mice. **Methods:** In DS mice (n=4), the CeA was stimulated under light anesthesia by injecting current (500 μAmp, 50 Hz) with monopolar electrodes guided stereotactically while measuring breathing using head-out plethysmography. Electrode locations were verified post hoc by electrolytic lesion and histology. A 3D map was generated of where in the CeA breathing was modulated. A second cohort of DS mice were electrolytically lesioned in the rostral CeA (n=17) or served as control (n=5). Body temperature was increased with a heat lamp by 0.5°C per minute until a seizure was induced, or a maximum of 42.5°C was reached. The body temperature at which the first seizure or any fatal seizure occurred were recorded. A third cohort of DS mice (n=10) underwent HC kindling, after which propagation of seizures and mortality were evaluated. In a cohort of *Scn8a* mice (n=6) audiogenic seizures were induced. Immunohistochemistry was performed for c-Fos expression. WT littermate mice (n=4) served as controls. Confocal and epifluorescence images were obtained for analysis. **Results:** CeA stimulation modulated breathing, induced apnea, or increased breathing frequency in a site-specific manner. Apnea was inducible for the duration of the electrical stimulation. The temperature of the first seizure was the same for both lesioned (n=17) and control (n=5) DS mice. Respiratory arrest and death occurred in 23% of lesioned mice (n=4/17), vs 80% in control mice (n=4/5). HC stimulation could induce apneas even if the seizure was not propagated by the CeA. Tonic seizures were not necessary to induce apnea. C-Fos expression in the CeA of *Scn8a* mice in which a seizure had been induced was higher compared to control mice. **Conclusions:** Our results demonstrate that the amygdala plays a role in modulating breathing and a tonic phase is not necessary to induce fatal apneas. Also, the rostral amygdala is highly activated by audiogenic seizures. While the amygdala may not play a significant role in seizure induction, it likely plays a role in fatal seizures ending in respiratory arrest.

Disclosures: E. Bravo: None. **I.L. Anania:** None. **F.A. Teran-Garza:** None. **M.S. Crotts:** None. **B.J. Dlouhy:** None. **G.B. Richerson:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.04/E16

Topic: B.08. Epilepsy

Support: France Alzheimer AAP JC 2022

Title: Acceleration of the amyloid plaque deposition induced by early epileptic seizures: study in the Tg-f344-AD transgenic rat

Authors: *W. GRABON, V. BLOT, B. GEORGES, S. RHEIMS, L. BEZIN;
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Abstract: The occurrence of epileptic seizures and Alzheimer's disease (AD) are strongly interconnected. The link between the two pathologies was first considered as unilateral, as dementia has been experimentally shown to lead, in its late phase, to the occurrence of epileptic events. But recent data suggest that this link is more complex. Indeed, clinical studies show that people whose first epileptic seizure occurs between the ages of 50 and 60 have a three times higher risk of developing dementia compared to the general population, suggesting that epilepsy occurring at this stage might itself precipitate the development of amyloid pathophysiology. Therefore, the present study aims to investigate whether early epilepsy can accelerate the installation of the mechanisms underlying AD in a model of transgenic rats predisposed to develop AD: Tg-F344-AD rats. This strain models a symptomatology as close as possible to the human family form, with a slow development of the disease that makes it possible to induce early seizures, before AD onset. In this study, seizures were induced using the kindling pentylenetetrazole (PTZ) model, which allows pharmacological induction without surgical intervention while controlling the number of induced generalized tonic-clonic seizures (GTCS). Amyloid burden was quantified by immunohistochemistry one month following the induction of 5 GTCS. Our results show on the one hand that rats predisposed to develop AD are more sensitive to the induction of seizures and on the other hand that the deposition of amyloid plaque is significantly accelerated after seizure induction. These results demonstrate the direct effect of seizures on the amyloid features of AD. Neuroinflammatory processes are pathophysiological mechanisms common to epilepsies and AD. These inflammatory processes could thus partly underlie the bidirectional link between the two pathologies and will be further explored. Our results show that at 4 months, the increased presence of amyloid plaques in Tg-F344-AD rats subjected to PTZ-induced seizures is not associated with higher mRNA levels of prototypical markers of inflammation. Similarly, we did not observe an increase in these markers in Tg-F344-AD rats between 4 and 6 months of age, which could mean that the early presence of plaques does not cause sustained neuroinflammation. It may be however that a transient level of inflammation is essential for the onset of amyloid pathology. Our ongoing studies investigate whether astrocyte and microglia cell activation, as well as the level of monocyte infiltration, correlates with the presence of amyloid deposits.

Disclosures: **W. Grabon:** None. **V. Blot:** None. **B. Georges:** None. **S. Rheims:** None. **L. Bezin:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.05/E17

Topic: B.08. Epilepsy

Title: Cell type specific rescue of seizures in a mouse model of DNM1-associated developmental and epileptic encephalopathy

Authors: *P. MATHKAR[1], A. N. SHORE[1], M. P. MCCABE[2], R. O'CONNOR[3], W. N. FRANKEL[4], M. C. WESTON[1];
[1]Fralin Biomed. Res. Inst., Virginia Polytechnic Inst. and State Univ., Roanoke, VA; [2]Dept. of Neurolog. Sci., [3]Univ. of Vermont, Burlington, VT; [4]Columbia Univ. Med. Ctr., New York, NY

Abstract: SfN Abstract:Title: Cell type specific rescue of seizures in a mouse model of DNM1-associated developmental and epileptic encephalopathyAuthors: Pranav P. Mathkar, Amy N. Shore, Matthew P. McCabe, Robert O'Connor, Wayne N. Frankel, Matthew C. Weston Dynamin-1 (*Dnm1*) is a large GTPase protein found in presynapse, where it facilitates synaptic vesicle fission from membrane during clathrin-mediated endocytosis. *De novo* missense mutations in *Dnm1* cause developmental and epileptic encephalopathies (DEEs), a group of complex conditions that includes Lennox-Gastaut Syndrome (LGS) and Infantile spasms, among others. LGS is a severe epilepsy disorder characterized by a triad of features, multiple types of refractory seizures, global developmental delay, and abnormal electroencephalogram (EEG) patterns. A mouse model with a spontaneous mutation (A408T), termed "fitful," recaptures major phenotypes of the condition. Heterozygous fitful mice display spontaneous seizures and handling-induced seizures, along with behavioral anomalies such as hyperactivity and altered postures. Previous work showed *Dnm1*^{Ftfl} has pleiotropic effects on excitatory and inhibitory neurons and neurotransmission. Hemizygous expression of *Dnm1*^{Ftfl} in excitatory cortical and hippocampal neurons causes behavioral abnormalities, whereas expression of *Dnm1*^{Ftfl} in inhibitory neurons causes severe seizures and death, with corresponding alterations in inhibitory synaptic function. We hypothesized that the expression of *Dnm1*^{Ftfl} in GABAergic neurons is not only sufficient, but also necessary, for seizure onset. To test this hypothesis, we selectively removed *Dnm1*^{Ftfl} from different neuronal populations and assessed the seizure phenotype. Targeted removal of *Dnm1*^{Ftfl} from medial ganglionic eminence (MGE)-derived interneurons rescued handling-induced seizures, whereas removal of the variant from excitatory neurons exacerbated seizures. Cellular studies showed that *Dnm1*^{Ftfl} causes both alterations in inhibitory synaptic transmission and GABAergic neuron loss. Future studies will determine whether these cellular phenotypes are also reversible, the relationship between altered synaptic transmission, cell loss, and neural activity, and whether removal of *Dnm1*^{Ftfl} from subpopulation also prevents seizures. These studies provide crucial insight on the burden of *Dnm1* mutation on cellular

physiology and interneuron survival, and the ability of targeted genetic manipulations to treat symptoms of neurological disease.

Disclosures: **P. Mathkar:** None. **A.N. Shore:** A. Employment/Salary (full or part-time):; Virginia tech. **M.P. McCabe:** None. **R. O'Connor:** None. **W.N. Frankel:** A. Employment/Salary (full or part-time):; Columbia University. **M.C. Weston:** A. Employment/Salary (full or part-time):; Virginia tech.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.06/E18

Topic: B.08. Epilepsy

Support: NRF-2020R1A6A1A03043283

Title: Photobiomodulation improves the synapses and cognitive function and ameliorates epileptic seizure by inhibiting downregulation of Nlgn3

Authors: *N. HONG[1], H. KIM[2], K. KANG[3], J. PARK[4], S. MUN[5], H.-G. KIM[6], B. KANG[10], P.-S. CHUNG[7], M. LEE[8], J.-C. AHN[9];
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Abstract: Temporal lobe epilepsy (TLE) remains one of the most drug-resistant focal epilepsies. Glutamate excitotoxicity and neuroinflammation which leads to loss of synaptic proteins and neuronal death appear to represent a pathogen that characterizes the neurobiology of TLE. Photobiomodulation (PBM) is a rapidly growing therapy for the attenuation of neuronal degeneration harboring non-invasiveness benefits. However, the detailed effects of PBM on excitotoxicity or neuroinflammation remain unclear. We investigated whether tPBM exerts neuroprotective effects on hippocampal neurons in epilepsy mouse model by regulating synapse and synapse-related genes. In an *in vitro* study, we performed imaging analysis and western blot in primary hippocampal neurons from embryonic (E17) rat pups. In an *in vivo* study, RNA sequencing was performed to identify the gene regulatory by PBM. Histological stain and immunohistochemistry analyses were used to assess synaptic connections, neuroinflammation and neuronal survival. Behavioral tests were used to evaluate the effects of PBM on cognitive functions. PBM was upregulated synaptic connections in an *in vitro*. In addition, it was confirmed that transcranial PBM reduced synaptic degeneration, neuronal apoptosis, and

neuroinflammation in an *in vivo*. These effects of PBM were supported by RNA sequencing results showing the relation of PBM with gene regulatory networks of neuronal functions. Specifically, Nlgn3 showed increase after PBM and silencing the Nlgn3 reversed the positive effect of PBM in *in vitro*. Lastly, behavioral alterations including hypoactivity, anxiety and impaired memory were recovered along with the reduction of seizure score in PBM-treated mice. Our findings demonstrate that PBM attenuates epileptic excitotoxicity, neurodegeneration and cognitive decline induced by TLE through inhibition of the Nlgn3 gene decrease induced by excitotoxicity.

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Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Program #/Poster #: PSTR526.07/E19

Topic: B.08. Epilepsy

Support: NIH R01NS112538
NIH 5R35NS116852

Title: Functional network connectivity, at the single neuron level, evolves during epileptogenesis

Authors: *K. P. LILLIS[1], L. A. LAU[2], K. J. STALEY[3];
[1]Neurol., Harvard Med. School, MGH, Boston, MA; [2]Neurol. Res., Massachusetts Gen. Hosp., Melrose, MA; [3]Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Seizures arise not only as a consequence of abnormal activity at the single neuron level, but also by the abnormal propagation of activity through brain networks. Large-scale measures of brain connectivity ("macro networks") reveal distinct patterns of anatomical and functional connectivity in patients with refractory epilepsy. "Functional" macro networks, in patients with epilepsy, are inferred from correlated activity measured with electrical recordings or resting-state functional MRI. At the cellular level, the dysfunction of small-scale networks ("micro networks", interactions between neurons) underlies the pathological macro networks observed at the whole brain level. Here, we quantify changes in functional connectivity of micro networks during both ictogenesis and epileptogenesis. We imaged neuronal activity in an *ex vivo* model of post-traumatic epileptogenesis: the organotypic hippocampal slice culture. Slices were prepared from P7 mice that were transduced intracerebroventricularly on P0 with a soma-targeted version of the genetically encoded calcium indicator GCaMP8m. We then used a novel imaging system constructed inside of a tissue culture incubator, to image slices continuously beginning shortly after the injury of slicing and continuing through the onset of spontaneous recurrent seizures (after ~7 days *in vitro*). Every 4 hours, a movie of calcium dynamics with cellular resolution and a field of view spanning the entire epileptic network was acquired.

"Resting-state" functional network connectivity was quantified by identifying neurons that fired together at a rate above chance during non-epileptiform activity. On the time scale of *ictogenesis*, we found that edge density increased in the minutes leading up to seizure onset, indicating an increase network synchrony. On the time scale of *epileptogenesis,* we also observed a resting-state edge density increase of >100% ($p<0.01$) during the first 3 days *in* vitro, when spontaneous seizures first emerged. Quantitative analysis is ongoing, but preliminary results indicate that incubating slices in chondroitinase ABC, which is anticipated to enhance inhibition, decreases functional network connectivity in epileptic slices. These results are consistent with short- and long-term increases in functional connectivity associated with ictogenesis and epileptogenesis respectively. Uncovering the mechanisms that underlie these variations in functional cellular network connectivity will be critical to identifying drug targets to disrupt the pathological activity they produce.

Disclosures: K.P. Lillis: None. **L.A. Lau:** None. **K.J. Staley:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

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Program #/Poster #: PSTR526.08/E20

Topic: B.08. Epilepsy

Support: Ester Floridia Neuroscience Research Foundation (grant 1502 to VC)

Title: Selective activation of alpha 3-containing GABA-A receptors blocks absence seizures and increases sleep spindles

Authors: *V. CRUNELLI[1], T. P. MORAIS[1], F. DAVID[2], Z. ATHERTON[1], F. DELICATA[1], G. DI GIOVANNI[3], T. A. JACOBSON[4], J. S. LARSEN[4], K. SANDAGER-NIELSEN[4]; [1]Cardiff Univ., Cardiff, United Kingdom; [2]Integrative Neurosci. and Cognition Ctr., CNRS, Paris, France; [3]Univ. of Malta, Univ. of Malta, Msida, Malta; [4]Saniona A/S, Glostrup, Denmark

Abstract: GABA-A receptors (GABA-ARs) containing the alpha 3 subunit are implicated in a number of physiological and pathological activities. In particular, compared to wildtype controls, mice with whole-brain knockout (KO) of the alpha 3 subunit have a lower EEG power in the 10-15 Hz band at NREM-REM transitions and a larger power in the 11-13 Hz band in the waking EEG (Neuroscience 154,2008,595-605). Moreover, alpha 3 KO mice have a similar seizure susceptibility in a model of temporal lobe epilepsy but show a decrease in pharmacologically induced absence seizures (ASs) compared to wildtype mice (Proc Natl Acad Sci 106,2009,7630-7635). Notably, there is a compensatory effect in thalamic GABA-AR synaptic potentials of alpha3 KO mice, i.e. the amplitude and the decay of mIPSCs in the nucleus reticularis thalami (NRT) is higher and faster, respectively, than those of wildtype mice. This increased NRT inhibition was suggested to underlie the decrease of ASs in the alpha 3 KO mice. However, a causal link could not be established because i) other compensatory anti-absence mechanisms

might be present in these KO mice, and ii) alpha3 subunit-containing GABA-ARs are highly expressed not only in the NRT but also in cortical layer 6 neurons (J Comp Neurol 359,1995154-194) that are critical for AS initiation (Arch Neurol 62,2005,371-376). In this study, we investigated the effects of SAN711, a selective PAM at alpha 3-containing GABA-ARs, on spontaneous absence seizures in male GAERS rats and sleep spindles in male Wistar rats. Systemically injected SAN711 dose-dependently blocked absence seizures, an effect that occurred within 20 min of SAN711 administration and lasted for more than 3 hours. The maximal effect was observed with 10 mg/kg (80±4%), whereas 3 mg/kg elicited a 36±5% reduction. Moreover, the highest dose of SAN711 increased the number (22±7%) and duration (34±5%) of sleep spindles without affecting their frequency and power. These effects might be mediated by alpha 3-containing GABA-ARs in the NRT and/or cortical layer 6 neurons. These results demonstrate for the first time that direct activation of alpha 3-containing GABA-ARs controls spontaneous absence seizures and spindles of natural sleep.

Disclosures: V. Crunelli: None. **T.P. Morais:** None. **F. David:** None. **Z. Atherton:** None. **F. Delicata:** None. **G. Di Giovanni:** None. **T.A. Jacobson:** A. Employment/Salary (full or part-time):; Employee, Saniona A/S. **J.S. Larsen:** A. Employment/Salary (full or part-time):; Employee, Saniona A/S. **K. Sandager-Nielsen:** A. Employment/Salary (full or part-time):; Employee, Saniona A/S.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.09/E21

Topic: B.08. Epilepsy

Support: This work was supported by the Ester Floridia Neuroscience Research Foundation (grant 1502 to VC)

Title: Activation of astrocytic TrkB-T1 receptors rescues absence seizures and their comorbid memory deficits

Authors: *T. MORAIS[1,2], M. SOTTOMAYOR[3,4], C. PINA[3,4], A. M. SEBASTIÃO[3,4], G. DI GIOVANNI[1], S. H. VAZ[3,4], V. CRUNELLI[2];
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Abstract: Absence Seizures (ASs) are genetic generalized seizures involving brief lapses of consciousness, accompanied by spike-and-wave discharges in the EEG. Studies in Childhood Absence Epilepsy cohorts (where ASs is the only seizure type) have recently show that 30% of children with ASs are pharmaco-resistant and 60% show neuropsychiatric comorbidities, which may precede the epilepsy diagnosis and can persist even after full pharmacological control of

seizures.
In humans, drugs that increase GABA levels elicit ASs in normal individuals while exacerbates them in absence epilepsy patients, and MR spectroscopy has shown higher GABA levels in a child with unilateral spike-and-wave discharges. Moreover, tonic GABA-A inhibition is increased in AS models. Altogether these data indicate that an increase, rather than a reduction, of GABA-A inhibition underlies the appearance of ASs, and indirect evidence shows that a reduction in GAT1-mediated GABA uptake, and the consequent increase in extracellular GABA levels, underlies the increased tonic GABA-A inhibition.We have now directly measured GAT1 function in two ASs animal models (the Genetic Absence Epilepsy Rats from Strasbourg, GAERS, and the Stargazer (STG) mice) performing GABA uptake in key brain regions for ASs and comorbidities and investigated recognition and memory deficits in GAERS. Moreover, since Brain Derived Neurotrophic Factor (BDNF) is known to increase GAT1 activity in normal animals, we studied the effect of exogenously applied BDNF on ASs and memory impairments in freely moving GAERS rats.GAERS and STG, compared to their respective non-epileptic control animals, have GAT1-mediated GABA uptake reduced in the brain areas important for ASs and their comorbidities, whereas GAT3 activity is normal. Furthermore, BDNF exogenous administration increased the deficient GAT1-mediated GABA uptake, rescued ASs, hippocampal LTP and comorbid memory deficits. The effect of BDNF was shown to be mediated by activation of astrocytic TrkB-T1 receptors. Our work reveals for the first time a single target, i. e. TrkB-T1 receptors, that can rescue both ASs and their comorbidities, and thus highlights the existence of a common molecular/cellular pathway that can controls both ASs and their comorbid memory impairments.

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Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.10/E22

Topic: B.08. Epilepsy

Title: Cognitive comorbidities of absence seizures are independent of anxiety

Authors: M. NEUPARTH-SOTTOMAYOR[1], C. C. PINA[1], T. P. MORAIS[2], M. FARINHA-FERREIRA[1], D. S. ABREU[1], F. SOLANO[1], F. MOURO[1], M. GOOD[3], A. M. SEBASTIÃO[1], *G. DI GIOVANNI[2], V. CRUNELLI[4], S. H. VAZ[1];
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Abstract: Typical absence seizures (ASs) are brief periods of lack of consciousness, characterized by 2.5-4 Hz spike-wave discharges (SWDs) in the thalamocortical network in the

EEG, which are highly prevalent in children and teenagers. Together with ASs, 60% of these young epileptic cohorts show neuropsychological comorbidities, including cognitive, memory and mood impairments, even after the seizures are pharmacologically controlled. Similar cognition and memory deficits have been reported in different, but not all, genetic animal models of ASs. Since cognitive alteration may be subtle, not easily detected and task-specific, their presence may be confounded by an anxiety-like phenotype. In this work, three anxiety and seven memory tests were used to compare anxiety and memory in the same animals from the well-established inbred model of Genetic Absence Epilepsy Rats from Strasbourg (GAERS), their inbred strain of Non-Epileptic Control (NEC) strain (that lack ASs) and normal outbred Wistar rats. Additionally, the Stargazer (STG) mice and their wild-type littermates were also studied. GAERS do not exhibit higher anxiety-like behavior and neophobia compared to both NEC and Wistar rats. In contrast, GAERS show decreased spontaneous alternation in the Y-maze, spatial working memory and cross-modal object recognition compared to both NEC and Wistar rats. Notably, GAERS preferentially used egocentric strategies to perform spatial memory tasks. Moreover, STG mice did not exhibit increased anxiety but had deficits in spatial reference memory, compared to wildtype mice. In summary, these results provide solid evidence of memory deficits in GAERS rats and STG mice, which do not depend on an anxiety or neophobic phenotype. Moreover, the presence of differences between NEC and Wistar rats stresses the need of using both outbred and inbred control rats in behavioural studies of inbred models of ASs.

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Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Program #/Poster #: PSTR526.11/E23

Topic: B.08. Epilepsy

Support: CONACyT Grant A3-S-26782
CONACyT scholarship 753802

Title: Study of the effect of cannabinol on the synaptic release of glutamate from the cortex of patients with drug-resistant epilepsy

Authors: *C. MARTINEZ AGUIRRE[1], F. CARMONA CRUZ[1], M. A. ALONSO VANEGAS[2], M. CUÉLLAR-HERRERA[3], L. ROCHA[1];
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Abstract: Rationale: Drug-resistant epilepsy (DRE) is associated with high extracellular levels of glutamate, condition that facilitates the excitotoxicity and neuronal damage. On the other hand, studies support that cannabinol (CBN) regulates the calcium homeostasis, an ion involved in glutamate release. However, at present it is unknown if CBN modifies the glutamate over-release in the brain of patients with DRE. The aim of this study was to investigate if CBN reduces the evoked glutamate release in cortical synaptic terminals obtained from patients with DRE. **Methods:** Synaptic terminals (synaptosomes) were obtained from neocortex of patients with DRE submitted to epilepsy surgery (n=8). Immediately after resected, the tissue was immersed in saccharose (0.32 M), oxygenated by bubbling (0.5 l/h) and transported (<45 min) from the surgery room to the laboratory. Synaptosomes were highly purified by Percoll-sucrose density gradient and resuspended in artificial cerebrospinal fluid. Synaptosome-homogenates were divided and incubated with CBN at different concentrations (100 nM, 1 µM, 10 µM, 100 µM or 1 mM) during 15 min. Then, membranal depolarization was evoked by addition of KCl (33 mM). Synaptosomes were centrifugated and the supernatant was used to estimate the glutamate release by HPLC. The results were compared with values obtained under basal conditions as well as KCl without CBN exposure. **Results:** Basal glutamate release was 40.86±9.9 nmol/mg of protein. The KCl-induced depolarization augmented the extrasynaptosomal glutamate concentration (422 %, p=0.007 vs basal). Synaptosomes pre-incubated with CBN showed lower glutamate release (100 nM, 88%, p=0.0056; 1 µM, 84%, p=0.0162; 10 µM, 88%, p=0.006 vs KCl alone). At higher concentrations, this effect was persistent in 50% of the patients, while in the rest of the patients, the glutamate release was similar to the observed by the high KCl alone. **Conclusions:** Acute exposure to CBN reduces glutamate release from cortical synaptic terminals obtained from patients with DRE. Further studies are required to determine if this effect is also evident with chronic CBN exposure, as well as if this effect is dependent of specific clinical conditions.

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Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

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Program #/Poster #: PSTR526.12/E24

Topic: B.08. Epilepsy

Title: Synaptamide Phosphonate-GAO-3-02-potentiates GABAergic transmission in the rat lithium-pilocarpine model of temporal lobe epilepsy via activation of CB2 receptor

Authors: *A. BELMEGUENAI[1,2], L. BEZIN[1,2], J. BODENNEC[1,2], V. MUTEL[2], S. BODENNEC[2];
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Abstract: Synaptamide Phosphonate (GAO-3-02) is a first-in-class antiepileptic drug candidate being developed by GAOMA Therapeutics. We have recently reported that GAO-3-02 displayed robust anti-seizure effects across validated seizure and epilepsy models. However, the mechanisms by which GAO-3-02 exerts its effects are not fully understood. To understand its potential molecular mechanism of action, standard whole cell patch clamp electrophysiological techniques were used to characterize the effects of GAO-3-02 on GABAA-mediated currents in recordings from the CA1 neurons in acutely-obtained hippocampal slices from lithium-pilocarpine-induced status epilepticus (SE)-treated rats. In this study, we also investigated the possible roles of the cannabinoid receptor CB2, present in the hippocampus, in the effects produced by GAO-3-02 using pharmacological approaches. We found that bath application of GAO-3-02 dose-dependently increased the amplitude of evoked inhibitory postsynaptic currents (eIPSCs) with an EC50 value of 60 nM. This effect was blocked by a CB2 receptor antagonist (SR144528). Further results demonstrated that bath-application of CB2 receptor agonist (JWH133) potentiated the amplitude of eIPSCs. However, bath perfusion of GW842166X, a potent and selective CB2 receptor agonist, which is undergoing clinical development, failed to significantly increase the current amplitude of eIPSCs. The present study suggests that GAO-3-02 enhances GABAergic transmission onto CA1 pyramidal neurons through activating CB2 receptor. Our results may provide a cellular and molecular mechanism that helps explain the anti-seizure effects of GAO-3-02 in the rat lithium-pilocarpine model of temporal lobe epilepsy.

Disclosures: **A. Belmeguenai:** 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.; GAOMA Therapeutics, France. F. Consulting Fees (e.g., advisory boards); GAOMA Therapeutics, France. **L. Bezin:** 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.; GAOMA Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics. F. Consulting Fees (e.g., advisory boards); GAOMA Therapeutics. **J. Bodennec:** 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.; GAOMA Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics. F. Consulting Fees (e.g., advisory boards); GAOMA Therapeutics. **V. Mutel:** A. Employment/Salary (full or part-time):; GAOMA Therapeutics. **S. Bodennec:** A. Employment/Salary (full or part-time):; GAOMA Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GAOMA Therapeutics.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.13/E25

Topic: B.08. Epilepsy

Support: NIH/NINDS R01NS115800
Iowa Neuroscience Institute

Title: Blocking $GABA_A$ receptors decreases ictal-like events in the neonatal and adolescent neocortex

Authors: *R. LANGTON[1,2], J. GLYKYS[1,2,3];
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Abstract: Neurons differ in their response to GABA depending on their developmental age. Early in brain development, GABA has depolarizing actions on neurons, whereas in the mature brain, GABA is mainly inhibitory. Higher intraneuronal chloride concentrations in the neonatal period mostly mediate this difference. We studied the effect of bicuculline, a $GABA_A$ receptor antagonist, on the generation of interictal and ictal-like events (events longer than 10 sec) in the neocortex of neonatal (postnatal day, P8-10) and adolescent (P25-30) mice *in vitro*. Neocortical seizure-like activity was induced with 4-aminopyridine (4-AP). We observed that neocortical ictal events were present in both ages with a similar duration, but they were more frequent in the adolescent neocortex. Bicuculline perfusion decreased the frequency of ictal events in both ages. The interictal event frequency was similar between ages in 4-AP, and bicuculline perfusion reduced the number of events in both ages. Significantly, during bicuculline perfusion, the length of interictal events increased compared to baseline in both age groups but did not reach the threshold of ictal duration. In conclusion, blocking $GABA_A$ receptor-mediated inhibition led to longer interictal events in the neonatal neocortex but did not transform interictal to ictal events as ictal events became less frequent. Thus, $GABA_A$ receptor-mediated activity is necessary to maintain ictal-like activity in neonatal and adolescent slices in the 4-AP *in vitro* model of seizure-like activity.

Disclosures: R. Langton: None. **J. Glykys:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Topic: B.08. Epilepsy

Support: KAKENHI JP (21K15247) to YT
KAKENHI JP (21H03606, 22H05698, 19H01142, 21H03532, 20H04341, 19K12190) to TT

Title: Investigating the role of the CA2 area in seizure propagation with a voltage-sensitive dye (VSD)-based assay in hippocampal slices: The effects of seizurogenic drugs

Authors: *Y. UTSUMI[1], M. TAKETOSHI[2], Y. TOMINAGA[2], T. TOMINAGA[3];
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Abstract: The central nervous system's (CNS) toxicity is a critical factor to consider during non-clinical and clinical drug development. Seizures and convulsions are common findings and leading causes of drug development attrition. We have previously reported a new method of testing susceptibility to seizures and exploring the mechanism of drug-induced seizures using a VSD-based assay in hippocampal slices (specifically the CA1 area). Four seizurogenic drugs (picrotoxin, gabazine, 4-aminopyridine, and pilocarpine) were tested in the previous report. Picrotoxin and gabazine induced seizure-like prolonged depolarizing activity in the stratum radiatum (SR) and stratum pyramidale (SP), while applying 4-aminopyridine led to transient delayed depolarization in the SR. In contrast, pilocarpine reduced the EPSP and then the response in the SP. The differential effect on CA1 activity suggests that the system can evaluate drugs on different neuronal mechanisms, thus permitting the study of CNS toxicity.Seizurogenic drugs often induce oscillatory responses accompanied by long-lasting depolarizing responses. The CA2 area showed a specific oscillatory activity during this study using the VSD-based high-speed real-time imaging methods. This study shows the oscillatory activity of the CA2 area. Using a fast real-time VSD imaging technique (up to 10kHz framerate), the excitatory oscillatory activity starts in the CA2 area. This area is essential for social memory and has been implicated in temporal lobe epilepsy (TLE) and proposed as a novel therapeutic target for epilepsy. However, little is known about whether changes in CA2 properties promote the onset or propagation of seizures. The attempt to use VSD imaging to investigate whether these seizurogenic drugs have an epileptogenic focus in CA2 should shed light on the possible causes of seizures. It may reveal new targets for testing seizurogenic drugs.

Disclosures: **Y. Utsumi:** None. **M. Taketoshi:** None. **Y. Tominaga:** None. **T. Tominaga:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Program #/Poster #: PSTR526.15/E26

Topic: B.08. Epilepsy

Support: NIH Grant 1R37NS115439

Title: Persistent seizure induced transcriptional and post-translational down regulation of GluA2 is selective to neurons activated by early-life seizures

Authors: *S. B. DUTKO[1], B. XING[2], A. J. BARBOUR[2], K. HOAG[2], X. LI[2], D. M. TALOS[2], F. E. JENSEN[2];
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Abstract: Early-life seizures (ELS) can cause permanent cognitive deficits and network hyperexcitability, but it is unclear whether ELS involves persistent alterations to specific neuronal populations and if these changes can be targeted to mitigate network dysfunction. We have previously shown that seizures cause early decreases in GluA2 subunit of the -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), associated with increased AMPAR function and calcium permeability in hippocampal area CA1 in mouse models of ELS. To determine whether these changes are long lasting and/or affecting selective neuronal populations, we used the targeted recombination of activated populations (TRAP) approach to genetically label neurons activated during an ELS induced by kainic acid (KA), in immature postnatal day (P) 10 mice. The ELS-TRAPed neurons were highly enriched in the hippocampal CA1 region and remained preferentially susceptible to reactivation by later life seizures in adulthood. Using single-nucleus RNA-sequencing (snRNA-seq) we observed a specific and enduring decrease in the expression of Gria2 mRNA, which is responsible for encoding the GluA2 subunit protein, within ELS-TRAPed neurons and not in surround neurons (n_{KA} = 8 mice, n_{Sal} = 2 mice, $P < 0.0001$). These results were confirmed using RNAscope in situ hybridization (RNA-ISH; n_{KA} = 3 mice, n_{Sal} = 3 mice, $P < 0.05$). These changes persisted for several weeks subsequent to the seizures. In addition to changes in Gria2, there was also persistent downregulation of synaptic GluA2 expression and increased dendritic phosphorylated GluA2 at Ser880, also observed selectively in the ELS-trapped neurons and not in surrounding neurons (n_{KA} = 9 mice, n_{Sal} = 5 mice, P <). As downregulation of GluA2 induces increased synaptic AMPAR function and has been implicated in epileptogenesis in prior models, this data reveals that ELS-induced network hyperexcitability is due to both transcriptional Gria2 changes as well as post-translational modification of GLuA2 subunit protein. Importantly, these changes are specific only to neurons that were previously activated with the original early life seizure, suggesting that these neurons may play a critical role in the long-lasting network hyperexcitability after ELS.

Disclosures: S.B. Dutko: None. **B. Xing:** None. **A.J. Barbour:** None. **K. Hoag:** None. **X. Li:** None. **D.M. Talos:** None. **F.E. Jensen:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

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Topic: B.08. Epilepsy

Support: National Natural Science Foundation of China 31871085
National Natural Science Foundation of China 31700902
Natural Science Foundation of Shanghai 21ZR1407300

Title: Neural circuits underlying piriform cortex-kindling-induced seizure

Authors: Y. TAO, Y. ZHAO, H. ZHU, Y. XUE, ***R. WU**;
Inst. of Brain Sci., Fudan Univ., Shanghai, China

Abstract: Epilepsy, a chronic neurological disorder characterized by recurrent unprovoked seizures, affects 1% of the population. Understanding neural network behavior is crucial for comprehending epileptogenesis, seizure propagation, and epilepsy treatment. Previous studies have shown that the piriform cortex (PC) acts as a key seizure-trigger zone or, even, epileptogenesis. However, the neural mechanisms involved in the PC's role in epilepsy occurrence and development are still not well understood. In this study, we aimed to: 1) determine the specific functional role of PC neurons in seizure occurrence and regulation, 2) refine our understanding of the anatomical and functional connections of PC, as well as neural reorganizations in the PC-kindling model, and 3) investigate the fundamental circuit mechanism of PC-kindling-induced seizure. To achieve these goals, we utilized functional magnetic resonance imaging (fMRI) in combination with neural modulation (i.e. optogenetics and chemogenetics), multi-channel electrophysiological recording, multi-channel fiber photometry, iDISCO immunolabeling, neural tracing, and behavior assessment, to reveal the neural mechanism underlying PC-kindling-induced seizure in mice. Our results showed that the activation of PC^{vglut1} neurons caused a hyperexcitable state and seizure-like behavior and that different neurons in the PC presented differentiated modulations of seizure. PC-kindling led to whole-brain and long-term activations, and functional connectivity was enhanced at rest in the PC-kindling model. The PC-Ent-Hip circuit served as a key pathway in seizure propagation. Overall, our multimodal study provided new information, from the cellular level to the network level, that extends our understanding of epilepsy neuropathology and may shed new light on epilepsy-targeted intervention and treatment.

Disclosures: Y. Tao: None. **Y. Zhao:** None. **H. Zhu:** None. **Y. Xue:** None. **R. Wu:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR526.17/E28

Topic: B.08. Epilepsy

Support: NIH Phase 2 Grant R44MH119621

Title: "epilepsy in a dish assay" (eida): a suite of assays to discover therapeutics for epilepsy using isogenic hipsc-neuron/astrocytes/microglia 2d and 3d tri-cultures and kinetic image cytometry

Authors: *M. HASAN[1], K. GORDON[2], C. RINES[1], N. SUAREZ[2];
[1]Vala Sciences, Inc., San Diego, CA; [2]Vala Sci. Inc., San Diego, CA

Abstract: Epilepsy is a neurological disorder marked by unprovoked emergence of seizure or synchronous neuronal activities. The gradual shift of normal neuronal network activity toward abnormal seizure subserving state or epileptogenesis is orchestrated by multiple cell types including neurons, astrocytes, and microglia. In our work, we present an isogenic, highly pure neuron-astrocyte-microglia tri-culture system termed “Epilepsy in a Dish Assay (EiDA)”. Neuronal activity, network dynamics, and effect of compounds in EiDA can be observed with automated high-speed kinetic image cytometer (KIC) and integrated liquid handler with high temporal and spatial-single cell resolution. Paired with Vala’s automated image analysis suite, CyteSeer, these high-speed videos can be conveniently and reliably translated into quantifiable phenotypes including network dynamics, activity, and cytotoxicity. As a proof of concept, we have separately differentiated pure populations of excitatory neurons, astrocytes, and microglia from a single human induced pluripotent stem cell (hiPSC) line and successfully created EiDA with defined cell identity and composition with high replicability and throughput. Optical recording of Ca^{2+} activity using fluorescent calcium dyes with KIC in EiDA demonstrated that 4-Aminopyridine (4-AP) induced an increase in neuronal activity and synchronization. Application of anti-epileptic drugs showed reduction of synchronized network hyperactivity in a dose dependent manner. Vala’s high resolution structured illumination microscope (SIM) was also used to image and analyze cellular morphology, processes, and proteins with high throughput to yield valuable mechanistic insights. We further created 3D substrate-adhered tri-cultures from these cells termed “3D EiDA”. Initial study of 3D EiDA exhibit enhanced in-vivo representation, sustained spontaneous seizure-like activity periods, and physiologically relevant pharmacodynamics in response to anti-epileptic drugs. Together, 2D, and 3D EiDA with Vala’s image acquisition and analysis capabilities present an effective high-throughput compatible modality to assay therapeutics for epilepsy with high translational accuracy.

Disclosures: M. Hasan: None. **K. Gordon:** None. **C. Rines:** None. **N. Suarez:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

Location: WCC Halls A-C

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Program #/Poster #: PSTR526.18/E29

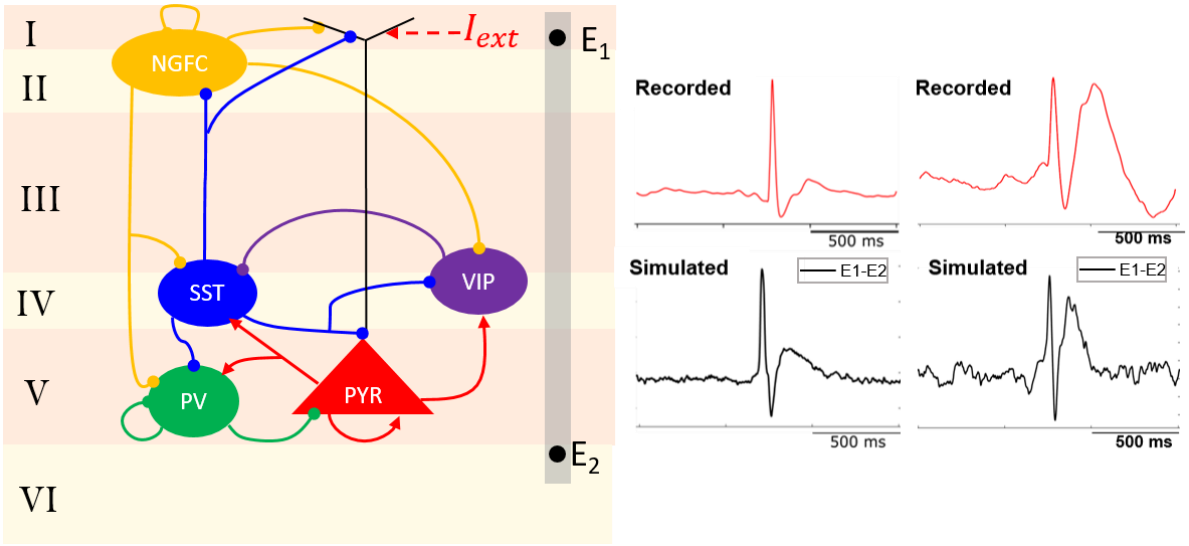
Topic: B.08. Epilepsy

Support: ERC Synergy Grant No 855109

Title: Modeling interictal epileptic discharges in partial epilepsies

Authors: *E. KÖKSAL ERSÖZ[1], R. LAZAZZERA[1], I. MERLET[1], B. MERCADAL[2], R. SANCHEZ-TODO[2], G. RUFFINI[2], F. BARTOLOMEI[3], P. BENQUET[1], F. WENDLING[1]; [1]INSERM, LTSI – UMR 1099, Rennes, France; [2]Neuroelectrics Barcelona, Barcelona, Spain; [3]INSERM & Inst. De Neurosciences Des Systè, Marseille, France

Abstract: In partial epilepsies, interictal epileptic discharges (IEDs) are paroxysmal events observed in epileptogenic zones and connected non-epileptogenic zones. IEDs are short, recurrent, and aperiodic events with a large pattern variability in terms of shapes, including spikes and spike-waves (SWs). IEDs can be generated by both the epileptic zone and irritative zone from where they can propagate to adjacent "healthy" brain regions. IEDs can show distinctive spatiotemporal features depending on the recording site in the same patient. As the occurrence of the IEDs across different brain regions can be used to determine the epileptogenic network and even to predict the surgical outcome.
Here, an example set of IEDs with SW morphology recorded by stereo-electroencephalography (SEEG) electrodes is considered. The recorded IEDs are classified with respect to their morphologies, durations, and generation sites. A neural mass model is used to mimic the spatiotemporal features of the IEDs. Laminar contributions of the glutamatergic and GABAergic post-synaptic potentials and intra-/inter-column synaptic interactions are considered to the simulated SEEG signals.
The study predicts that the epileptogenic zones generate IEDs due to being hyperexcited, IEDs in the irritative zone appear as an inhibitory response to the afferent inputs from epileptogenic zones. The early spike component of the simulated IEDs originates from fast glutamatergic and GABAergic signaling, whereas the wave component is a slow GABAergic response. The synaptic kinetics, inter-column organization, and network interactions shape the observed signals.
This study suggests that a combination of signal analysis and computational models provides an efficient ground for exploring IEDs in partial epilepsies (Elif Köksal-Ersöz et al 2022 J. Neural Eng. 19 055005).



Disclosures: **E. Köksal Ersöz:** None. **R. Lazazzera:** None. **I. Merlet:** None. **B. Mercadal:** A. Employment/Salary (full or part-time):; Neuroelectrics Barcelona. **R. Sanchez-Todo:** A. Employment/Salary (full or part-time):; Neuroelectrics Barcelona. **G. Ruffini:** A. Employment/Salary (full or part-time):; Neuroelectrics Barcelona. **F. Bartolomei:** None. **P. Benquet:** None. **F. Wendling:** None.

Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Program #/Poster #: PSTR526.19/E30

Topic: B.08. Epilepsy

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NIH award K08-NS069783
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Title: Seizure source localization from ictal intracranial EEG data using dynamic mode decomposition

Authors: *M. MCCUMBER[1], K. TYNER[1], S. DAS[1], M. ALFATLAWI[1], W. C. STACEY[2], G. C. SMITH[2], S. V. GLISKE[1];
[1]Univ. of Nebraska Med. Ctr., Omaha, NE; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Epilepsy is a common neurological disorder characterized by recurring, unprovoked seizures. For thirty percent of patients, medication alone does not control seizures. Resective surgery remains the next best option for patients with focal refractory epilepsy. Yet, surgery does not always result in seizure freedom. Improvements in identification of what tissue to resect may have a positive impact on patient care. The purpose of this study was the localization of seizure activity from intracranial EEG data using dynamic mode decomposition (DMD) to assist in resective surgery planning and improve surgery outcomes in epilepsy patients. Analysis was performed on retrospective data through the identification of patients with sEEG implantation and Engel Class 1 outcomes from the University of Michigan intracranial EEG database. One seizure was selected at random per patient, and seizures with low data quality were redacted. Channels known to be extraparenchymal or to have poor data quality were redacted. A band pass filter was applied between 1 and 40 Hz. Individual Component Analysis was performed to reduce noise. A common average reference filter was applied. The data was down sampled to 128 Hz to reduce computational cost. A two-minute window of data was selected for analysis centered around clinically documented seizure onset. The data were then quantified using our DMD method to provide a low-dimensional representation of the high-dimensional complex seizure data (blind source separation). The real part of the dominant mode was extracted for each DMD update. The dominant mode was selected as the seizure was expected to be the dominant brain activity at the time. A matrix was created for each seizure with a row per recording channel and a column per each DMD update. Each matrix was visualized as a heatmap. Channels in which the ten highest values occurred were compared to clinical notes to test the association between the DMD results and the clinically documented seizure onset locations. Using the binomial cumulative distribution, we assessed whether the percentage agreement was better than random chance, based on the total number of channels and the number of onset/early-spread channels. Statistically significant agreement ($p < 0.001$) was found in three stereo EEG patients with additional analysis ongoing. We concluded that DMD can identify the “seizure mode” of activity and may therefore aid in the analysis of seizure localization. Further analysis will show

the extent to which DMD can assist in surgical planning and contribute to the optimization of resective surgery outcomes.

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Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Program #/Poster #: PSTR526.20/E31

Topic: B.08. Epilepsy

Support: NIH/NINDS Grant R21NS116937

Title: Alterations in 5-hmC DNA Methylation Patterns in the Hippocampus of an Experimental Model of Refractory Temporal Lobe Epilepsy

Authors: *R. BAHABRY[1], R. M. HAUSER[3], R. SÁNCHEZ[4], S. SINT JAGO[1], L. IANOV[2], K. RILEY[5], L. VER HOEF[5], F. LUBIN[1];
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Abstract: Epigenetic DNA methylation (DNAme) mechanisms have been shown to play a critical role in regulating gene expression changes in the epileptic hippocampus. While research has largely focused on the role of the 5-methylcytosine (5-mC) form of DNAme in temporal lobe epilepsy (TLE), the involvement of 5-hydroxymethylcytosine (5-hmC) modification, catalyzed by the ten-eleven translocation (TET) enzymes, remains unexplored. Here, we investigated the role of TET-mediated 5-hmC in TLE. Human samples used for mass spectrometry analysis (MS) were obtained from patients with epilepsy, and controls were obtained from non-epileptic post-mortem samples. Seizures were induced in Male Sprague Dawley rats by administering Kainic Acid (KA) intraperitoneally. Eight weeks following status epilepticus (SE), hippocampal tissue was isolated and processed for MS and 5-hmC DNA immunoprecipitation followed by sequencing (hMeDIP-seq). To examine the effect of *Tet1* on seizures, *Tet1* small interfering RNA (siRNA) was used to knockdown *Tet1* expression, and *Tet1* was overexpressed via lentivirus. Behavioral seizure severity was assessed using the Racine scale. Post-SE, hippocampal 5-mC/5-hmC levels were measured via enzyme-linked immunosorbent assay (ELISA). We found that bulk hippocampal 5-hmC levels were significantly reduced in both patients with TLE and in the kainate rat model of TLE. We found no significant changes in bulk 5-mC levels in the epileptic hippocampus. hmeDIP-seq analysis showed 5-hmC loss within intergenic regions and significant changes in gene bodies and promoters in the epileptic hippocampus. Gene Ontology analysis suggests that 5-hmC enrichment at critical gene pathways such as GABA signaling and ion transport. Reducing *Tet1* expression in the hippocampus

resulted in reduced 5-hmC levels and was associated with a decreased seizure threshold, suggesting that blocking *Tet1* is sufficient to increase seizure susceptibility. In contrast, *Tet1* overexpression in the hippocampus increased 5-hmC levels, leading to a delayed onset of SE and improved seizure resiliency.Together, these findings indicate that TET1/5-hmC changes play a crucial role in the epigenetic regulation of gene processes in TLE. Future studies will assess the effect of manipulating TET1/5-hmC on epileptic seizures and determine the cell type-specific changes in *Tet1* expression contributing to abnormal gene transcription programs in TLE.

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Poster

PSTR526. Epilepsy: Mechanisms and Interventions

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Program #/Poster #: PSTR526.21/E32

Topic: B.08. Epilepsy

Support: NIH NS050229

Title: Tau phosphorylation in a rat model of temporal lobe epilepsy surveyed with mass spectrometry

Authors: O. O. ESTES, N. A. EKSTROM, F. A. CONCEPCION, M. N. KHAN, ***N. P. POOLOS**;
Univ. of Washington, Seattle, WA

Abstract: Tau is an intracellular protein known to undergo hyperphosphorylation and subsequent neurotoxic aggregation in Alzheimer's disease (AD). It has been suggested that tau may undergo similar changes in human epilepsy as well. We sought to study this question in a well-validated animal model of temporal lobe epilepsy (TLE) using antibodies which assay phosphorylation at three canonical loci known to be hyperphosphorylated in AD (AT8, AT270, and 1H6L6 Abs). We used the rat pilocarpine post-status epilepticus (SE) model of TLE. We first measured tau expression at a relatively late time point 4 months post-SE using western blotting with homogenates from the whole hippocampal formation and compared to that from age-matched naïve animals. We found that total tau expression in chronic epilepsy was unchanged, but there was a significant reduction in phosphorylation levels at the AT8 loci (45% decrease, $p<0.0001$). No change in phosphorylation was seen at the AT270 and 1H6L6 tau loci, nor was there change in AT8-assayed levels in somatosensory cortex, outside of the seizure onset zone. These findings suggest that tau is not hyperphosphorylated at three canonical loci associated with AD but rather shows dephosphorylation at one locus, and that change in AT8 phosphorylation was specific to the hippocampus where seizures arise in this model. However, tau has many phosphosites, not all associated with AD pathogenesis. We then used mass spectrometry to survey all tau phosphosites at once in a shotgun fashion. We were able to detect

phosphorylation at 40 unique tau phosphosites. Of these, only three showed changes in phosphorylation state in chronic epilepsy: S189 (60% decrease in epilepsy vs. naïve, p=0.005), T203 (33% decrease, p=0.04) and S253 (55% decrease, p=0.04). No phosphosite demonstrated significant hyperphosphorylation in chronic epilepsy. We conclude that tau in an animal model of TLE studied does not show hyperphosphorylation at any locus. Instead, at least three sites demonstrated significant dephosphorylation. This suggests that changes in tau expression may play a different role in epilepsy than in AD. Further study is needed to understand how these changes may impact neuronal excitability in chronic epilepsy.

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Poster

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Program #/Poster #: PSTR526.22/E33

Topic: B.08. Epilepsy

Title: The effect of miRNA-134 inhibition in an intra-amygdala kainic acid model of temporal lobe epilepsy

Authors: H. KOOIJKER[1], C. CHAU[2], T. TSAI[2], T. YANG[2], T. BRAGGE[3], S. BÄCK[4], ***C. S. PERITORE**[5];
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Abstract: Temporal lobe epilepsy (TLE) is the most prevalent type of epilepsy, in where the amygdala (AMY) plays an important role in the epileptogenic pathways. It is also a more reliable animal to human translational model of epilepsy than the traditional intra-hippocampal injection (IHK) of kainic acid. So far, the most powerful tool in seizure monitoring is via electroencephalogram (EEG) and video (video-EEG) monitoring. Emerging evidence has shown beneficial effects of the use of antisense oligonucleotides (ASO), such as antagomirs against miRNA-134 to reduce the frequency of spontaneous seizures in preclinical animal models of TLE.To develop a reliable mouse model of TLE to assess the anticonvulsant effects of ASOs, 10 adult male C57Bl/6 mice were implanted with infusion guide cannulas over the AMY and lateral ventricle (LV) as well as an EEG telemetry device (DSI, HD-X02) with screw electrodes placed on the skull for seizure monitoring via video-EEG. ASO treatment is delivered directly into the LV. After 10 days of surgery recovery, a 5-day baseline EEG was recorded before kainic acid (KA) infusion into AMY to induce *status epilepticus* (SE). Animals were rescued from their SE via intraperitoneal administration of Lorazepam (6 mg/kg) 40 minutes after KA infusion. Two weeks after SE, the animals were administered with an ASO or vehicle into their LV. Throughout the study, video-EEG was recorded for 3-6 days a week.The results show that TLE

can be reliably induced via KA infusion into the AMY in an adult mouse model. A reduction in spontaneous seizures after ASO treatment confirms the validity of the study design for future studies to investigate not only TLE but also potential therapeutic candidates given via LV infusion.

Disclosures: H. Kooijker: None. **C. Chau:** None. **T. Tsai:** None. **T. Yang:** None. **T. Bragge:** None. **S. Bäck:** None. **C.S. Peritore:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.01/E34

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

Support: IDSAF foundation

Title: Spatial transcriptomic profiling of neurovascular changes in Alzheimer's disease with Stereo-seq

Authors: *Z. ZHAO[1], **S. XIA**[2], T. GE[1], F. GAO[3], R. SONG[1];
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Abstract: Alzheimer’s disease (AD) is an age-dependent neurodegenerative disease that progressively deteriorates brain function, it is the most common form of dementia that impacting millions of people globally. The signature hallmarks of AD include amyloid plaques and Tau tangles, synaptic loss and neuronal dysfunction, vascular impairment, neuroinflammation and neurodegeneration. Although amyloid and Tau mediated proteinopathies have been considered as the main drivers, piling evidence from both patients and animal models have clear indicated that the disease is more complex at genetic, molecular, and cellular levels. Advancements in single-cell technologies have significantly refined our understanding of the molecular, cellular, and genetic traits in central nervous system (CNS). Spatial transcriptomics now offer unparalleled insights for spatial decoding of the pathologies and their impact on transcriptomics. In this study, we utilize a cutting-edge technology called spatial enhanced resolution omics-sequencing (Stereo-seq) to explore the regional differences and selective vulnerability among different brain cell types in Alzheimer’s mouse model. This technique is based on DNA nanoball (DNB)-patterned array/chip and can generate whole-transcriptome maps of mouse brain unprecedentedly with a resolution up to 0.5 μm. More importantly, it is comparable with immunohistological procedures for pathological mapping on the same section. These advantages are unparalleled and particularly beneficial for mapping the regional and cell-type specific susceptible to amyloid or tau proteinopathies throughout the brain regions and over the disease progression. More importantly, we delved into the spatial interactions between different cell types and A-beta plaque deposition at single cell resolution by employing a series of analysis

pipeline. In conclusion, this work provides us in-depth understanding of Alzheimer's disease, and highlights the promising capabilities of the Stereo-seq technology for investigating neurological diseases.

Disclosures: **Z. Zhao:** None. **S. Xia:** None. **T. Ge:** None. **F. Gao:** None. **R. Song:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.02/E35

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

Support: NIH 5R42AG052249-03

Title: A transcriptomic analysis to investigate the role of sigma-2 receptor modulators CT1812 and CT2168 in a mouse model of Alzheimer's disease

Authors: ***J. CALDWELL**[1], E. CHO[1], N. KNEZOVICH[1], A. O. CAGGIANO[2], V. DICARO[1], M. E. HAMBY[1];
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Abstract: Amyloid-beta (Aβ) oligomers bind to receptors on neurons and cause synaptotoxicity and cognitive decline in Alzheimer's disease (AD). Sigma-2 receptor (S2R) modulators, such as our investigational therapeutic CT1812, can displace Aβ oligomers from binding to neuronal synapses and clear the oligomers to cerebrospinal fluid. We have previously demonstrated that decreasing binding of Aβ oligomers to neuronal synapses can restore cognitive activity in a transgenic mouse model of AD. To further investigate the biological processes of S2R modulators and their molecular mechanism of action, we performed RNA sequencing analysis in an *in vivo* AD mouse model, hAPPsl, treated with our investigational therapeutic, CT1812, and a chemically distinct S2R modulator, CT2168.5-month-old non-transgenic mice (nTg) were dosed with vehicle and hAPPsl transgenic mice (Tg) were dosed with vehicle or either CT1812 (10 mg/kg) or CT2168 (5 mg/kg), given orally, once daily for 7 days. Unbiased RNA sequencing analysis (N=10 per group) was conducted to evaluate differentially expressed genes (DEGs) between nTg and Tg mice, and to assess the effect of CT1812 and CT2168 compared to vehicle. STRING and MetaCore pathway analyses were performed using gene lists of $p \leq 0.05$.In hippocampal brain tissue, treatment with CT1812 altered the expression of 2031 mRNA transcripts, and treatment with CT2168 altered 365 transcripts ($p \leq 0.05$). Pathway analysis using STRING and MetaCore revealed that the transcripts altered are mainly involved in membrane trafficking, cytoskeleton remodeling, autophagy, inflammation and WNT/β-catenin signaling. Comparative analysis between the two compound treated groups identified 127 DEGs ($p \leq 0.05$). Comparison between Tg, CT1812, and CT2168 groups identified 20 common DEGs, including *TGF-β2, Nde1, and Mcur1*, potentially associated with AD, as well as having opposite directional fold changes between control and compound treated groups underlying a possible

regulatory activity of our compounds. Understanding the mechanism of action of S2R modulators, like CT1812 and CT2168, in an AD mouse model may be a promising approach to improve therapeutic intervention for Alzheimer's patients. Our transcriptomic analysis further elucidates how S2R modulators can impact relevant pathways and modulate gene expression of genes associated with the Alzheimer's phenotype. Overall, these findings support continued clinical development of CT1812 for AD (NCT04735536, NCT03507790).

Disclosures: J. Caldwell: None. **E. Cho:** None. **N. Knezovich:** None. **A.O. Caggiano:** None. **V. DiCaro:** None. **M.E. Hamby:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.03/E36

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

Title: Spatial analysis of Alzheimer's human brain samples with Xenium high throughput in situ gene expression profiling

Authors: *A. KIM[1], J. SICHERMAN[2], A. KALAIMANI[3], B. NGUYEN[3], J. BERRIDGE[3], H. CHIRRA[3], K. BELHOCINE[3], M. OLIVEIRA[3], R. GANTT[3], S. TAYLOR[3], S. MOHABBAT[3], F. WAGNER[3];
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Abstract: Alzheimer's Disease (AD) is a complex neurodegenerative disorder characterized by progressive cognitive decline and memory loss. Despite extensive and ongoing research, the underlying mechanisms of AD remain poorly understood. In this study, we applied 10x Genomics Xenium In Situ technology with the Human Brain Panel, supplemented with an add-on custom panel designed for Alzheimer's Disease, to profile gene expression in post-mortem brain samples from AD patients. This spatial transcriptomics approach allowed us to map gene expression patterns with high resolution, revealing distinct molecular signatures in different brain regions affected by AD. Our results provide new insights into the molecular changes associated with AD and highlight the potential of spatial transcriptomics for advancing our understanding of complex neurological disorders.

Disclosures: A. Kim: A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **J. Sicherman:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **A. Kalaimani:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **B. Nguyen:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **J. Berridge:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **H. Chirra:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **K. Belhocine:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **M. Oliveira:** A.

Employment/Salary (full or part-time):; 10X GENOMICS Inc. **R. Gantt:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **S. Taylor:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **S. Mohabbat:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc. **F. Wagner:** A. Employment/Salary (full or part-time):; 10X GENOMICS Inc.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.04/E37

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

Support: NIH/NIA (R01AG059848)
Chan Zuckerberg Initiative (Ben Barres Early Career Acceleration Award; grant ID 199150)
Stanford Alzheimer Disease Research Center (NIH/NIA P30 AG066515)

Title: Identifying resistant and vulnerable neuronal populations in the human Alzheimer's disease neocortex by single-nuclear RNA sequencing

Authors: *A. SANKARAESWARAN[1], M. OTERO-GARCIA[1], J. PAN[1], K. VALLEJO[1], J. FORES MARTOS[1], Y. LIU[2], V. DOAN[1], J. E. OBERHAUSER[1], I. COBOS[1];
[1]Stanford Univ., Palo Alto, CA; [2]Stanford Univ., Palo Alto, CA, United States, CA

Abstract: In Alzheimer's Disease (AD), specific brain regions exhibit selective vulnerability to neurodegeneration. To investigate cell-type-specific vulnerability and define the transcriptomic signatures associated with resistance, we conducted single-nucleus profiling of the human AD brain across the full spectrum of disease progression, along with age-matched healthy controls. Neurons were enriched using fluorescence-activated nuclear sorting (FANS) with NeuN immunocytochemistry. Our multi-region, multi-stage dataset comprises 429,316 nuclei (after quality check) from of 46 donors. We profiled neocortical areas affected relatively early (prefrontal [BA9] and precuneus [BA7]) and later (primary visual [BA17] during disease progression. We annotated 18 excitatory neuron, 19 inhibitory neuron, 4 astrocyte, 2 oligodendrocyte, and 4 microglial states. Additionally, we employed the 10x Genomics Visium platform to spatially map the cortical layers occupied by each of the neuronal populations identified by snRNAseq. Our custom gene expression panel, consisting of 197 marker genes, in addition to the standard 10x Visium targeted Human Neuroscience panel (1,186 genes), serves as a cost-efficient tool for mapping neuronal vulnerability in the human AD brain. Our analysis revealed a population of layer 4 excitatory neurons expressing *RORB*, *CUX1/2* and *EYA4* in the BA9 and BA17 that exhibited resistance. In contrast, the ratio of excitatory to inhibitory neurons remained consistent throughout disease progression, indicating degeneration in both major neuronal classes in AD. Differential gene expression analysis using mixed models revealed early changes in interneurons, specifically in a subtype expressing *KIT* and *LAMP5*. Cell-cell communication networks demonstrated reduced interactions between KIT/LAMP5 interneurons

and excitatory neurons in early AD, while interactions of this interneuron subtype with reactive astrocytes increased in late AD. Overall, our study provides a census of vulnerable and resistant neuronal populations in AD progression, highlighting the resistant of layer 4 excitatory neurons in neocortical regions that are affected early and late, and are also resistant to tau pathology.

Disclosures: A. Sankaraeswaran: None. **M. Otero-Garcia:** None. **J. Pan:** None. **K. Vallejo:** None. **J. Fores Martos:** None. **Y. Liu:** None. **V. Doan:** None. **J.E. Oberhauser:** None. **I. Cobos:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.05/E38

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

Support: NIA R01AG065836

Title: A multi-omics approach reveals novel astrocyte dysfunction in a murine model of Alzheimer disease

Authors: *J. LI[1], X. WEI[1], H. SONTHEIMER[2], M. L. OLSEN[3];
[1]Virginia Tech. Neurosci. PhD Program, Blacksburg, VA; [2]Dept. of Neuroscience, Univ. of Virginia, Charlottesville, VA; [3]Sch. of Neuroscience, Virginia Tech., Blacksburg, VA

Abstract: Alzheimer’s disease (AD) is the most common age-related neurodegenerative disease, with 58 million individuals affected worldwide. Pathological sequelae include abnormal aggregation of β-amyloid (Aβ) peptides and neurofibrillary tangles (NFTs), neuronal loss and astrocyte and microglial activation. Astrocytes play an important role in brain health and express high levels of the three AD causative genes *APP, PSEN1 and PSEN2* and are the primary cell type expressing the strongest risk factor gene in late onset Alzheimer disease, *APOE*. Research obtained evaluating astrocyte gene expression in bulk, single cell, single nuclei and spatial transcriptomic RNA sequencing in human AD tissue, IPSC derived astrocytes and in AD animal models reveal alterations in genes driving inflammation, cell senescence, morphology and territory size. It is not clear how these transcriptomic changes relate to protein expression and astrocyte cell function. Here, we applied a multi-omics unbiased, transcriptome and proteome approach in hAPPJ20 mice (a commonly used AD animal model, with vascular Aβ accumulation) to evaluate the astrocyte transcriptome and proteome across healthy aging and AD disease progression (3, 6, 12 and 18 months) in male and female animals. Our data indicate robust astrocyte gene expression differences early as 3 months, and astrocyte reactivity, as indicated by several reactivity markers (*Gfap, Serpina3n, and Hspb6*), prior to quantifiable plaque burden. Gene Ontology (GO) enrichment analysis indicate global inflammation, disrupted astrocyte function at the synapse and vascular dysfunction at the 3 months time point in males. Similar pathways were identified at protracted timepoints in females, suggesting sex specific

adaptations across disease progression. Intriguingly, both sexes demonstrate gene dysregulation associated with cell apoptotic pathways at 12-18 months, suggesting astrocytes are undergoing apoptotic cell death during disease progression. Ongoing work includes integrating data from our studies with publicly available data in other AD murine models and human AD tissue. Further immunohistochemical and biochemical approaches will be applied to link changes in gene and protein to astrocyte function in AD.

Disclosures: **J. Li:** None. **X. Wei:** None. **H. Sontheimer:** None. **M.L. Olsen:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.06/E39

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

Support: NIH 5R42AG052249-03

Title: A proteomic analysis to elucidate the role of sigma-2 receptor modulators CT1812 and CT2168 in a mouse model of Alzheimer’s disease

Authors: *E. CHO[1], J. CALDWELL[1], K. PANDEY[3], D. DUONG[4], N. T. SEYFRIED[4], A. O. CAGGIANO[2], V. DI CARO[1], M. E. HAMBY[1];
[1]Cognition Therapeutics, Inc., Pittsburgh, PA; [2]Cognition Therapeutics, Inc., Purchase, NY; [3]Emtherapro, Atlanta, GA; [4]Emory Sch. Med., Emory Univ., Atlanta, GA

Abstract: Sigma-2 receptor (S2R) modulators can modulate amyloid-beta (Aβ) oligomer binding to neuronal synapses. The S2R modulator CT1812 is a first-in-class investigational therapeutic, currently in Phase 2 clinical trials for Alzheimer’s disease (AD). CT1812 selectively displaces Aβ oligomers from synapses and clears them from the brain into the cerebrospinal fluid, restoring cognitive performance in a transgenic mouse model of AD. To better understand and identify the biological processes that S2R modulators can impact, a proteomic analysis was performed in an *in vivo* mouse model of AD treated with CT1812 and a chemically distinct S2R modulator, CT2168.
5-month-old non-transgenic mice (nTg) were dosed with vehicle and hAPPsl transgenic mice (Tg) were dosed with vehicle or, either CT1812 (10 mg/kg) or CT2168 (5 mg/kg), given orally, once daily for 7 days. Unbiased proteomics (n = 10 per group), using tandem-mass tag mass spectrometry (TMT-MS), was conducted to examine differences in the hippocampus proteome between Tg and nTg mice, and to assess effect of CT1812 and CT2168 compared to vehicle in Tg animals using differentially expression analysis ($p \leq 0.05$). STRING and MetaCore pathway analyses were performed using protein lists of $p \leq 0.05$.
In the brain, treatment with CT1812 and CT2168 altered the expression level of proteins compared to Tg mice, 219 and 316 proteins, respectively ($p \leq 0.05$). Comparative analysis between compound treatments identifies 23 common proteins differentially expressed. Among

them, 9 proteins including GALT, NCAM1, and OPA1 showed fold change of protein expression in all three comparison groups (Tg vs. nTg, CT1812-Tg vs. vehicle-Tg, and CT2168-Tg vs. vehicle-Tg). Pathway analysis by STRING and MetaCore indicated the role of CT1812 and CT2168 in altering key pathways and functions including metabolism of lipid and proteins, synapse organization (pre-synapse and post synapse), neuron development, vesicle transport, autophagy, and remyelination.
This proteomic analysis data has demonstrated that two chemically different S2R modulators, CT1812 and CT2168, are highly related to biological networks and pathways in an AD model. These new findings can lead to further understanding of the molecular mechanism of action by which S2R modulators may be a promising therapeutic approach for Alzheimer's disease patients.

Disclosures: E. Cho: None. **J. Caldwell:** None. **K. Pandey:** None. **D. Duong:** None. **N.T. Seyfried:** None. **A.O. Caggiano:** None. **V. Di Caro:** None. **M.E. Hamby:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.07/E40

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

Support: NIH Grant AG037868

Title: A ROBUST AND HIGHLY INTEGRATED SUBSET OF MESSENGER RNAS SHOW ENHANCED AND STOCHASTIC ASSOCIATION WITH RNA INTEGRITY NUMBER AND IS EXACERBATED IN ALZHEIMER'S DISEASE VS CONTROL POST-MORTEM HUMAN BRAIN TISSUE

Authors: *K. M. BRETLAND[1], E. S. JOHNSON[1], S. LEE[2], E. M. BLALOCK[1];
[1]Pharmacol. and Nutritional Sci., [2]Sanders Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY

Abstract: RNA Integrity Numbers (RINs) have become a standard in the field to assess RNA quality, especially prior to transcriptional profiling measurements. Researchers often establish some lowest acceptable RIN and/or adjust transcriptional profile results based on sample RINs. Linear regression is a popular RIN correction method, but our lab recently showed that the relationship between mRNA level and RIN in control post-mortem autopsy brain tissue is poorly described by a linear relation. Instead, once batch effects had been removed by ComBat in R, post-mortem human brain tissue transcriptional profiling datasets with disambiguated RINs downloaded from the Gene Expression Omnibus (GEO) showed a stochastic pattern, with a group of 383 genes showing highly significant and synchronous downregulation in 16 of the 264 profiles, with a bias towards increased occurrence at lower RINs. A study by Miller et al., 2017 found that, in Alzheimer's disease (AD) profiles, correcting for lower RIN removed many AD-

significant genes. Here, we gathered post-mortem human brain control vs AD data sets from GEO and found that this effect, while stochastically RIN-associated in control samples becomes RIN-independent and highly consistent in AD samples. Further, in both AD and control tissue, the effect is persistent within subjects across brain regions. Finally, these 383 genes showed a graded decline with increasing AD severity. Since RIN itself was not significantly correlated with post-mortem interval or brain pH, it is reasonable to suggest that reduced RIN in AD subjects may be a property of the disease rather than of tissue handling.

Disclosures: K.M. Bretland: None. **E.S. Johnson:** None. **S. Lee:** None. **E.M. Blalock:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.08/E41

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

Support: AG057565-IL
AG066198-RK
AG077636-RK
AG075992-IL

Title: Transcriptional responses to ABCA7 deficiency in AD mouse model expressing human APOE3 and APOE4 isoforms

Authors: *S. Y. PATIL[1], Y. LU[1], I. LEFTEROV[2], R. KOLDAMOVA[4], N. F. FITZ[3];
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Abstract: Title: Transcriptional responses to ABCA7 deficiency in AD mouse model expressing human APOE3 and APOE4 isoforms.Authors: Snehal Patil, Yi Lu, Iliya Lefterov, Radosveta Koldamova and Nicholas F. Fitz
Introduction: ATP-binding cassette subfamily A member 7 (*ABCA7*) polymorphisms have been established as genetic risk factors for late-onset Alzheimer's disease (AD) through multiple Genome-Wide Association Studies, though exact mechanisms are unknown. ABCA7 is a membrane-associated protein important in transport, though the exact substrates are not established. *Abca7* is primarily expressed in neurons and microglia and thought to be involved in lipid metabolism and inflammatory response. In an AD mouse model, haploinsufficiency of *Abca7* caused increased production and decreased clearance of Aβ, thus contributing to the pathophysiology of AD. Inheritance of ε4 allele of Apolipoprotein E (*APOE*) is the major genetic risk factor for late-onset AD, also impacting lipid metabolism, inflammation and Aβ pathology. While *APOE* and *ABCA7* are two major genetic risk factors for late-onset AD, surprisingly little is known about the interplay contributing to AD pathogenesis.
Aim: In this study, we hypothesis that *Abca7* deletion would have a differential impact on the

brain transcriptomics dependent on APOE isoform in an AD mouse model.
Methods: APP/PSEN1dE9 mice expressing human APOE3 or APOE4 isoforms were crossed with *Abca7* knockout and *Abca7* wild-type mice. The four groups (APP/E3, APP/E4, APP/E3/Abca7^{ko}, APP/E4/Abca7^{ko}) included 6 mice (equal sex distribution) that were 8 months of age. Whole cortical brain tissue was used for bulk RNA sequencing and analysis of the results was performed using edgeR.
Results: 1470 genes were differentially expressed in APP/E3/ABCA7^{ko}-APP/E3 and 1066 genes in APP/E4/ABCA7^{ko}-APP/E4. Biologic processes related to synapse organization, regulation and function including axon and dendritic spine formation were significantly downregulated in ABCA7^{ko} as compared to the APP/E3 and APP/E4 whereas those associated with immune response including microglial cell activation, and inflammatory response were significantly upregulated in APP/E4/ABCA7^{ko} vs APP/E3/ABCA7^{ko}.
Conclusions: We conclude that loss of function of ABCA7 differentially affects the brain transcriptome depending on APOE isoforms.

Disclosures: S.Y. Patil: None. **Y. Lu:** None. **I. Lefterov:** None. **R. Koldamova:** None. **N.F. Fitz:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.09/F1

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

Title: Unravelling the potential implication of m6A RNA Modification and proteins with PrLDs in Alzheimer's Disease: Insights into Neuronal RNA Granules

Authors: *S. BOULAASSAFRE;
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Abstract: Unravelling the potential implication of m6A RNA Modification and proteins with PrLDs in Alzheimer's Disease: Insights into Neuronal RNA Granules*Soukayna Boulaassafre[1], Hassan Ainani[1], Abdellatif El Khayari[1], Rachid El Fatimy[1]*
[1]Institute of Biological Sciences (ISSB-P), Faculty of Medical Sciences (FMS), Mohammed VI Polytechnic University (UM6P), 43150, Ben-Guerir, Morocco
Abstract Alzheimer’s disease (AD) is a neurodegenerative disorder with no preventive or curative treatment. The AD is associated with several dysregulated pathways especially counting axonal transport, that latter supplies synapses with several essential components including RNAs. A large proportion of RNAs localized in synapses are transported from the soma to neuronal extensions along microtubules in a highly organized structure called neuronal RNA Granules (NRGs). The NRGs have heterogeneous composition combining different RNAs, proteins, and RNA-binding proteins (RBPs). the genesis and implication of NRGs in neurodegenerative diseases are still not fully understood. In this study, we propose that NRGs

impaired axonal transport may be involved in AD pathogenesis. Through our *in-silico* investigation, we showed that N6-methyladenosine (m6A), the most abundant epitranscriptomic modification in eukaryotes, and prion-like domain proteins (PrLDs) could be a key component, and their alteration might affect NRGs homeostasis in AD. First, we showed that a high proportion of NRGs mRNAs and NRGs-associated mRNAs targeted by fragile X mental retardation protein (FMRP), one of the most enriched RNA-binding proteins in NRGs, are likely to be methylated. Afterwards, genes encoding the m6A methylation regulatory complex and proteins with PrLD present in NRGs were studied in 16 available RNA-sequencing and microarray datasets of mice and humans. Our analysis demonstrates that some of those genes are dysregulated in AD compared to normal controls. This includes m6A methylation regulatory complex like METTL3, YTHDF2, and interestingly RBPs with Prion-like domains (Proteins with low complexity domain). Furthermore, we identified dysregulated methylated genes like both in NRGs and synapses and revealed a range of their related altered pathways that might be linked to NRGs and synaptic disturbance leading to AD pathology. Finally, based on our comprehensive analysis, we proposed a working model elucidating the potential dysregulation of NRGs homeostasis in AD pathogenesis, paving the way for NRGs as a new potential therapeutic target in AD.
Keywords: Neuronal RNA Granules, N6-methyladenosine, FMRP, Proteins with prion-like domain, Alzheimer's disease.

Disclosures: S. Boulaassafre: None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.10/F2

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

Support: OSU Foundation
NIH Grant 2R21AG060206
OSU College of Science

Title: Identifying mechanisms of selective vulnerability of CA1 region in 5xFAD mice with the use of spatial transcriptomics

Authors: *M. R. FRISCHMAN, T. M. HAGEN, K. R. MAGNUSSON;
Oregon State Univ., Corvallis, OR

Abstract: Alzheimer's Disease (AD), the leading cause of dementia, has no viable treatment and little research done at the preclinical stage, which stands to be a promising window for treatment. In this study, we examined preclinical differences in hippocampus subregions using spatially-resolved transcriptomic profiling with Nanostring's GeoMx DSP. Eight one-month-old 5xFAD (C57BL/6 background) heterozygote mice and wild-type littermates had brains harvested and

fresh-frozen. Cryostat sections through the dorsal hippocampus were mounted onto slides and shipped to Nanostring, Inc., where they were treated with indexing oligo-labeled cDNA probes. The expression of mRNA was measured separately within the cell body layers of the CA1, CA3, and DG hippocampal subregions of each mouse. The overall data was analyzed with t-distributed stochastic neighbor embedding (t-SNE), and each subregion was analyzed for differential transcript expression and gene-set pathway differences between wild-type and 5xFAD. t-SNE analysis showed strong clustering by subregion, and differential transcript expression analysis showed 231 differentially expressed transcripts (DETs) in the CA1, 179 DETs in the CA3, and 165 DETs in the DG subregions between wildtype and 5xFAD heterozygotes. Gene-set pathway differences were found in the CA1 and DG subregions, with 6 pathway changes in the CA1, 4 of which were downregulations of mitochondrial-associated pathways in the heterozygotes, and 10 in the DG subregions. The extensive heterogeneity observed revealed numerous spatially-distinct transcripts and pathways dysregulated in the preclinical state and most strongly implicates mitochondrial changes in the CA1 subregion in the early progression of AD.

Disclosures: **M.R. Frischman:** None. **T.M. Hagen:** None. **K.R. Magnusson:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.11/F3

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

Support: Alzheimer's Association Grant: AARG-22-919611
Washington Royalty Research Foundation
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ACT study: U19 AG066567
NSF Graduate Research Fellowship Program: DGE-1762114

Title: Messenger RNA and circular RNA mislocalization to synapses are key features of Alzheimer's disease

Authors: *S. SMUKOWSKI[1,2], C. DANYKO[2], J. SOMBERG[2], E. KAUFMAN[2], M. M. COURSE[2,3], P. N. VALDMANIS[2,1];
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Abstract: Localized translation is an essential mechanism of synaptic plasticity whereby synaptic signals between neurons stimulate the rapid translation of proteins from mRNAs compartmentalized within synapses. For this to work, RNAs need to be properly trafficked along the cytoskeleton to distal locations. Neuron cytoskeletal “traffic jams” have become an appreciated component of Alzheimer’s disease (AD) pathology therefore making it likely that

RNA cytoskeletal transport is perturbed in AD. To investigate differences in RNAs localized to synapses in AD, we acquired human brain samples from AD patients and cognitively healthy controls. From these, we isolated synaptic particles (synaptosomes) and RNA sequenced their contents. We compared sequencing data between the synaptosomes and pre-fractionated homogenate determining a set of ~3,400 mRNAs enriched at synapses. Next, we compared RNA in AD vs. control among synaptosome fractions cross examining the data against AD vs. control in homogenate. This analysis identified 1,198 mislocalized transcripts - differences in synaptosome expression independent of global expression changes. Next we pursued an analysis of circular RNAs (circRNAs) from our data which have been an exciting new area of study in the context of neurodegenerative disease since they have been found to play regulatory roles in the expression of other transcripts. Comparing synaptosome sequences to homogenate, we confirmed previous research showing the vast majority of circRNAs preferentially localize to synapses in contrast to bulk homogenate. Comparing circRNAs between AD and control synaptosomes, we found 110 differentially expressed circRNAs. Furthermore, we discovered multiple circular isoforms arising from the same gene, some having contrasting expression changes. Among this set of differentially expressed circRNAs, we identified two circular isoforms from the gene *GSK3B*. One isoform is a circle of exons 7-9, which was found to be upregulated in AD and the other is a circle of exons 9-10 which was downregulated. This is a noteworthy discovery given that GSK3B has an established role in tau hyperphosphorylation. To determine if *circGSK3B* isoforms had impacts on protein expression and phosphorylation, we transfected plasmids that expressed *circGSK3B* isoforms into neuron-differentiated SH-Sy5y cells. We found that the *circGSK3B* 9-10 substantially reduced tau phosphorylation compared to the 7-9 isoform. Altogether, this research sets the stage for investigating the impact of circRNAs on localized translation and synaptic plasticity as well as roles in neurodegeneration which may reveal new therapeutic targets.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.12/F4

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

Support: NIH Grant IR01AG068581

Title: Spatial proteomic characterization of amyloid plaques in brain tissue using laser capture microdissection and mass spectrometry.

Authors: *Y. JIAO;
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Abstract: Spatial proteomic characterization of amyloid plaques from Alzheimer's disease tissue using laser capture microdissection and mass spectrometry.*Y. Jiao[1], H. Sun[1], M. Chu[1], S. Yang[1], J. Peng[1] [1]Department of Structural Biology, St. Jude Children's Research Hospital. Alzheimer's disease (AD) is characterized by the accumulation of pathological deposits, such as amyloid aggregates, in the brain. Analyzing the proteomic composition of these plaques is crucial for advancing biomedical research. Laser capture microdissection (LCM) enables the isolation and collection of specific cells from tissue samples. Our aim is to combine LCM with mass spectrometry to characterize amyloid plaques and analyze their proteomic features. Previously, we successfully established a proteomics platform utilizing 18-plex tandem mass tag (TMT) coupled with two-dimensional liquid chromatography and tandem mass spectrometry (LC/LC-MS/MS) capable of processing samples at the sub-microgram level. In this study, we have developed a method that employs laser capture microdissection (LCM) to capture amyloid plaques from fresh frozen human and mouse brain tissue. The captured plaques are then subjected to profiling using TMT proteomics. Our method involved the following steps: (i) Collection of 12 µm tissue sections from fresh frozen brain tissue. (ii) Fixation and staining of the sections with X-34 dye to label the amyloid plaques. (iii) LCM capture of 300 amyloid plaques and 300 non-plaque areas from the same tissue section. (iv) Short digestion time with a high level of trypsin to yield low protein levels in the plaques (~2ng/plaque). Proteomic analysis was performed using the TMT-LC/LC-MS/MS method. Our results are as follows: 1. We established an optimal method for collecting amyloid plaques, as well as other aggregated proteins such as Tau and α-Synuclein, and neurons from human and mouse brain tissue samples. 2. We developed a deep proteomic platform for profiling LCM samples, enabling the quantification of 100,000 peptides and 8,000 proteins from an initial protein amount of 0.6 µg per sample (corresponding to 300 LCM punches). 3. In the AD mouse model, we observed a significant upregulation of 177 proteins and downregulation of 2 proteins within amyloid plaques compared to non-plaque regions. Several key proteins, including Abeta, Apoe, Mdk, and Ntn1, are consistently present in the plaque areas. Conclusion: Our approach provides an optimized platform for in-depth profiling of LCM-captured amyloid plaques. This method enables a comprehensive understanding of the proteomic composition of these plaques, contributing to the advancement of Alzheimer's disease research.

Disclosures: Y. Jiao: None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.13/F5

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

Support: NIH RF1AG074569
P30 AG047266

Title: Disease stage and brain region specific characterization of beta-Amyloid deposits in postmortem human brains

Authors: *W. TSERING[1], J. PHILLIPS[1], G. HERY[1], K. LOLO[1], T. GOLDE[2], S. PROKOP[1]; [1]Univ. of Florida, Gainesville, FL; [2]Emory Univ., Atlanta, GA

Abstract: Disease stage and brain region specific characterization of beta-Amyloid deposits in postmortem human brains

Background: Alzheimer's disease (AD) is neuropathologically characterized by extracellular amyloid-β (Aß) plaques and intercellular neurofibrillary tangles (NFT), but how these two pathological hallmarks interact spatially and temporally during disease progression is still far from understood. Neuritic plaques (NP) are a specific subtype of Aß deposits which encompass abnormal neuronal processes with accumulation of various cellular organelles, termed dystrophic neurites (DN). DN contain numerous proteins including tau, BACE1, APP, LAMP1, RTN3 and Ubiquitin (Sadleir et al., 2016; Sharoar et al., 2021). NP have been suggested to be the place of origin for tau seeds that can spread to different brain regions (He et al., 2018) and are associated with a strong microglia response. While NP are an integral part of the current neuropathological diagnostic criteria for AD, a plethora of other, morphologically distinct Aß plaque types (diffuse, dense-core, fibrillar, coarse grained) have been described in AD patient brains, but the disease stage and brain region specific distribution of these subtypes in relation to NP and their pathological significance have not been formally investigated.**Method:**We used immunohistochemistry (IHC) combined with silver staining on post-mortem brain tissue specimens to assess the disease stage and brain region specific distribution of NP and other Aß deposits in cases with low, intermediate and high AD neuropathological changes in three disease relevant brain regions (hippocampus, frontal cortex and occipital cortex). In addition, we analyzed the association of Aß deposit subtypes with a local microglia response using IHC for Aß (Ab5) and microglia (Iba1) combined with Gallyas silver staining and assessed the presence of other proteins co-depositing with Aß using IHC. We also did unbiased spatial transcriptomic analysis to understand gene expression level differences in different Aß plaque types. **Result:**We identified brain region and disease stage specific differences in the distribution and ratio of NP and other Aß-deposits and correlated these findings with local microglia activation and co-depositing proteins.**Conclusion:** Characterization and quantification of neuritic plaque and co-depositing proteins in different brain regions and AD stages will guide effective therapeutic treatment for Alzheimer's disease.

Disclosures: W. Tsering: None. **J. Phillips:** None. **G. Hery:** None. **K. Lolo:** None. **T. Golde:** None. **S. Prokop:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.14/F6

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

Support: other support

Title: Single-nucleus analysis of accessible chromatin uncovers cell type specific epigenomic alterations in Alzheimer's and Pick's brain

Authors: *S. DAS, Z. SHI, S. MORABITO, E. MIYOSHI, V. SWARUP;
Dept. of Neurobio. and Behavior, Univ. Of California, Irvine, Irvine, CA

Abstract: Pick's disease (PiD), a behavioral variant of frontotemporal dementia, shares many features with the most common type of neurogenerative dementia 'Alzheimer's disease'(AD). From both pathological and cognitive point of view PiD is difficult to differentiate from Alzheimer disease, especially when the patients have progressed to an advanced stage. What determines the differences and similarities between PiD and AD progression, especially at the epigenetic level, are largely unknown. Therefore, we performed snATAC-seq on postmortem human frontal cortex tissues from late-stage AD (n=12), PiD (n=7) and their corresponding age matched cognitively healthy controls to identify disease specific dysregulation in epigenomics. Profiling of 83,938 PiD nuclei and 114,784 AD nuclei led to identification of cell type specific, disease enriched putative enhancers and their linked promotors, as well as the cis-regulatory landscape at GWAS loci in specific cell types. Additionally, to complement our analysis of cis regulatory element, we constructed cell type specific, and disease enriched trans regulatory elements in AD and PiD. Our findings indicate a potential dysregulation of CTCF interaction with other genes, including UBE3A. To validate the finding *in-vitro,* we performed CRISPER knockout of a part of the enhancer region of the target gene in iPSC derived neurons. Overall, this work expands our understanding of epigenomic changes in AD and PiD, especially in regard to cell-type-specific genomic loci with disease risk.

Disclosures: S. Das: None. **Z. Shi:** None. **S. Morabito:** None. **E. Miyoshi:** None. **V. Swarup:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.15/F7

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

Title: Evaluating gene markers associated with neurodegenerative diseases using 10x Genomics Xenium In Situ Technology

Authors: *H. A. OH, P. MARKS, D. RIORDAN, Z. MA, H. SASAKI, R. HENLEY, S. TAYLOR, F. MESCHI;
10x Genomics, Pleasanton, CA

Abstract: Development of effective therapeutics for neurodegenerative diseases such as Alzheimer's disease (AD) and Huntington's disease (HD) depends on understanding of

underlying cellular and molecular mechanisms. While the field has made significant advances in genome-wide transcription analysis using single cell RNA-seq, often native spatial context is lost, which can limit biological interpretations especially in neurodegenerative diseases where spatial-specific disease progression occurs. Here, we use Xenium In Situ analysis with a high-plex panel that includes over 1000 genes covering all major cell types in the human brain and key markers that are known to be important in the study of neurodegenerative diseases. Coronal FFPE brain sections (5 μm) were placed on 12 x 24 mm Xenium slides and underwent deparaffinization and decrosslinking. Next, DNA barcoded probes were hybridized to mRNA targets. The DNA probes have two target binding regions, one at each end of the oligo such that they form a circular structure when bound to the mRNA. The non-hybridized portion of the probe contains the barcode identifying the target transcript. After successful hybridization, the two target binding regions were ligated to form a circular DNA probe, which was subsequently amplified through rolling circle amplification, producing many copies of the circularized probe. The rolling circle products were then detected through multiple rounds of imaging with fluorescently labeled detection probes to generate a unique optical signature for each gene in each cycle. These images were visualized and analyzed with the Xenium Explorer software. The highplex panel used in this study allowed the identification of the expected cell types in the healthy and diseased brain samples. Further, we were able to explore the unique gene expression profiles of cells in the AD and HD brain samples with subcellular spatial resolution. These findings demonstrate that Xenium is a powerful tool that provides critical insights for identifying risk genes, and possible development of therapeutic targets for neurodegenerative diseases.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.16/F8

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

Support: AG057565-IL
AG066198-RK
AG077636-RK
AG075992-IL

Title: Single-cell transcriptional and chromatin architecture changes in response to bexarotene-activated nuclear RXR in the brain of APP/PS1 mice

Authors: *D. D. O'SULLIVAN, C. GIRARDI, Y. LU, R. KOLDAMOVA, I. LEFTEROV; Envrn. and Occup. Hlth., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Background: Treatment with the RXR-specific agonist Bexarotene exerts neuroprotective effects in Alzheimer's disease (AD) mouse models by improving cognition and increasing Aβ clearance. At the transcriptional level, ligand-activated RXR receptors regulate gene networks linked to neural development, neuroinflammation, and metabolism. This study aimed to reveal the association between changes in chromatin architecture and transcriptional activity in brain of Bexarotene-treated APP/PS1 mice.
Methods: APP/PS1de9 mice were treated with bexarotene (100 mg/kg/d, 10 days, oral administration) or vehicle (corn oil, DMSO 1%). Mouse brains were dissociated and used for cDNA library generation on a 10X platform for single-cell resolution. Dimensionality reduction and unsupervised clustering were performed using the *Seurat* pipeline to resolve cell populations and for differential expression analysis, followed by gene ontology search to reveal cell population-specific transcriptional responses to Bexarotene. Changes in chromatin architecture were evaluated in single-cell ATAC-seq libraries generated from the brains of the same mice. scATAC-seq data were processed and integrated with scRNA-seq using *ArchR* pipeline.
Results: RNA-seq: We identified clusters relative to astrocytes, microglia oligodendrocytes, neurons, blood vessel cells, and macrophages. In astrocytes, Bexarotene increased expression levels of Abca1, Apoe, Rora, Mertk, and Clu, and in microglia - Apoe, Trem2, and Tyrobp. Cluster-specific differential expression analysis showed astrocytic modulation of gene ontologies linked to brain development, lipid and cholesterol metabolism, and secondary transcription modulation. Expression levels were interpreted with changes in chromatin architecture and possible activation states of transcription factors in promoter regions and remote genomic locations.
Conclusions: Results revealed that well-known RXR-controlled gene ontologies in the brain are upregulated in primary brain cells. Bexarotene-induced *Apoe,* increased expression within microglia and astrocytes, is restricted to populations functionally associated with Aβ trafficking. We also show bexarotene-mediated transcriptional responses in brain barriers. The results of our study provide further insight into how the activation of the RXR-controlled network can be used for restoring brain homeostasis in the context of amyloidosis, neuroinflammation, and neuronal damage.

Disclosures: D.D. O'Sullivan: None. **C. Girardi:** None. **Y. Lu:** None. **R. Koldamova:** None. **I. Lefterov:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

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Program #/Poster #: PSTR527.17/G1

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

Support: NIH Grant AG057565
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NIH Grant AG075992

Title: Single-cell RNA-seq identifies transcriptional responses to ABCA1 deficiency in human APOE expressing mice

Authors: Y. LU[1], ***J. KIM**[2], A. MAMUN-OR-RASHID[1], N. F. FITZ[1], I. M. LEFTEROV[1], R. KOLDAMOVA[1];
[1]Envrn. and Occup. Hlth., Univ. of Pittsburgh, Pittsburgh, PA; [2]Univ. of Pittsburgh Grad. Ctr. For Neurosci., Pittsburgh, PA

Abstract: Background: ATP-binding cassette transporter A1 (ABCA1) regulates cholesterol and phospholipid efflux to lipid-poor apolipoproteins and is essential for the generation of HDL. Various mutations of *ABCA1* are causatively linked to Tangier disease, characterized by lack of HDL and prevalent cardiovascular disease. Recent GWAS studies identified gene variants of *ABCA1* that increase the risk of Late Onset Alzheimer's disease (AD). In mouse brain, *Abca1* deficiency leads to decreased APOE protein level. In AD model mice, lack of *Abca1* increases Aβ deposition and cognitive deficits.**Methods:** We used APP/PSEN1dE9 mice expressing human APOE3 or APOE4 (APP/E3 or APP/E4) haplodeficient on *Abca1* (APP/E3/het, and APP/E4/het) and their non-APP WT littermates (referred to as E3, E4, E3/het, and E4/het). The mice were 6-8 months old, with a total of 4 mice per group (2 males and 2 females). Using brain tissue and 10x Genomics platform we generated sequencing libraries, performed single-cell RNA sequencing and assessed gene expression profiles. Sequencing results were preprocessed by Cellranger (v 6.1, 10x Genomics), and analyzed using *Seurat* pipeline (v 4.1.1, R 4.3.0).**Results:** With a total of 18,6254 cells after filtering (UMI >500, genes/cell > 200, mitochondrial genes <10%), we identified 16 clusters (comprised of astrocytes; microglia; endothelial cells; oligodendrocytes; vascular cells; neurons; macrophages; ependymal and choroid plexus epithelial cells). The main effects of *Abca1* deficiency were observed in microglia (26596 cells) and astrocytes (42629 cells). After re-clustering of microglia, we identified two clusters associated with homeostatic and two clusters of disease associated microglia (DAM). Lack of one copy *Abca1* led to a significant increase of number of cells in DAM clusters in both APP/E4 and APP/E3 mice but did not have a significant effect in non-APP mice. *Abca1* deficiency also increased the number of differentially expressed DAM genes between APP/E3 and APP/E4. The results for the most prominent Abca1 targets were validated using Fluorescence in situ hybridization.**Conclusions**: The results demonstrate that *Abca1* deficiency differentially affects microglia transcriptome depending on APOE isoform and amyloid pathology.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.18/G2

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

Support: Nancy and Buster Alvord Endowed Fund in Neuropathology
NIH P30 AG066509

Title: Large format highly multiplexed in situ single-cell protein profiling of human brain

Authors: *V. M. RACHLEFF[1], T. PHAN-EVERSON[2], G. ONG[2], A. ROSENBLOOM[2], S. BONNETT[2], E. PIAZZA[2], P. DANAHER[2], T. RANE[2], A. WARDHANI[2], M. KORUKONDA[2], C. BROWN[2], C. KANG[2], J. LYSSAND[2], J. JUNG[2], G. GEISS[2], D. W. RUFF[2], D. DUNAWAY[2], J. BEECHEM[2], C. D. KEENE[1];
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Abstract: The brain is a complex heterogeneous organ, where the ability to explore protein-driven activities at high resolution within spatial context of both immediate and far-ranging neuroanatomy is necessary to develop a comprehensive understanding of brain structure, function, and alterations, including aging, injury, inflammation and reactivity, and neurodegeneration. Alzheimer's disease (AD) is characterized by the spatiotemporal progressive deposition of pathologic peptides, amyloid beta (Aβ) and hyperphosphorylated tau (pTau). pTau burden, in the form of neurofibrillary degeneration, corresponds strongly with cognitive impairment and progresses generally from medial temporal lobe structures to neocortex. The middle temporal and superior temporal gyri represent a critical transition zone in disease progression, separating pTau accumulation associated with aging and pre-clinical AD from advanced stages of dementia-associated pTau pathology. The CosMx™ Spatial Molecular Imager (SMI) introduces highly multiplex protein and > 6000 plex RNA multi-omic exploration of FFPE tissues using universal multi-analyte readout reagents. For spatial omics applications, it is critical to focus efforts across transitional zones (one or more cortical gyri), but high plex cycling imagers are limited to ~100-300 mm^2 of imaging area, insufficient to study advancing neurodegeneration across anatomical boundaries. Here we present a novel Large Surface Area flow cell with > 1600 mm^2 of imaging area, a > 5-fold increase. Combining the Large Surface Area flow cell imaging capabilities with an antibody based > 68 plex CosMx Human Neural Cell Profiling and Alzheimer's Pathology Protein Panel, we demonstrate spatial imaging of neural cell typing (GFAP, Iba1, NeuN), disease specific targets (APP, Aβs), and key post translational modifications (phosphorylated Tau at multiple sites), tracking the spread of tauopathies across AD associated brain regions. Applying the large format CosMx SMI technology to AD tissues sheds light on key unanswered questions such as how pTau species, comorbid proteinopathies (such as TDP-43), and immune cells differentially impact vulnerable cell types and dynamically change with disease progression. Critically, this technology provides the unique ability to identify vulnerable cells across brain regions for additional transcriptomic (> 6000 plex CosMx RNA assay) investigation to understand the earliest changes as neurodegeneration advances within the brain, to identify pathways involved in initiation and progression of pathology, and to uncover novel therapeutic targets for AD and related dementias.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

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Program #/Poster #: PSTR527.19/G3

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

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Title: Human induced neuron modeling for the study of early-onset Alzheimer's disease

Authors: *P. VALDES[1], A. B. CALDWELL[1], Q. LIU[2], K. W. HENRY[3], M. Q. FITZGERALD[1], K. MURALIDHARAN[4], S. RAMACHANDRAN[1], C. M. KARCH[5], D. (DIAN)[5], S. YUAN[6], S. L. WAGNER[2], L. S. B. GOLDSTEIN[3], W. C. MOBLEY[2], D. R. GALASKO[2], S. SUBRAMANIAM[1];
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Abstract: Early-onset Alzheimer's disease (EOAD) occurs at an early age at onset (AAO) before 65 years of age, constituting 5-6% of all AD cases and is a complex disease that remains poorly understood. Patient-derived induced pluripotent stem cells (iPSCs) have been used to model and study both penetrative familial EOAD (EOFAD) mutations *PSEN1*, *PSEN2* or *APP* and have been seldom used for sporadic forms of EOAD that display more heterogeneous disease mechanisms. We examined iPSC-derived neurons from both EOFAD patients harboring mutations in $PSEN1^{A79V}$, $PSEN2^{N141I}$, *and* APP^{V717I} and non-familial, EOAD at an early AAO. RNA-seq for EOFAD and non-familial EOAD patients as well as ATAC-seq for EOFAD patients were carried out to mechanistically characterize the gene expression and chromatin accessibility changes, respectively. Differential expression and enrichment analysis, TF activity identification, and co-expression module detection were performed for EOFAD RNA-seq whereas clustering and surrogate neuron marker classification was performed for non-familial EOAD RNA-seq. Differential peak analysis and annotation, TF motif footprinting and differential motif accessibility, and peak functional enrichment were performed for EOFAD ATAC-seq. This approach allowed us to identify the correlation between gene expression and chromatin accessibility associated with key disease EOFAD endotypes. We then identified limitations with our non-familial EOAD neuron model to study sporadic AD, providing evidence that these neurons are less terminally differentiated after integration of multiple RNA-seq neuron datasets. Common endotypes were identified across the three EOFAD mutations such as dedifferentiation of a mature neuron to a less differentiated quasi-neuron state and repression of mitochondrial function and metabolism. Integrative analysis allowed us to ascertain the master transcriptional regulators associated with these endotypes, including REST, ASCL1, and ZIC family members (activation), as well as NRF1 (repression). Our non-familial EOAD study showed a modest difference in expression profiling and a limited number of differentially expressed genes (DEGs) between diseased and control subjects. Using iPSC-derived neurons

demonstrated that EOFAD mutations share common regulatory changes within endotypes with varying severity, leading to the reversion to a less-differentiated neuron state. Extending the usage of these neurons to non-familial EOAD may not serve as ideal to study sporadic AD. Overall, we have demonstrated that human neuron modeling can be applied to different forms of EOAD to understand the disease etiology better.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.20/G4

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

Support: NIH/NINDS Grant RF1NS117486

Title: Transcriptomic Analysis of a CADASIL mouse model relevant to Alzheimer’s Disease

Authors: ***L. LJUNGQVIST BRINSON**, S.-H. CHOI, J. PARK, A. C. SILVA;
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Abstract: Several studies suggest an interplay between vascular dysfunction and Alzheimer’s Disease (AD). This dysfunction causes inadequate energy absorption and waste disposal leading to an increase in beta-amyloid buildup and oxidative stress in the brain, which further exacerbates the condition. But our understanding of the exact sequence of events is still unravelling. Here, we investigated how this relationship affects RNA expression and regulation by analyzing the transcriptome of a mouse model of cerebral small vessel disease involving Notch3 signaling, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Cortical Notch3$^{C456R\ +/-}$ (Heterozygous Knock-In) and $^{-/-}$ (Wildtype) mouse samples (n=4 for each genotype, 12 months) were sequenced and expression profiles including raw read counts were obtained. Transcripts were analyzed using RStudio. Transcript raw read counts were converted into Transcripts per Million (TPM) using transcript length, and Log2(TPM+1) was calculated to filter out transcripts which did not have any values above 3. After transcripts were filtered, raw read counts were analyzed through DESeq2 package. Adjusted p-value was used in significance calculations (p-adj $\geq$ 0.05), and 58 transcripts were identified. Transcript Heatmap was generated using the ComplexHeatmap package. Transcript Volcano Plot was generated using the EnhancedVolcano package (pCutoff = 0.05, FCcutoff = 1). Preliminary DESeq2 analysis showed that 58 transcripts were significantly differentially expressed in Notch3 Het samples compared to WT. Transcripts further filtered by Log_2 Fold Change yielded 10 upregulated and 13 downregulated transcripts. Genes associated

with the differentially expressed transcripts have been shown to play a role in cerebrovascular function, glucose homeostasis, lipid metabolism, and mitochondrial function. These preliminary results show promise into understanding how disruption of BBB function via Notch3^{C456R} causes changes in expression of transcripts involved in energy utilization. Future directions will include sequencing from Hippocampal and Thalamic regions which show enhanced beta-amyloid building in Notch3^{C456R} mice and will include samples from the 5xFAD strain and a strain of Notch3^{C456R} crossed with 5xFAD. This research was funded by NIH/NINDS grant RF1NS117486.

Disclosures: L. LjungQvist Brinson: None. **S. Choi:** None. **J. Park:** None. **A.C. Silva:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.21/G5

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

Support: NIH/NIA grant #AG061831 to PD

Title: Spatial transcriptomics identifies regional disruptions in brain circadian gene expression in a mouse model of Alzheimer’s disease

Authors: *A. GELBER[1], H. ROMERO[1], D. BURROWS[2], E. A. MUKAMEL[4], P. DESPLATS[3];
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Abstract: Disruptions in circadian rhythms are a common symptom of Alzheimer’s disease (AD), which occur early in disease progression and may contribute to the neurodegenerative process. However, the molecular mechanisms underlying pathological changes to circadian rhythms in AD is unknown. Here we used brain-wide spatial transcriptomics to characterize diurnal transcriptional rhythms across multiple brain regions, and investigate their progressive disruptions in a mouse model of AD.
Using Visium spatial transcriptomics, we investigated transcriptional changes with spatiotemporal resolution in APP23 transgenic mice, which overexpress human APP with the Swedish mutation. Sagittal brain slices from APP23 and littermate control mice (n=65) were collected at 4 Zeitgeber times. Slices were clustered into anatomical brain regions using the spatially aware community detection algorithm PRECAST. Finally, DESeq2 was used on pseudobulk clusters to perform harmonic regression to identify significantly rhythmic genes while controlling for covariates (sex, genotype, and age).
We first characterized rhythmic gene expression in non-transgenic animals. This revealed a set of common rhythmic genes across all major brain regions, including the core clock genes, as well as region-specific sets of rhythmic genes. Moreover, AD-associated brain regions, such as the

granular layer of the dentate gyrus, exhibited markedly more rhythmic genes than other brain areas.
We then examined changes to rhythmic gene expression in APP23 mice. We found a substantial increase in the number and amplitude of diurnally rhythmic genes in AD mice across the brain, particularly in the hippocampus and cortex. For example, Bdnf, a neurotrophic factor critical for learning and plasticity, exhibited reduced expression and became rhythmic in AD. Increased rhythmicity is apparent early in the onset of amyloid deposition and persists with increased pathology. Finally, we applied cellular deconvolution (CSIDE) to characterize cell-type specific changes to rhythmic gene expression, which identified several disease-associated cell types linked to circadian disruption in AD.
This study represents the first application of spatial transcriptomics to comprehensively characterize both brain-wide and regionally specific rhythmic gene expression patterns in non-transgenic and AD model mice. Our findings provide evidence of large-scale dysregulation of rhythmic gene expression associated with AD pathology, which identifies novel molecular pathways linked to circadian disruption early and late in AD and offers insights into the potential role of these disruptions in disease progression.

Disclosures: **A. Gelber:** None. **H. Romero:** None. **D. Burrows:** None. **E.A. Mukamel:** None. **P. Desplats:** None.

Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.22/G7

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

Support: NIH RF1AG063540
NIH RF1AG051437
Ellison Research Fund
Weill Neurohub
DIAN U19 AG032438

Title: Transcriptomic changes in peripheral immune cell populations associated with early-onset familial Alzheimer’s disease

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Abstract: More than forty million people worldwide are estimated to be afflicted by Alzheimer's disease (AD), and AD is the leading cause of disability and decreased health span in people over age 65. A subset of AD patients have deterministic variants in one of three genes: amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) or presenilin 2 (*PSEN2*), and are classified as having early-onset familial AD (EOFAD). Individuals with EOFAD develop symptoms earlier than those with late-onset AD (LOAD), but the two groups share pathological features, suggesting a similar underlying disease mechanism. EOFAD presents an important opportunity for understanding AD etiology and identifying biomarkers for early disease detection, as individuals with causative variants can be studied in a pre-symptomatic phase of disease. Here, we studied gene expression in the peripheral immune compartment in a cohort of *APP*, *PSEN1*, or *PSEN2* variant-carrying pre-symptomatic individuals (n = 24) and family member controls without disease-causing variants (n = 13). In parallel, we analyzed peripheral immune cell gene expression in a cohort of individuals with LOAD (n = 22) and age-matched controls (n = 9). In both cohorts, we used bulk RNA sequencing to analyze the gene expression of CD14+ peripheral blood mononuclear cells (PBMC). We found an effect of variant status on gene expression and biological pathways - including lipid metabolism and endolysosomal transport - in the EOFAD cohort, when compared to the familial controls. In the LOAD cohort, a subset of genes showed differential expression between AD cases and controls. Single-cell RNA sequencing (scRNAseq) in cryopreserved PBMCs in a cohort of 12 asymptomatic individuals with *PSEN1* variants and healthy controls was used to further explore the impact of variant status on all peripheral immune cell populations. We demonstrated changes in immune cell composition between AD variant carriers and controls as well as alterations in gene expression. The gene expression alterations are linked with biological pathways of leukocyte migration and oxidative stress. We are currently doubling this cohort to further explore these effects. If validated, these findings could help to elucidate immune activation that precedes symptomatic onset of Alzheimer's disease.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.23/G8

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

Support: AG072599
AG074004
AG061869
AG014449

AG067598
AG077103

Title: Epichaperomics: an approach to determine protein interaction dysfunctions and the impact of sex differences across the Alzheimer's disease spectrum

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Abstract: Background: Understanding the impact of sex differences across the Alzheimer's disease (AD) spectrum from no cognitive impairment to mild cognitive impairment to AD remains elusive. Network dysfunctions in memory and executive function circuits play a crucial role in dementia progression. This knowledge gap hinders therapeutic interventions, especially precision medicine, for mitigating stressors in individuals at risk for AD. While recent technologies have shed light on the molecular and genetic basis of sex differences in aging and AD, functional insights remain limited with descriptive 'omics data.
Hypothesis: To address this knowledge gap, we propose utilizing epichaperomics, an 'omics platform, to directly access protein-protein interaction (PPI) network perturbations and their functional outcomes across the AD spectrum. By applying epichaperomics to well-characterized postmortem human brains, we aim to uncover sex-dependent dysfunctions and gain insights into gender and phenotype relationships and associations with neuropathology not accessible through other 'omics platforms.
Results: We initiated epichaperomics studies in postmortem human brains across the AD spectrum to identify sex-dependent vulnerability mechanisms. Preliminary evaluations in AD patients revealed more severe PPI network perturbations in ApoE e4 carriers compared to e2/e3 carriers, with the influence of sex and ApoE genotype requiring further investigation on a larger cohort of well-characterized subjects. Epichaperomics analysis identified ~3,000 proteins with AD-specific or both AD-specific and sex-specific interactions. We also performed global proteomic analysis to assess protein stoichiometry, confirming that changes in connectivity observed through epichaperomics were independent of protein expression. Clear sex differences were observed in key brain functions, such as synaptic transmission, altered in AD, demonstrating the feasibility of epichaperomics and suggesting novel sex differences in specific pathways.
Conclusions: Epichaperomics is well-suited for identifying defects in key proteins, pathways, and networks including molecular mechanisms underlying sex differences in synaptic plasticity, brain bioenergetics, and vulnerable neural circuits during the onset and progression of AD. This 'omics approach is a valuable tool for gaining unbiased system-level insights into how sex differences influence vulnerability to stressors associated with each patient's disease, as it detects stressor-induced PPI dysfunctions and provides molecular insights for therapeutic intervention.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.25/G9

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

Support: NIH Grant AG076805
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NIH Grant AG064049
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NIH Grant AG066530

Title: The Multi-Scale Brain Logic of Alzheimer’s Disease

Authors: C.-T. LEE[1], A. HECK[2], E. LAI[1], B. KELLER-FELIX[1], K. YOUNG[2], A. ROSENBLOOM[2], E. BOOKER[1], A. WARDHANI[2], M. WALTER[2], G. ONG[2], R. KHAFIZOV[2], M. KORUKONDA[2], Z. LEWIS[2], T. PHAN-EVERSON[2], S. A. BONNETT[2], C. WILLIAMS[2], P. DANAHER[2], R. LIU[2], J. CEJA NAVARRO[2], C. KANG[2], J. REEVES[2], G. GEISS[2], D. W. RUFF[2], A. WILLIAMS[1], P. KOSURI[1], R. A. RISSMAN[3], A. HINIKER[4], J. M. BEECHEM[2], ***K.-F. LEE**[1];
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Abstract: We are interested in understating where and how Alzheimer’s Disease (AD) is initiated in the brain without any cognitive impairment-during a period called prodromal phase lasting 15-25 years in humans- before progressing to clinically diagnosed dementia. We believe selective vulnerability in cell bodies of both neurons and non-neuronal cells and/or synaptic sites is at the heart of this logic. A multi-model whole brain approach at multiple scales is needed to unlock this logic. Several lines of evidence led us to test the hypothesis in multiple AD models that selective vulnerability is initiated at the locus coeruleus and progresses to the entorhinal cortex followed by the hippocampus and the prefrontal cortex. Importantly, these four brain regions project to each other and multiple targets to form specific hubs. Thus, establishing atlas for both neurons and non-neuronal cells selectively susceptible to proteinpathies (e.g., Aβ plaques and Tau tangles) for these four brain regions will be one of steps toward uncover underlying disease mechanisms. Taken together, spatial transcriptomics and proteinomics are crucial in the multi-scale approach to unlock the logic. Here we employ the CosMx^{TM} Spatial Molecular Imager (SMI) platform for high plex detection of 6000 RNAs and 40 proteins in the same FFPE human brain sections in spatial context. This proteogenomic assay involves first detecting proteins with oligonucleotide barcode-conjugated antibodies and then exposing sections to protease digestion and detecting RNAs with barcoded RNA probes. Detection of each analyte relies on barcode readout on the SMI instrument via several rounds of reporter binding and fluorescence imaging utilizing universal SMI readout reagents that work for both RNA and

protein assays. With the SMI proteogenomic approach it is ultimately possible to use high plex protein data from a large area of tissue to identify smaller regions of interest on the same slide for 6000-plex RNA profiling, which offers a more comprehensive view of the neurobiological picture in healthy vs. diseased brain sections. Whereas the protein panel focuses on neural cell typing and neurodegenerative disease pathology (including phospho-tau variants, amyloid beta, and amyloid precursor protein), the ultra-high plex RNA panel includes >4900 neuroscience-related genes, covering >80 pathways and enabling robust neuronal and glial cell typing as well as exploration of key ligand-receptor interactions. Thus, through the proteogenomic approach, we demonstrate the use of SMI to create a spatial atlas of vulnerable brain cell types, define neighborhoods, and probe numerous pathways and cellular phenotypes.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.26/G10

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

Support: K99AG065645
R00AG065645

Title: A multi-omics approach to investigate the synapse dysfunction in Alzheimer’s disease

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Abstract: Background: Synapse dysfunction is an early event of Alzheimer’s disease (AD). It is caused by multiple cellular and pathological factors such as Amyloid beta, p-tau, inflammation, and aging. However, the exact molecular mechanism of synapse dysfunction in AD is largely unknown. Therefore, to understand the molecular basis of synapse dysfunction in AD, we conducted a high throughput multi-omics analysis of the synaptosome fraction in postmortem brain samples from AD patients and cognitively normal individuals. **Methods**: We extracted synaptosomes from the postmortem brain of healthy control (HC) individuals (n=14) and AD patients (n=27). We then performed miRNA and mRNA high throughput analysis on synaptosomal RNAs. Additionally, we used synaptosome samples from HC (n=10) and AD

(n=10) individuals for mass spectroscopy proteomic analysis. Furthermore, we used an integrated transcriptomic and proteomic approach to understand the molecular interactions of deregulated synapse miRNAs, mRNAs, and proteins at the AD synapse. **Results**: MiRNA high throughput analyses showed significant deregulation of several miRNAs and identified several novel potential candidate miRNAs in the synaptosome fraction in AD compared to HC. Similarly, several protein-coding and novel transcripts were also deregulated in AD compared to HC synaptosomes. The mass spec analysis showed significant deregulation of several synaptic proteins in AD compared to HC synaptosomes. Further multi-omics analysis of synapse miRNAs-mRNAs-proteins revealed the involvement of omics targets in several biological processes and molecular functions such as signal transduction, protein binding, GABAergic synapse, and synaptic vesicle cycle, etc. **Conclusion**: Our study unveiled synapse-centered novel omics candidates that could be potential synapse biomarkers and therapeutic targets for synapse dysfunction in AD.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.27/H1

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

Support: IDeA under NIGMS under NIH #P20GM103408
COBRE under NIGMS under NIH #P20GM109095

Title: Physical Exercise Induces Changes in Hippocampal DNA Methylation Patterns of Transgenic Alzheimer’s Disease Rats

Authors: *A. SCHULLER[1], S. HALL[2], L. MONTROSE[1];
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Abstract: Physical exercise has been shown to have neuroprotective effects across the population, including for Alzheimer’s disease (AD) patients. While the benefits of regular activity are well established in cardiovascular, immune, and metabolic systems, the molecular mechanisms underlying this phenomenon in the central nervous system remain elusive. Here, we sought to explore the effects of regular physical exercise on DNA methylation patterns in the hippocampus of an AD transgenic rat model, Tg-F344, which expresses amyloid-ß and tau pathology, micro- and astro-gliosis, and loss of neurons in the hippocampus and cortex. Following treadmill training for one hour/day, 5 days/week from 12 to 16 months of age, male and female AD rats were sacrificed and DNA was extracted from bilateral hippocampus (n=4/group). Reduced-representation bisulfite sequencing was utilized to examine DNA

methylation profiles between exercised and control groups, not comparing across sex due to baseline DNA methylation variance. Differentially methylated regions were identified and annotated to genes using a standard bioinformatics approach. 104 genes were associated with differentially methylated CpGs in exercised females relative to controls and 93 genes were associated with differentially methylated CpGs in exercised males relative to controls. These annotated genes were then mapped using Gene Ontology and KEGG pathways analysis. Pathways for response to hormone, endogenous stimulus, organic substance, chemical, as well as protein and receptor binding were associated with differentially methylated CpGs in exercised females relative to controls. Alternatively, pathways for cellular protein modification, protein modification process, macromolecule modification, protein phosphorylation, metal ion binding, cation binding, and calcium binding were associated with differentially methylated CpGs in exercised males relative to controls. The results of these analyses demonstrate that physical exercise affects DNA methylation patterns in AD transgenic rats at pathways which are implicated in disease pathogenesis, although there may be different effects between male and female subjects in this context. Future directions for this work will include examination of DNA hydroxymethylation using novel epigenetic assessment techniques such as nanopore sequencing.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.28/H2

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

Support: U54AG054345
U54AG054349

Title: Model Ad Explorer: An Interactive, Open Access Dashboard for Exploring Phenotypic and Gene Expression Data from Alzheimer's Mouse Models

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Abstract: The search for a suitable mouse model that accurately represents the complexities of Alzheimer's disease (AD) in humans is a major obstacle to the progress of fundamental research findings in the field. The identification of ideal model systems faces many challenges including

the absence of standardized phenotypic measures, the absence of mice that completely replicate late-onset human disease characteristics, and various licensing requirements. In response to these challenges, the MODEL-AD consortium has successfully engineered novel strains of mice that provide a closer representation of late-onset, sporadic Alzheimer's Disease (AD). These mouse models undergo a standardized and rigorous pipeline to phenotype mice at multiple ages, and are readily available to the research community, without any limitations or restrictions. Furthermore, the data collected from these models is made openly accessible through the AD Knowledge Portal. In order to streamline the assessment of phenotypic data and models, we have created the Model AD Explorer (https://modeladexplorer.org/), an interactive dashboard designed to facilitate the exploration of summarized phenotypic data. The explorer includes a feature that enables the comparison of gene expression between mouse models and human cases of Alzheimer's disease (AD). Users can access information on the Jackson Laboratory website for any mouse models of interest. Future updates to the Model-AD Explorer will encompass new phenotypic data from supplementary MODEL-AD mouse strains. To further standardize analyses, we developed an RNAseq pipeline to process RNAseq data from the MODEL-AD consortium housed on the AD Knowledge Portal. To account for CRISPR/Cas9 gene edits, a customized mouse Ensemble reference genome (https://useast.ensembl.org/Mus_musculus/Info/Index) was modified and benchmarked using the Reform tool for CRISPR/Cas9 reference genome editing (https://gencore.bio.nyu.edu/reform/) and expanded to allow for multiple gene edits across the MODEL-AD project’s lifetime (https://github.com/Sage-Bionetworks/customReferenceMODEL-AD/blob/main/createCustomReference.py). By utilizing a customized version of Nextflow Tower to fit the needs of sensitive biomedical data at Sage Bionetworks, MODEL-AD fastq samples are processed in the cloud via AWS Batch using the rnaseq nf-core Nextflow pipeline (https://nf-co.re/rnaseq). Differential gene expression analysis is performed in sex, age, and tissue stratifications between modified and control mouse genotypes from automated metadata pulling. Outputs will be incorporated into the Model AD Explorer.

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Poster

PSTR527. Alzheimer's Disease and Dementia: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR527.29/H3

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

Support: NIH/NIA 1R01AG069433

Title: Single-nuclei analysis of hippocampi from wild-type mice after intracerebroventricular injection of neural stem cell-derived exosomes

Authors: *S. SAIEVA[1], J. GUPTARAK[1], W.-R. ZHANG[1], K. M. JOHNSON[2], M.-A. MICCI[2], G. TAGLIALATELA[1];
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Abstract: Neural stem cells (NSC) and adult neurogenesis modulate synaptic plasticity and cognitive function. NSC are also known to secrete exosomes, namely microvesicles containing cell-specific cargos of biomolecules, that are taken up by other cells, thus modulating their function. Also, exosomes are considered a fundamental mechanism of communication within CNS with roles in homeostasis and plasticity. Alzheimer's Disease (AD) is characterized by a reduced number of NSC, while improvement of learning and memory processes are associated with NSC increase. However, an increased neurogenesis does not correlate with preserved cognition, thus indicating that mechanisms involving increased numbers of NSC promote memory preservation. We and others have shown that NSC-derived exosomes (NSCexo) injected in brains of mice ameliorated the cognitive decline and decreased the binding of toxic oligomers to synapses. Our hypothesis is that the maintenance of a sufficient number of NSC allows the secretion of an adequate amount of NSCexo, that in turn deliver their protective action through the release of their content in CNS. The exact mechanisms by which NSCexo provide protection to synapse is still under investigation: preliminary deep-sequencing analyses show that elements of CNS immune response seem to modulate NSCexo-mediated neuroprotection, in particular microglia. When we injected fluorescent-labeled NSCexo in mice brain we observed that, in the hippocampus, microglia seem to engage with exosomes consistently throughout the hippocampus, compared to neurons and astrocytes, thus suggesting their involvement in activating mechanisms that lead to neuronal and synaptic protection. Notably, NSCexo induce microglia activation, measured by levels of CD68 immunostaining. Next, we sought to identify specific genes regulated by NSCexo that may be responsible for the cognitive resilience and the overall improvement of CNS function by investigating single-nuclei gene expression on hippocampi isolated from mice injected with NSCexo, compared to not-injected mice. These investigations allowed us to determine cell-specific changes in gene expression after NSCexo injection, thus indicating which cellular mechanisms are more likely to drive exosomes-mediated neuroprotection.
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Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.01/H4

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

Support: FONDECYT 1200908

Title: Changes in the cGAS-STING1 pathway induced by β-Amyloid oligomers to conditionate the IR stress and synaptic dysfunction.

Authors: *O. FLORES, J. PANES, O. RAMIREZ MOLINA, R. DURAN, M. MEZA, J. GAVILAN, J. FUENTEALBA, P. A. GODOY;
Fisiología, Univ. of Concepcion, Concepción, Chile

Abstract: One of the main pathological agents postulated as responsible for Alzheimer's disease (AD) is the beta-amyloid peptide (Aβ), to which different toxic effects have been attributed, such as decreased cytosolic ATP levels, increased levels of intracellular calcium ions and Reactive Oxygen Species (ROS). In addition, data from our laboratory have shown that Aβ-peptide treatments in PC-12 cells and primary hippocampal culture induce changes in mitochondrial morphology and dynamics. Both, increased ROS production and changes in mitochondrial morphology. can culminate in different mitochondrial elements leaking into the cytoplasmic milieu, such as mitochondrial DNA (mtDNA). It has been described that mtDNA can trigger inflammatory reaction through the enzyme cyclic GMP-AMP synthase (cGAS) and the interferon response stimulator cGAMP-interactor 1 (STING1). cGAS recognizes the mtDNA present in the cytoplasm, this triggers the production of cyclic GMP-AMP second messenger (cGAMP) which promotes the activation of STING1 at the interface between the endoplasmic reticulum and the Golgi apparatus and ultimately the production of proinflammatory cytokines to the extracellular milieu. The aim of this work was to study the effect of Aβ peptide on the activation of the cGAS-STING1 pathway using SH-SY5Y cells differentiated to neurons and primary culture of mice hippocampal neurons. Importantly, in our hands we observed that cGAS is predominantly found in neurons that the glial cells, interestingly most located in the cell nucleus. Furthermore, chronic treatments with Aβ peptide (0.5μM, 24 and 48h) induced changes in cGAS distribution (changes on cytosol/nucleus ratio: 2/1) compared to that obtained in untreated conditions, while the cGAS expression shown to increase a time dependent manner near to 20-30%. Our results suggest that Aβ peptide may modulate cGAS activity in neurons and thereby contribute to the proinflammatory environment, which could open new windows in the understanding of inflammatory events associated with AD.

Disclosures: O. Flores: None. **J. Panes:** None. **O. Ramirez Molina:** None. **R. Duran:** None. **M. Meza:** None. **J. Gavilan:** None. **J. Fuentealba:** None. **P.A. Godoy:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.02/H5

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

Support: NIH/NIA 1R01AG061891-01A1
NIH/NIA 1R01AG062547
NIH/NIA 1R01AG071496

Title: cGAS-STING axis regulates aβ42-induced tau pathology in 3D human neural cell culture models

Authors: *T. R. KALATTURU[1], S. KWAK[1], M. HEBISCH[1], S. C. SINHA[2], R. E. TANZI[1], D. KIM[1];
[1]Dept. of Neurol., Genet. and Aging Res. Unit, Inst. for Neurodegenerative Diseases, Massachusetts Gen. Hospital, Harvard Med. Sch., Charlestown, MA; [2]Weill Cornell Med. Col., New York, NY

Abstract: Alzheimer's disease (AD) is a growing public health concern affecting more than 55 million people worldwide. Previously, our laboratory developed three-dimensional (3D) human neural cell culture models of Alzheimer's disease, recapitulating robust amyloid-β42 (Aβ42) (amyloid plaque-like) and Aβ42-triggered hyperphosphorylated tau (neurofibrillary tangle-like) accumulation and aggregation. In the follow-up transcriptomic analysis, we found that Aβ42 accumulation increased a group of genes coding for antiviral response proteins of the cGAS-STING pathway in human neural cells. To investigate the role of the cGAS-STING signaling axis on regulating AD pathology, we treated chemical modulators of cGAS-STING signaling (2′3′ cyclic GMP-AMP (cGAMP), activator; (TDI-8246) cGAS inhibitor) into 3D AD models with high Aβ42 accumulation for 6 weeks. We found that cGAMP treatment dramatically increased tau phosphorylation and accumulation, while the cGAS inhibitor treatment decreased tau pathology without significantly affecting Aβ42 levels. We also found that Aβ42 accumulation induced release of mitochondrial double-stranded DNAs (dsDNAs) into the cytosol in 3D-differentiated human neural cells. Since cytosolic dsDNA is known to trigger the cGAS-STING signaling axis in cells, our data strongly suggest a pathogenic cascade that Aβ42 accumulation leading to mitochondrial damages, cytosolic ds-mtDNA release, cGAS-STING activation, and tau phosphorylation and accumulation in 3D human neural cells. We are currently studying the precise molecular mechanism of how the cGAS-STING signaling axis regulates Aβ42-induced tau pathology in human neurons.

Disclosures: T.R. Kalatturu: None. **S. Kwak:** None. **M. Hebisch:** None. **S.C. Sinha:** None. **R.E. Tanzi:** None. **D. Kim:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.03/H6

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

Support: AGCF was supported by National Council of Science and Technology as part of the PhD program “Doctorado en Ciencias Biologicas y de la Salud, UAM”, with CVU number: 888624

Title: Evaluation of mitochondrial function and oxidative stress in the hippocampus and cerebral cortex of rats with obesity induced by two hypercaloric diets

Authors: *C. AGUILAR-GAMAS[1,2,3], E. MARTINEZ-ABUNDIS[1], N. GOMEZ-CRISOSTOMO[1], E. DE LA CRUZ-HERNADEZ[1], N. LOPEZ-DIAZGUERRERO[2];
[1]Lab. de investigacion en enfermedades metabolicas e infecciosas, Univ. Juarez Autonoma De Tabasco, Comalcalco, Mexico; [2]Dept. de ciencias de la Salud, [3]Doctorado en Ciencias Biológicas y de la Salud, Univ. Autónoma Metropolitana, Ciudad de Mexico, Mexico

Abstract: Evaluation of mitochondrial function and oxidative stress in the hippocampus and cerebral cortex of rats with obesity induced by two hypercaloric diets. Aguilar-Gamas C.F[1,2,3], Gómez-Crisostomo[1], N.P, Lopez-Diazguerrero N.E[2], de la Cruz-Hernández E[1], Martínez-Abundis E[1]*. [1]Universidad Juárez Autónoma de Tabasco, Multidisciplinary Academic Division of Comalcalco. Laboratory for Research in Metabolic and infectious diseases; [2]Universidad Autónoma Metropolitana Iztapalapa, Health Sciences Department. [3]Doctorado en Ciencias Biológicas y de la Salud, UAM. México. * lulimtz@yahoo.com.mxObesity mainly results from a sedentary lifestyle and excessive consumption of sugars and fat; consequently, people may undergo hyperglycemia, hypertension, and dyslipidemia, propitiating an environment of oxidative stress among mitochondrial dysfunction in the central nervous system. For this study, we fed weaned male Wistar rats recently with a high sucrose (HSD) or a high-fat content diet (HFD) for 12 months. The body weight, blood pressure, glucose tolerance, spatial memory, and abdominal fat percentage were determined. Subsequently, mitochondria were isolated from the hippocampus (HP) and cerebral cortex (CC) for the measurement of the mitochondrial respiratory chain (MRC) complexes activity and protein amount. In contrast, oxidative stress was determined as protein carbonylation and TBARS in mitochondria and homogenates from HP and CC. After 12 months of hypercaloric diets consumption, animals from both groups developed obesity, low glucose tolerance, and hypertension. Additionally, the brain mass of these rats was lower than the Control group, whereas their spatial memory was impaired. According to the MRC, in HP, increased activity of complex I was observed in both groups; nonetheless, greater activity in complex II was detected only in the HSD group, while in CC, the activity of complex I increased, and complex III decreased in the HSD group. It is important to note that activity changes did not correlate with changes in the protein quantified. Finally, an increase in protein carbonylation was observed in the HP mitochondria from both HSD and HFD groups, but only in the homogenate from HFD, whereas carbonylation increased only in the CC mitochondria of HSD. In conclusion, consumption of both hypercaloric diets induced obesity, decreased brain mass, and cognitive impairment. However, the alterations in either activity and amount of the MRC complexes and the state of oxidative stress were more extended in the HSD group, suggesting the consumption of high sucrose diet may be worse for the CNS than a HFD diet.

Disclosures: C. Aguilar-Gamas: None. **E. Martinez-Abundis:** None. **N. Gomez-Crisostomo:** None. **E. de la Cruz-Hernadez:** None. **N. Lopez-Diazguerrero:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.04/H7

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

Support: JPMJSP2153

Title: Identification of small molecule inhibitors of amyloid beta aggregation from a mushroom library using quantum dot imaging

Authors: *T. GEGEN[1], M. SWAMY[2], R. SASAKI[1], K. SHIMAMORI[1], M. KURAGANO[1], K. MONDE[2], K. TOKURAKU[1];
[1]Muroran Inst. of Technol., Muroran city, Japan; [2]Hokkaido Univ., Sapporo, Japan

Abstract: Alzheimer's disease (AD) is a brain disorder that results in loss of memory and cognitive function. Enormous costs of AD care have long been a social issue. The amyloid cascade hypothesis postulates that AD is caused by abnormal accumulation of amyloid β (Aβ) aggregates in various areas of the brain. Therefore, it is important to find inhibitors to prevent the aggregation of Aβ. In recent years, with the spread of foods with functional compounds, attention has been focused on the health effects of foods. Our group previously reported daurichromenic acid from *rhododendron dauricum* as a moderately potent inhibitor of Aβ aggregation from plant library. In this study, we chose a small library of mushrooms (over 200) to identify potent Aβ aggregation inhibitors. To screen mushroom extracts, we used quantum dot imaging based automated real-time microliter-scale high throughput screening (MSHTS) system. Out of 212 mushroom extracts, 11 extracts showed Aβ aggregation inhibition among them *Ganoderma applanatum* and *Fuscoporia obliqua* showed better inhibition activity. We went ahead to perform MSHTS assay guided fractionation of methanol extract *Ganoderma applanatum* (200 grams). Initially, methanol extract was fractionated using polar and non-polar solvents. Ether and ethyl acetate fractions displayed Aβ aggregation inhibitory activity. The ether fraction was subjected to sub fractionation using silica gel column chromatography followed by MSHTS assay. Finally, we were able to get active fraction with inhibitory activity and EC_{50} is 0.00718 ± 0.00045 mg/ml. Currently, the final active fraction is analyzing using ^{1}HNMR, ^{13}C NMR spectroscopy and high-resolution mass spectrometry for structural characterization. In future, after getting a new Aβ aggregation inhibitor, we would like to perform structural activity relationship studies and also elucidate the mechanism behind its inhibitory activity.

Disclosures: T. Gegen: None. **M. Swamy:** None. **R. Sasaki:** None. **K. Shimamori:** None. **M. Kuragano:** None. **K. Monde:** None. **K. Tokuraku:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.05/H8

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

Support: NIH Grant R21AG061674-01
Garrison Family Foundation

Title: The role of mitochondria and autophagy/mitophagy system in Alzheimer's disease progression in APP23 and Tau-P301L transgenic mouse models.

Authors: *M. MANCZAK, X. YIN, D. BURUGU, J. LAWRENCE, V. NEUGEBAUER; Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Mounting evidence suggests that Aβ and phosphorylated Tau promotes mitochondrial dysfunction and synaptic damage that are largely involved in Alzheimer's disease (AD) pathogenesis and progression. Mitophagy constitutes a key cellular pathway in mitochondrial quality control. Failure in the removal of dysfunctional mitochondria induces cellular stress and is linked to human diseases such AD. The failure of mitochondria function and mitophagy-autophagy- lysosomes systems are closely linked to AD progression, which is why we studied mitochondria and autophagy/mitophagy pathway in APP23 mice carrying human Swedish double mutation (APP751*K670N/M671L) and in Tau mice carrying human (TauP301L) mutation. Using real-time PCR, immunoblotting, and immunostaining analyses, we measured genes related to the mitochondrial dynamics, mitochondrial biogenesis, mitophagy, and mitophagy receptors and adaptors in both transgenic mice models. We also estimated mitochondria function by quantifying energy level and ROS production. APP23 mice exhibited deficiency of Pink1 and Parkin1 suggesting that amyloid pathology may closely correlate with Pink-Parkin-dependent pathway, which selectively identifies terminally damaged mitochondria for elimination via the mitophagy-lysosomal pathway.In Tau-P301L mice we observed earlier deficits in mitochondrial function compared to APP23 mice. Tau mice also showed strong downregulation of Parkin1, suggesting that Tau pathology is mediated by distinct mechanisms, predominantly by a Parkin-dependent pathway. Further investigations are required to clarify the precise mechanisms between Amyloid/Tau pathology, mitochondrial dysfunction, and autophagy-mitophagy machinery and their pathological roles in AD progression.

Disclosures: M. Manczak: None. **X. Yin:** None. **D. Burugu:** None. **J. Lawrence:** None. **V. Neugebauer:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.06/H9

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

Support: EY032488 (XF)
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the James Fickel Alzheimer's Disease Research Fund (XF)
the National Eye Institute, Center Core Grant for Vision Research (P30EY031631) at Augusta University

Title: Amyloid beta accumulation and glutathione homeostasis disruption synergistically triggers neuronal cell death

Authors: *K. RADEEN[1,2], C. HAO[1], Z. WEI[1], X. FAN[1];
[1]Cell. Biol. and Anat., Med. Col. of Georgia at Augusta Univ., Augusta, GA; [2]Natl. Inst. of Biotech., Dhaka, Bangladesh

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by Aβ plaques and neurofibrillary tangles, with aging being the primary risk factor. Age-related accumulation of oxidative stress is considered a major causative factor in AD and other neurodegenerative disorders. Glutathione (GSH), the most abundant antioxidant in the body, declines in the brain with age and is further depleted in patients with AD and dementia. Despite this, the molecular mechanisms of GSH in brain aging and AD are not fully understood. This study aimed to investigate neuronal-like cell death under conditions that mimic brain aging. The study utilized SH-SY5Y neuroblastoma cells either treated with synthetic β amyloid oligomers or having an inducible overexpression of APP695 and APP Swedish/Indiana ($APP_{sw/Ind}$) mutations. The level of Aβ1-42 and Aβ1-40 was monitored using ELISA and western blot, while intracellular GSH synthesis was disrupted by deleting the *de novo* synthesis enzyme GCLC via Crispr/Cas9 or inhibiting GCLC activity with buthionine sulfoximine (BSO). Cell death and viability were evaluated through cell morphology imaging and the cell counting kit-8 (CCK8). The mechanism of cell death was further evaluated using caspase inhibitor (Z-VAD-FMK) and lipid peroxidation inhibitor (Ferrostatin-1 and Liproxstatin-1), while C11-BODIPY was used to measure cell membrane lipid peroxidation. After 48 hours of DOX-induced expression, $APP_{sw/Ind}$-SH-SY5Y cells produced more than 100-fold of Aβ1-42 compared to APP695-SH-SY5Y cells. Treatment with 350μM BSO resulted in cell death in $APP_{sw/Ind}$-SHSY5Y cells 36 hours later, but not in APP695-SH-SY5Y cells. Cell viability analysis showed that 46 hours after BSO treatment, $APP_{sw/Ind}$-SHSY5Y had over 75% reduction in cell viability compared to APP695-SH-SY5Y cells. Interestingly, lipid peroxidation inhibitors, Ferrostatin-1 (Fer-1) and Liproxstatin-1 (Lip-1), largely rescued cell death, restoring cell viability to 76% and 74%, respectively. Activated caspase 3 was not detected, and the caspase 3 inhibitor Z-VAD-FMK failed to prevent BSO-induced cell death in $APP_{sw/Ind}$-SHSY5Y cells. However, co-treated with N-acetylcysteine (NAC) prevented cell death. These findings suggest that the accumulated Aβ and decreased intracellular GSH may trigger neuronal-like cell death via lipid peroxidation-mediated mechanisms.

Disclosures: K. Radeen: None. **C. Hao:** None. **Z. Wei:** None. **X. Fan:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.07/H10

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

Title: Acute toxicity of amyloid beta oligomers involves chronic hyperphosphorylation of Tau: evidence from in vitro and in vivo models.

Authors: *N. CALLIZOT, A. HENRIQUES, L. ROUVIÈRE, B. EL WALY, S. LIEGER, E. STEIDL, P. POINDRON;
NeuroPharmacology, Neuro-Sys, Gardanne, France

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease with no cure. It is the most common cause of dementia. People affected with AD show disorientation, cognitive impairment, memory loss, behavioral changes. AD is characterized by the deposition of extracellular senile plaques. The field has now reached a general consensus that soluble, non-fibrillar amyloid beta (Aβ) oligomers (AβOs) are the primary neurotoxic molecular assemblies responsible for the cognitive dysfunction and progressive neurodegeneration that occur in AD. AβO-induced neurotoxicity is characterized by sequential loss of synapses, followed by axonal damage and neuronal death. Mice at 18-month of age were bilaterally injected with a solution of Aβ1-42 containing a defined proportion of oligomers, in the CA1 area of the hippocampus. Short- and long-term spatial memories were evaluated several days after injury in the Y-maze and in the Morris water maze test. Toxicity of AβO was histologically investigated over the time (up to 6 weeks) on mitochondrial stress, the hyperphosphorylation of Tau protein, neuroinflammation and neuronal death. In parallel, same AβO preparation was applied on primary culture of hippocampal neurons and the sequential toxic events were carefully studied over the first 48h with a focus on the mitochondria, the activation of GSK3b and the lysosomal burden. After 1 week, we showed that AβO-injected mice displayed clear deficit in spatial memory and in learning (assessed respectively by Y-maze and MWM), these deficits were observed over the 6 weeks of study. Neurodegenerative processes (loss of synapses followed by neuron death) were evidenced by immunostaining and associated with a large activation of microglial cells and astrocytes. In culture, primary hippocampal neurons injured with AβO showed early mitochondrial stress and lysosomal burden with hyperphosphorylation of Tau. Over time, progressive neuronal loss and neuroinflammation were evidenced, translating spreading of the hippocampal lesion. In addition, histopathology was associated with increased NfL levels in the CSF of AβO-injected animals. A chronic treatment with donepezil was able to mitigate cognitive deficits and neuronal loss. Altogether, intra-hippocampal injections of AβO in aged mice led to behavioural and histologic phenotypes highly similar to pathological hallmarks of Alzheimer's disease. This new animal model of Alzheimer's disease represents a valuable tool for studying the toxicity of AβO and evaluate protective effects of drug candidates.

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Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.08/I1

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

Support: NSERC N00675

Title: Effects of soluble amyloid beta$_{(1-42)}$ on evoked synaptic responses and AMPA receptor phosphorylation in the entorhinal cortex

Authors: *M. E. SUVANTO, L. M. BUYNACK, O. OLAJIDE, C. A. CHAPMAN;
CSBN, Concordia Univ., Montreal, QC, Canada

Abstract: The earliest symptoms of Alzheimer's Disease (AD) include deficits in episodic memory and spatial navigation, both likely tied to neurodegeneration first occurring in layer II of the entorhinal cortex (EC; Findley et al., 2019; Guo et al. 2010; Braak & Braak, 1998; Kocahan & Doğan, 2017). In the initial stages of AD, toxic accumulation of soluble amyloid beta peptides (Aβ) is thought to contribute to the onset of neurodegeneration (Selkoe and Hardy, 2016). In the hippocampus, application of soluble Aβ promotes activation of NMDA glutamate receptors and can enhance excitatory synaptic transmission by facilitating postsynaptic Ca^{2+} influx (Findley et al., 2019; Rudy et al., 2015; Li et al., 2009; Kamenetz et al. 2003; Mucke et al., 2000). Increased postsynaptic Ca^{2+} can lead to the phosphorylation of AMPA receptors which can enhance synaptic strength, and prolonged increases can contribute to excitotoxic cell death. Aβ-mediated excitotoxicity and the effects of Aβ on excitatory transmission have been studied extensively in the hippocampus, but less is known about the effects of Aβ on excitatory synaptic transmission in layer II of the EC where early degeneration also occurs. Previously we have shown that incubating acute horizontal brain slices from in 100 nM Aβ$_{(1-42)}$ (n=24) versus a dimethyl sulfoxide control (n=25) for 45 minutes to 3 hours results in an enhancement of field excitatory postsynaptic potentials (fEPSPs) in the rat medial entorhinal cortex. All experiments were blinded. Co-incubation of slices in Aβ and the NMDA receptor blocker D-AP5 (50 μM; n=13 vs n=12 control) blocked the effect, showing that activation of NMDA receptors was required for the facilitation of synaptic strength. The facilitation of synaptic responses induced by Aβ likely results from activation of postsynaptic NMDA receptors that enhance Ca^{2+} influx, leading to the phosphorylation of AMPA receptors via activation of CaMKII. We have assessed tissue samples of the medial EC using Western blotting to measure the expression of CaMKII, AMPA receptors, and phosphorylated AMPA receptors following a one-hour incubation in either 100 nM Aβ$_{(1-42)}$ (n=6) or a dimethyl sulfoxide control (n=6). Preliminary data suggest an effect of Aβ on AMPA receptors, and current experiments are assessing alterations in AMPA receptor phosphorylation, CaMKII expression, and the dependence of these changes on the activation of NMDA receptors.

Disclosures: M.E. Suvanto: None. **L.M. Buynack:** None. **O. Olajide:** None. **C.A. Chapman:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

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Program #/Poster #: PSTR528.09/I2

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

Support: R01AG067048

Title: Arf5 regulates amyloid β oligomer-induced and tau-dependent activation of mTORC1 at the plasma membrane

Authors: *T. KIM[1], G. S. BLOOM[2], A. NORAMBUENA[3];
[2]Univ. of Virgina, [1]Univ. of Virginia, Charlottesville, VA; [3]Univ. of Virgina, Charlottesville, VA

Abstract: Alzheimer's disease (AD) is characterized by the accumulation of two types of insoluble fibrillar deposits in the brain—extracellular amyloid plaques composed of amyloid-β (Aβ) peptides and intraneuronal neurofibrillary tangles comprising the microtubule-associated protein, tau—as well as massive synaptic dysfunction and neuron death. A fraction of the neuron loss in AD occurs when neurons that are normally post-mitotic ectopically re-enter the cell cycle without dividing and eventually die. Our group has previously described that extracellular Aβ oligomers (xcAβOs) trigger neuronal cell cycle re-entry by activating mTORC1 at the plasma membrane (PM) in a tau-dependent manner (Norambuena, et al. 2017. Alzheimer's Dement 13: 152-167). However, the molecular mechanisms by which tau allows xcAβO-mediated activation of mTORC1 at the PM have not been identified. ADP-ribosylation factors (Arfs) are a family of 6 small GTPases (Arf1-6) regulating membrane trafficking, organelle structure and the cytoskeleton. Of these, Arfs 1, 4 and 5 have been reported to impact mTORC1 activity. Particularly, Arf5 was recently identified as a regulator of mTORC1 activity at the PM (Makhoul, et al. 2023. Mol Biol Cell 34: ar23) and as a potential interacting partner of phosphorylated tau (Radford, et al. 2023. J Neurochem 165: 563-586). Thus, we hypothesize that tau localization at the PM functionally interacts with Arf5 to facilitate xcAβO-mediated activation of mTORC1. xcAβO-mediated activation of mTORC1 was analyzed by western blots in NPC-derived human neurons expressing either WT full-length (2N4R) tau (Tau-WT) or WT full-length Tau fused to the PM targeting signal of HRas (Tau-HRas), which forces localization of tau at the PM. Baseline mTORC1 activity (measured as the change in the phospho-content of the ribosomal S6 protein) was ~3-fold higher in neurons expressing Tau-HRas compared to neurons expressing Tau-WT. Interestingly, xcAβO-mediated activation of mTORC1 increased by ~0.3-fold in Tau-WT expressing neurons compared to nearly 6-fold in neurons expressing Tau-HRas. Importantly, lentiviral-mediated delivery of 2 independent shRNA sequences targeting Arf5 completely blocked xcAβO-mediated activation of mTORC1 in Tau-WT and Tau-HRas expressing cells. Additionally, we are currently investigating the specific Tau domains involved in the process and performing mass spectrometry to identify additional Tau-HRas-interacting partners. Thus, Arf5 mediates xcAβO-induced and Tau-dependent activation of mTORC1 at the PM.

Disclosures: T. Kim: None. **G.S. Bloom:** None. **A. Norambuena:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.10/I3

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

Support: NIH R01 AG068215
NIH R01 AG068215-03S1
NIH T32 AG078110

Title: Sleep and circadian rhythms: insights into the relationship between amyloid-β pathology, biological sex, and Alzheimer's disease

Authors: *C. E. JOHNSON, V. BUZINOVA, L. GUO, S. BARTH, S. TURTON, S. PADGETT, T. JESSUP, H. WHITLOCK, T. MACHEDA, K. KOHLER, E. MANAUIS, B. OHARA, S. SUNDERAM, A. BACHSTETTER, M. J. DUNCAN, M. P. MURPHY;
Univ. of Kentucky, Lexington, KY

Abstract: Plaques composed primarily of the amyloid-β (Aβ) peptide are a defining neuropathology of Alzheimer's Disease (AD). It has been known for some time that there is a connection between disturbances in sleep and circadian rhythms that relates to the development of AD, and that this relationship may be driven primarily through Aβ. Recent studies from our group have demonstrated a range of interactions between biological sex, sleep-wake rhythm fragmentation, and AD neuropathology in multiple mouse lines. In this study, we focused on an APPxPS1 knock-in (KI) line, as they express the amyloid precursor protein at normal levels under the normal pattern of expression. Sleep was recorded from wild type (WT) and KI mice, of both sexes, using PiezoSleep cages (Signal Solutions LLC) for a minimum of one week (N = 127). An additional cohort of mice (N=128) were acclimated to a 12:12 light:dark cycle for two weeks, and then switched to housing in continuous darkness (D:D) for 24-48 hours. Animals were euthanized in groups of 16 at 3 hour intervals starting after the first 24 hours in D:D. In both studies, the numbers of male and female mice, WT and KI, were approximately the same. Aβ was measured in different fractions via custom 384-well ELISAs. Female mice slept less than male mice, had higher amounts of Aβ pathology, and were more susceptible to a manual sleep fragmentation protocol, exhibiting greater amounts of rebound sleep. A daily rhythm of Aβ was detected only in the most soluble extractable fraction, reached a daily minimum at 11 am (clock time), and peaking at 11 pm. Although the Aβ rhythm was essentially identical between WT and KI mice in a less disease affected area (cerebellum), it was lost in a more disease affected region (olfactory bulb). Interestingly, the Aβ rhythm was the same in both sexes. These data suggest that although some interactions between Aβ neuropathology, sleep, and circadian rhythms are linked to biological sex, the effect may not be uniform. Because two-thirds of AD cases are women, understanding the driving factors for these differences is of critical importance for disease diagnosis and treatment. Funded by NIH (R01 AG068215, R01 AG068215-03S1, and T32 AG078110).

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Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.11/I4

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

Support: NIH Grant AG059695
NIH Grant AG065651

Title: Induction of oxidative stress mechanisms by sporadic and familial forms of Aβ: rescue by antioxidants and Nrf2 activators

Authors: *M. VALLE, B. GETANEH, J. GHISO, A. ROSTAGNO;
New York Univ. Grossman Sch. of Med., New York, NY

Abstract: Cerebrovascular deposition of amyloid (Cerebral Amyloid Angiopathy, CAA) is a common finding in Alzheimer's disease (AD) present in >90% of cases. CAA has a broad impact in brain function, progressively affecting cerebral blood flow, altering BBB permeability, interfering with brain clearance mechanisms and triggering a cascade of harmful metabolic events affecting the integrity of the neurovascular unit. The extent of vascular compromise is exacerbated by the presence of genetic variants, particularly those located at positions 21-23 of Aβ, primarily linked to CAA. Among them, the AβE22Q Dutch mutation is associated with early onset and a very aggressive form of the disease, eventually leading to fatal cerebral hemorrhage. Current evidence supports a crucial role of mitochondria-mediated pathways dysregulation as a contributing factor to the pathogenesis of sporadic and familial forms of CAA. Since these organelles play essential roles in maintaining high levels of brain energy demands, they are the major consumers of oxygen and, therefore, the most important generators of reactive oxygen species (ROS). In the present work, we aimed to provide insight into the Aβ-compromised molecular pathways in microvascular endothelial cells and identify potential new targets for therapeutic intervention. Human brain microvascular endothelial cells were challenged with well-defined oligomeric assemblies of both wild type Aβ1-42 and E22Q variant synthetic homologues, evaluating ROS formation and concomitant production of lipid and protein oxidation products. Using a combination of immunofluorescence microscopy and biophysical/biochemical assays, our findings demonstrate the formation of oligomeric assemblies of both Aβ peptides and the induction of dose-dependent ROS formation that, in turn, causes lipid peroxidation and generation of protein carbonylation derivatives. Treatment with antioxidants and activators of Nrf2, a central regulator of the antioxidative response, significantly

attenuated ROS production and concomitant ROS-induced damage. Our data highlights the detrimental role of Aβ oligomeric assemblies for microvascular endothelial cells suggesting that modulation of oxidative stress is a complementary therapeutic strategy with the potential to preserve the neurovascular unit integrity.

Disclosures: **M. Valle:** None. **B. Getaneh:** None. **J. Ghiso:** None. **A. Rostagno:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.12/I5

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

Support: NIH grant AG059695
NIH grant AG065651

Title: Amyloid-β Oligomerization negatively influences brain efflux: contribution by the molecular heterogeneity of the brain deposits

Authors: *J. GHISO[1], E. CABRERA[2], A. ROSTAGNO[1];
[1]New York Univ. Grossman Sch. of Med., New York, NY; [2]Stony Brook Univ., Stony Brook, NY

Abstract: Extensive parenchymal and vascular amyloid deposits are classic pathologic hallmarks of Alzheimer's disease (AD). Aβ, the main component of these fibrillar deposits, is also a normal soluble constituent of biological and brain interstitial fluids. The mechanisms regulating the soluble-to-fibrillar conversion remain a topic of intense scrutiny, with soluble oligomeric intermediates being highlighted in recent years as particularly relevant for disease pathogenesis. This concentration-dependent process is highly reliant on homeostatic mechanisms regulating the steady state levels of Aβ, influencing the delicate balance between rate of synthesis, dynamics of aggregation, and clearance kinetics. Emerging data suggest that reduced Aβ clearance, particularly in the aging brain, plays a critical role in amyloid formation and AD pathogenesis. Historically, clearance studies have mostly centered in monomeric Aβ40 providing basic assessment of the participating mechanisms; however, the complex molecular/structural heterogeneity and aggregation proclivity of the brain Aβ peptidome have been largely overlooked. We have used stereotaxic cerebral injection in C57BL/6 wild-type mice to evaluate the brain clearance of well-defined radiolabeled monomeric and oligomeric assemblies of main components of the AD brain deposits including full-length Aβ40 and Aβ42 as well as N- and C-terminal truncated species. Our data clearly demonstrate that soluble forms with low oligomerization proclivity, including C-terminally truncated derivatives, are easily eliminated from the brain, consistent with their common presence in the interstitial and cerebrospinal fluids. On the contrary, oligomerization increases brain retention, a characteristic particularly evident in Aβ42 and enhanced in fragments truncated at Phe4. Consistent with these findings, the Aβ forms

with lower clearance propensity were found in the most insoluble parts of the brain deposits, as demonstrated by mass spectrometry and immunohistochemical studies. Overall, our data indicate that Aβ oligomerization negatively influences brain clearance mechanisms exacerbating amyloid formation and self-perpetuating the amyloidogenic loop, issues that should not be overlooked at the time of designing therapeutic strategies for AD

Disclosures: J. Ghiso: None. **E. Cabrera:** None. **A. Rostagno:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.13/I6

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

Title: Calcium-dependent suppression of macroscopic homomeric Kv1.2 and heteromeric Kv1.1/1.2 currents by amyloid beta (1-42) peptide: Implications for Alzheimer's disease

Authors: *D. JAMSHIDI[1], K. DEBOEUF[2], L. YAKLIN[2], J. FARLEY[2];
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Abstract: The roles of Aβ in the pathogenesis of Alzheimer 's disease (AD) include disruption of synaptic communication/function and synaptic plasticity mechanisms thought to be critical for learning and memory. Exactly how these abnormal processes arise is incompletely understood, but evidence suggests that dysregulation of intracellular Ca^{2+} $[Ca^{2+}]_i$ levels is involved in the alterations of neuronal excitability and synaptic remodeling and neurodegeneration in AD. Our lab has focused on the potential involvement of voltage-gated potassium channels (VGKCs) in these processes. VGKC members contribute to resting membrane potential, are activated during and following action potentials, and regulate Ca^{2+} influx. Inhibition of VGKCs can lead to synapto- and neuro-toxicity. Members of the Kv1.x family can be expressed as homomers or select heteromers; e.g., Kv1.1 is often co-expressed with Kv1.2. Our lab has shown that Aβ peptides suppress VGKC activity, particularly Kv1.1. Here we asked if Aβ suppression is specific to Kv1.1 homomers, whether it also occurs for other members of the Kv1.x family and/or heteromers, and the extent to which Aβ-suppression is calcium-dependent. Stage V/VI *Xenopus laevis* oocytes were injected with Kv1.2 cRNA for homomeric expression, and heteromeric expression was evaluated by coinjection of Kv1.1 and 1.2 cRNAs. The effects of bath application (1 μM) of Aβ(1-42) on macroscopic currents from homomeric Kv1.2 and 1.1/1.2 heteromeric channels were assessed using two-electrode voltage clamp (TEVC). Calcium dependency of Aβ-suppression was evaluated through exposure of oocytes to the calcium chelator BAPTA-AM. DMSO was used as a control. As seen for homomeric Kv1.1 channels, Aβ suppressed homomeric Kv1.2 current by ~50% in 30 min. This Aβ-suppression was reduced to 25% by BAPTA-AM. For heteromeric 1.1/1.2 channels, Aβ suppressed current by ~41% in 30 min. BAPTA-AM reduced this suppression to 11%. Additionally, Aβ-suppression of current through homomeric 1.2 channels was completely eliminated by cyclosporin A (CsA), a

calcineurin (CaN) inhibitor. Based on these results, we conclude that homomeric Kv1.2 and heteromeric Kv1.1/1.2 channels are both suppressed by Aβ(1-42) in a calcium/CaN-dependent manner, similar to Kv1.1 homomers. Suppression of homomeric and heteromeric Kv1.x channels could potentially lead to larger and longer action potentials, resulting in a greater influx of Ca^{2+} into presynaptic terminals of excitatory neurons, enhanced glutamate release, and greater depolarization of spines. These latter effects may contribute to synapto- and neuro-toxicity effects in AD.

Disclosures: **D. Jamshidi:** None. **K. Deboeuf:** None. **L. Yaklin:** None. **J. Farley:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.14/I7

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

Support: NIA 1R01AG070934-01 to B.P.G

Title: Role of gut-brain axis signaling in the central Aβ pathology of Alzheimer's Disease progression in Tg2576 mice

Authors: *T. K. DAS, B. P. GANESH;
Dept. of Neurol., McGovern Med. School, Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Abstract
Alzheimer's disease (AD) is the sixth leading cause of death in the United States. The exact cause and origin of AD are still unknown. Recently it was found that bidirectional communication between the gastrointestinal tract and brain through the "Gut-brain axis" has an important role in AD progression. The extrinsic connections between the gut and brain by the vagus nerve are poorly understood. Importantly, pathogenic bacteria produce amyloid-like proteins "curli" that form biofilms and show functional similarities to human amyloid beta (Aβ). Curli may trigger AD progression via signals originating from the gut to the brain. Therefore, we propose that curli causes neuroendocrine activation from the gut to the brain which promotes central Aβ pathology via gut-brain axis signaling.
6-9 months (pre-symptomatic) and 15-16 months (symptomatic) Tg2576 of AD mice and same-aged match WT littermate mice were used in this study. 16sRNA gene sequencing was used to investigate bacterial gut dysbiosis. Bacterial amyloid curli (CsgA), toll-like receptor (TLR2), and neuroendocrine marker PGP9.5 expression levels in response to curli in the lumen of the mice were analyzed by IHC and qRT-PCR analysis. Human 3D enteroids culture system was used to investigate the curli effects.
We found changes in the gut microbiota composition with elevated gram-positive bacterial (family *Lactobacillaceae*) colonization in association with a rise of curli ($p<0.001$) in the ileum

of pre- and symptomatic Tg2576 gut. It was associated with elevated TLR2 ($p<0.05$), and PGP9.5 ($p<0.01$) levels in the small intestine. TLR2 expression in symptomatic Tg2575 mice brains was also significantly increased. All data were compared to littermate WT control mice. Furthermore, TLR2 levels ($p<0.01$) were increased by stimulation with purified curli in an *in-vitro* 3D enteroid culture system.
Our study reveals that bacterial curli-burden is present in the gut prior to Aβ pathology in the brain. It activates TLR-specific gut-vagus-brain signaling within the epithelium and sub-mucosa of the gut. This interaction provides preliminary evidence for vagus nerve activation by bacterial curli from the gut to the brain. We are currently conducting a thorough investigation into the significance of curli burden in the gut and its impact on gut mucosa signaling in the advancement of Aβ pathology. Currently, all available AD treatment gets started after the clinical symptoms appear and it becomes futile. Therefore, investigation at the pre-symptomatic stage of AD is crucial to understand the initiation and progression of AD. It provides insights into novel preventative and therapeutic approaches in AD.

Disclosures: T.K. Das: None. **B.P. Ganesh:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.15/Web Only

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

Support: Russian Science Foundation (grant #19-74-30007)

Title: Human laminins interact with amyloid peptides and promote their aggregation

Authors: *O. KECHKO[1], S. RODIN[2], S. A. KOZIN[1], V. A. MITKEVICH[1], A. A. MAKAROV[1];
[1]Engelhardt Inst. of Mol. Biol., Moscow, Russian Federation; [2]Uppsala Univ., Uppsala, Sweden

Abstract: *Aims*. Alzheimer's disease (AD) development is characterized by amyloid plaques deposition in extracellular space. Laminins are a family of extracellular matrix glycoproteins that are the major component of the basement membrane. Emerging evidence suggests that laminins are involved in AD pathogenesis. Previously, laminin-111 was detected in amyloid plaques. Also mouse laminin-111 has been shown to interact with beta-amyloid and prevent its fibrilization. However, laminin-111 based treatment had no therapeutic efficacy. Deposition of laminin-111 in hippocampus could be not an early event of AD but rather its consequence. We hypothesized that abnormal binding of beta-amyloids to other laminins in the brain results in neurotoxicity and involved in AD development.
Methods. We investigated the interaction between unmodified beta-amyloid peptides (1-42) or the peptides with isomerized Asp7 and recombinant laminins using microscale thermophoresis and ELISA. We explored the change of beta-amyloid aggregation properties using fluorescent

microscopy, dynamic light scattering and nanoparticle tracking analysis.
Results. We observed a strong interaction between beta-amyloids and laminins-521, -511 and -111, but not with laminin-211, and determined that divalent metal ions influenced this interaction. Laminins significantly increased the mean size and number of amyloid aggregates. Laminin-dependent beta-sheet aggregates of unmodified and isomerized beta-amyloids had different size and morphology. Addition of zinc ions enhanced amyloid aggregation in the presence of laminins.
Conclusions. Our results bring new evidence that not only laminin-111 but a set of laminins presented in the brain interact with beta-amyloids and promote their aggregation. Pathological complex of laminins and beta-amyloid or its isoforms may be a seed of further neurotoxic oligomers formation and contribute to the disease progression.

Disclosures: O. Kechko: None. **S. Rodin:** None. **S.A. Kozin:** None. **V.A. Mitkevich:** None. **A.A. Makarov:** None.

Poster

PSTR528. Abeta Mechanisms of Toxicity

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR528.16/I8

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

Support: NS128039
DA048742
DA007304

Title: Oligomeric amyloid beta evokes sex-dependent plasticity of neuronal GIRK channel activity

Authors: *H. LUO[1], E. MARRON[2], B. GANSEMER[1], M. FREDERICK[3], S. A. THAYER[4], K. D. WICKMAN[5];
[2]Univ. of Minnesota, [1]Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Minnesota, Twin Cities, Univ. of Minnesota, Twin Cities, Minneapolis, MN; [4]U of MN, Univ. of Minnesota Med. Sch., Minneapolis, MN; [5]Univ. Minnesota, Univ. Minnesota, Minneapolis, MN

Abstract: Excitatory and inhibitory signaling (E/I) imbalance has been linked to synaptic and cognitive dysfunction in Alzheimer's Disease (AD). Oligomeric amyloid β (oAβ), a predominant pathogenic factor in AD, induces hyperexcitability by enhancing excitatory signaling. However, the impact of oAβ on inhibitory signaling and how that might contribute to AD pathology is less well-understood. Using whole-cell patch-clamp electrophysiology, we detected the suppression of a crucial inhibitory influence in hippocampal neurons - signaling via G protein-gated inwardly-rectifying K^+ (GIRK or Kir3) channels - following *in vitro* and *in vivo* oAβ incubation. In primary hippocampal cultures, suppression of GIRK channel activity preceded synapse loss and was observed in cultures from male but not female mice (2-way ANOVA with Šídák's

multiple comparisons, interaction: $F_{1,60} = 4.469$, *P = 0.0387; sex: $F_{1,60} = 1.526$, P = 0.2216; oAβ: $F_{1,60} = 4.122$, *P = 0.0468; male vehicle vs oAβ: **P = 0.0088, female vehicle vs oAβ, P = 0.9978; n = 14-19 per group). This male-specific neuroadaptation was mediated by the oAβ-induced activation of mGluR5. A similar suppression of GIRK channel activity was seen in CA1 pyramidal neurons after intra-hippocampal injection of oAβ into adult C57BL/6J male but not female mice (2-way ANOVA, interaction: $F_{1,25} = 3.971$, P = 0.0573; sex: $F_{1,25} = 3.107$, P = 0.0902; oAβ: $F_{1,25} = 2.914$, P = 0.1002; n = 7-8 per group). Enhancing GIRK channel activity via an adeno-associated virus (AAV) GIRK2 over-expression vector ameliorated oAβ-induced deficits in a novel object recognition task in both male and female C57BL/6J mice (for recognition index in novel object recognition task, 3-way ANOVA, GIRK overexpression: $F_{1,57} = 12.68$, ***P = 0.0008; sex: $F_{1,57} = 0.074$, P = 0.0869; oAβ: $F_{1,57} = 7.289$, **P = 0.0091; GIRK overexpression x sex: $F_{1,57} = 6.499$, P = 0.3418; GIRK overexpression x oAβ: $F_{1,57} = 0.044$, *P = 0.0135; GIRK overexpression x sex x oAβ: $F_{1,57} = 0.0016$, P = 0.9680; n = 7-12 per group). These data suggest that oAβ-induced suppression of GIRK channel activity may contribute to AD progression in males, and that upregulating GIRK channel activity may protect against oAβ-driven cognitive deficits in both males and females through distinct mechanisms.

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Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.01/J1

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

Support: JSPS KAKENHI Grant Numbers 18K07384
JSPS KAKENHI Grant Numbers 23K06840

Title: Brain derived p3-Alcβpeptide enhance the mitochondrial activity and protect neurons against Aβ toxicity

Authors: *S. HATA[1,2];
[1]Hokkaido Univ., Sapporo, Japan; [2]Natl. Inst. of Advanced Industrial Sci. and Technol. (AIST), Sapporo, Japan

Abstract: Neurotoxic amyloid-β (Aβ) protein accumulation is a key feature of Alzheimer's disease (AD), causing neurodegeneration and cognitive decline. Aβ is generated from the amyloid-β protein precursor (APP) through proteolysis by β- and γ-secretases. Neurons express a family of transmembrane proteins called alcadeins (Alcs) and calsyntenins (Clstns), including Alcα/Clstn1, Alcβ/Clstn3, and Alcγ/Clstn2. Alcβ/Clstn3 acts as a synaptic adhesion molecule and regulates synapse formation. Following primary cleavage by α-secretase, Alcβ undergo intramembrane proteolysis by γ-secretase, resulting in the secretion of p3-Alcβ peptide. To

investigate the effects of p3-Alcβ on neuronal cells, we used primary cultured neurons derived from the cerebral cortex and hippocampus, which are vulnerable to Aβ-induced damage. We found that p3-Alcβ37 and its shorter peptide, p3-Alcβ9-19, enhance mitochondrial activity and protect neurons from Aβ oligomer (Aβo) toxicity. We also explored the mechanism of p3-Alcβ's protective effect against Aβo toxicity by focusing on calcium ions. Aβo triggers excessive calcium influx, leading to neurodegeneration. Our analysis revealed that both p3-Alcβ9-19 and p3-Alcβ37 suppressed calcium influx by inhibiting abnormal activation of NMDA-type glutamate receptors by Aβo. Because mitochondrial dysfunction is common in the brain of AD patients alongside increased Aβ and reduced p3-Alcβ37 levels, administration of p3-Alcβ9-19 may be a promising treatment for protecting, and promoting brain functions in AD patients.

Disclosures: S. Hata: None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.02/J2

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

Title: Enhanced amyloidogenic metabolism of APP and Aβ accumulation in AD mice that lack Alcadein expression through the aging process

Authors: *K. HONDA;
Hokkaido Univ., Sapporo Hokkaido, Japan

Abstract: Generation and accumulation of amyloid-β (Aβ) protein in the brain are the primary cause of Alzheimer’s disease (AD) and their regulatory mechanisms are complicated and still controversial. Our previous observations indicated that amyloidogenic processing of Aβ protein precursor (APP) is enhanced in the absence of alcadein α (Alcα/Clstn1). Here, we generated mouse models expressing human $App^{NL\text{-}F/NL\text{-}F}$ (APP-KI mouse) in either Alcα- or Alcβ-deficient background and analyzed human APP processing and Aβ accumulation through aging. The Alcα-deficient APP-KI mice enhanced Aβ accumulation by increased amyloidogenic processing of APP through the aging process whereas Alcβ-deficient APP-KI mice neither affect APP metabolism nor Aβ accumulation at any age. The results showed amyloid plaque formation appeared in the earlier stage of aging in Alcα-deficient APP-KI mice compared to it in Alcβ-deficient APP-KI mice. These observations indicate that Alcα plays an important role in the suppression of the progression of AD by decreasing Aβ generation with age, while Alcβ suppresses the Aβ-induced neurotoxicity with different functions in the brain (see other presentations by Hata and Suzuki).

Disclosures: K. Honda: None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.03/J3

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

Support: NIH Grant NS110147
NIH Grant NS109075
VA Award JK6BX005239

Title: Cathepsin B deficiency improves memory deficits and reduces amyloid-beta in hAbetaPP mouse models representing the major sporadic Alzheimer's disease condition

Authors: *G. HOOK[1], M. S. KINDY[2], V. Y. HOOK[3];
[1]American Life Sci. Pharmaceuticals, La Jolla, CA; [2]USF, Univ. of South Florida, TAMPA, FL; [3]Univ. of Calif., San Diego, Univ. of Calif., San Diego, La Jolla, CA

Abstract: The lysosomal cysteine protease cathepsin B (CTSB) has been suggested as a biomarker for Alzheimer’s disease (AD) because elevated serum CTSB in AD patients has been found to correlate with cognitive dysfunction. Furthermore, CTSB gene knockout (KO) in non-transgenic and transgenic AD animal models showed that elimination of CTSB improved memory deficits. However, conflicting CTSB KO results on amyloid-β (Aβ) pathology in transgenic AD models have been reported. The conflict is resolved here as likely being due to the different hAβPP transgenes used in the different AD mouse models. CTSB gene KO reduced wild-type (Wt) β-secretase activity, brain Aβ, pyroglutamate-Aβ (pyroglu-Aβ), amyloid plaque, and memory deficits in models that used cDNA transgenes expressing hAβPP isoform 695. But in models that used mutated mini transgenes expressing hAβPP isoforms 751 and 770, CTSB KO had no effect on Wt β-secretase activity and slightly increased brain Aβ. All models expressed the AβPP transgenes in neurons. These conflicting results in Wt β-secretase activity models can be explained by hAβPP isoform specific cellular expression, proteolysis, and subcellular processing. CTSB KO had no effect on Swedish mutant (Swe) β-secretase activity in hAβPP695 and hAβPP751/770 models. Different proteolytic sensitivities for hAβPP with Wt versus Swe β-secretase site sequences may explain the different CTSB β-secretase effects in hAβPP695 models. But since the vast majority of sporadic AD patients have Wt β-secretase activity, the CTSB effects on Swe β-secretase activity are of little importance to the general AD population. As neurons naturally produce and process hAβPP isoform 695 and not the 751 and 770 isoforms, only the hAβPP695 Wt models mimic the natural neuronal hAβPP processing and Aβ production occurring in most AD patients. Significantly, these CTSB KO findings in the hAβPP695 Wt models demonstrate that CTSB participates in memory deficits and production of pyroglu-Aβ, which provide rationale for future investigation of CTSB inhibitors in AD therapeutics development.

Disclosures: **G. Hook:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Science Pharmaceuticals. **M.S. Kindy:** None. **V.Y. Hook:** E. Ownership Interest (stock, stock

options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Science Pharmaceuticals.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.04/J4

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

Support: NIAU19AG074866

Title: Early molecular events of Autosomal Dominant Alzheimer's disease in marmosets with PSEN1 mutations

Authors: L. BAILEY[1], G. HOMANICS[1], J. PARK[1], P. L. STRICK[1], D. SCHAEFFER[1], L. SCHAEFFER[1], J. OLUOCH[1], T. ZHANG[1], L. SCHULTZ[1], T. MURAI[1], L. MONGEAU[1], H. HUHE[1], B. STEIN[1], S. CHOI[1], S. HA[1], S. HACHEM[1], T. GUIMARÃES[1], A. THATHIAH[1], B. PATEN[2], D. DUONG[3], N. T. SEYFRIED[3], A. GREENWOOD[4], A. HABER[5], C. SPRUCE[5], G. W. CARTER[5], A. C. SILVA[1], *S. SUKOFF RIZZO[1];
[1]Univ. of Pittsburgh Sch. of Med., PITTSBURGH, PA; [2]Univ. of California Santa Cruz, Santa Cruz, CA; [3]Emory Univ., Atlanta, GA; [4]Sage Bionetworks, Seattle, WA; [5]The Jackson Lab., Bar Harbor, ME

Abstract: The lack of an adequate animal model of Alzheimer's disease (AD) that mirrors the natural onset and progression of the human disorder has been a substantial barrier to basic and translational research. A new consortium has been established with funding support from the National Institute on Aging aimed at the generation, characterization, and validation of Marmosets As Research Models of AD (MARMO-AD). MARMO-AD will study gene-edited marmoset models carrying genetic risk for AD, and wild-type (WT) genetically diverse aging marmosets from birth throughout their lifespan, using non-invasive longitudinal assessments. These include characterizing the genetic, molecular, functional, behavioral, cognitive, and pathological features of aging and AD. Here we report through CRISPR/Cas9 technology the successful generation of the first genetically engineered marmosets that carry knock-in (KI) point mutations of C410Y and A426P in the presenilin-1 (PSEN1) gene. In humans these mutations cause early-onset AD. As part of this characterization we conducted whole genome sequencing and validated the presence of the PSEN1 mutations as well as assessed variation at multiple other AD loci in the WT marmosets. Significant increases in the plasma Aβ42:40 ratio in PSEN1 mutation carriers was observed relative to age- and sex-matched WT controls which was also observed in culture media generated from fibroblasts of the same individuals. In the fibroblast cultures we detected differential gene expression for genes enriched in neuron development and Aβ regulation, providing strong evidence for the functional relevance of the engineered variants. We quantitatively compared changes in marmoset PSEN1 fibroblasts to similar molecular measures in postmortem brain tissue and iPSCs from human AD studies. Both gene and protein

expression changes in the undifferentiated fibroblasts correlated with changes in iPSCs from human AD carriers reprogrammed into neuronal lineages. We also observed both transcriptomic and proteomic changes in marmoset PSEN1 cells that match those in postmortem AD brains. These findings suggest an early emergence of disease processes well before the onset of frank neuropathology. These observations highlight the unique opportunity to comprehensively study these animals from birth throughout lifespan which will enable the ability for revealing the earliest primate-specific molecular and cellular events that are the root causes of AD onset and progression; and the utility of these models for evaluating the potential of novel therapeutic interventions to stop and ultimately prevent AD. Funding for these studies are supported by NIAU19AG074866.

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Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.05/J5

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

Support: National Institute on Aging Grant U19AG074866
National Institute of Neurological Disorders and 577 Stroke of the National Institutes of Health R21NS125372

Title: Imaging Alzheimer's disease biomarkers in genetically engineered marmosets expressing PSEN1 mutations using ^{11}C-PiB and ^{18}F-FDG positron emission tomography

Authors: *S. K. A. PELL[1], L. LETICA[1], D. SZCZUPAK[1], J. E. PARK[1], G. E. HOMANICS[1], P. L. STRICK[1], G. W. CARTER[2], S. J. SUKOFF RIZZO[1], D. J. SCHAEFFER[1], A. C. SILVA[1]; [1]Neurobio., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]The Jackson Lab., Bar Harbor, ME

Abstract: Imaging Alzheimer’s disease biomarkers in genetically engineered marmosets expressing PSEN1 mutations using ^{11}C-PiB and ^{18}F-FDG positron emission tomography
Sarah K. Pell, Leila Letica, Diego Szczupak, Jung Eun Park, Gregg E Homanics, Peter L Strick, Gregory W Carter, Stacey J Sukoff Rizzo, David J Schaeffer, Afonso C Silva

Marmoset monkeys (*Callithrix jacchus*) are a promising animal model to experimentally target the etiological features and progression of Alzheimer's disease (AD). The lissencephalic marmoset brain harbors primate-specific evolutionally divergent features while also being amenable to genetic engineering techniques. Recently, we generated marmosets carrying the PSEN1-C410Y and PSEN1-A426P mutations that cause early-onset, familial Alzheimer's disease. Here, we employed positron emission tomography (PET) to assess beta-amyloid (Aβ) plaque burden and glucose uptake in marmosets harboring PSEN1 mutations, comparing them against young, aging, and aged marmosets from our well-established outbred colony. Using protocols established by the Alzheimer's Disease Neuroimaging Initiative (ADNI) to diagnose AD clinically in human patients, we administered ~18.5 MBq (0.5 mCi) intravenous injections of the radiotracers ^{11}C-Pittsburgh compound B (PiB) and ^{18}fluorodeoxyglucose (FDG). The marmosets were anesthetized with isoflurane and imaged in a small-bore preclinical PET/CT system (Bruker Biospin Si78) first with a 90-minute dynamic ^{11}C-PiB acquisition, followed by a 60-minute ^{18}FDG acquisition and a 3D 200μM isotropic resolution CT image. In both healthy outbred aged marmosets and those harboring PSEN1 mutations, we found positive ^{11}C-PiB binding and retention (as indexed by standard update values) that were paired with ^{18}FDG patterns consonant to those typical of human patients with AD. This work demonstrates the utility of ^{11}C-PiB and ^{18}FDG for the phenotypic characterization of AD biomarkers in genetically engineered marmosets.
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Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.06/J7

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

Support: NIH-NS108189

Title: A new platform and paradigm to track age-dependent neurodegeneration in a murine model of Alzheimer's Disease (5xFAD mice)

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Abstract: Neurodegeneration in Alzheimer's disease (AD) contributes to cognitive decline, because of this, transgenic mouse models of AD that exhibit neuron loss may be useful for unraveling underlying AD pathophysiology. For example, 5XFAD mice carry mutant human

amyloid precursor protein (APP) and presenilin genes that cause early onset Familial Alzheimer's Disease (FAD). The 5XFAD transgene is expressed via the Thy1 promoter, leading to high levels of transgene expression in neurons in the cortex and hippocampus. Particularly high levels of transgene expression are seen in neurons in layer V of the cerebral cortex, and these neurons undergo extensive neurodegeneration as the mice age. Although previous studies have described cell loss, cellular events associated with this neurodegeneration are largely unknown, in part because methods for tracking death of layer V neurons are limited. Here, we describe a novel method for tracking degeneration of layer V pyramidal neurons using a new transgenic line created by crossing 5XFAD mice with $Rosa^{tdTomato}$ reporter mice. In these 5XFAD/$Rosa^{tdTomato}$ mice, AAV-based transduction with Cre permanently turns on tdT expression. When mice were 4-5 months old, layer V neurons were retrogradely transduced by injecting AAVrg/Cre into the spinal cord, which transduces large pyramidal neurons in layer V that give rise to the corticospinal tract. Cre-dependent activation of tdT expression provided a robust, selective and permanent marker for layer V neuron cell bodies, dendrites and axons. To track neurodegeneration, mice were perfused at 7-15 months of age. Neurodegeneration was evidenced by progressive appearance of large axonal swellings and fragmentation, dendritic dystrophy and loss of tdT-positive neurons. In contrast, in control $Rosa^{tdTomato}$ mice without 5XFAD, retrogradely-transduced layer V neurons expressing tdT exhibited normal morphology up to 26 months of age. A surprising feature of neurodegeneration in 5xFAD/$Rosa^{tdTomato}$ mice was the progressive appearance of tdT-positive profiles in cortical layer VI and below. TdT-positive profiles were large (20+µm in diameter), irregularly shaped, and appeared to be extracellular (no nucleus was evident with Hoescht stain). These tdT-positive profiles may be degeneration debris from layer V neurons that have died. Of note, tdT-positive profiles co-localized with and appeared to surround APP and thioflavin-positive particles. The ability to track timing morphological changes in layer V neurons in 5XFAD mice provides a unique platform to test potential therapeutic interventions that reduce, delay or prevent neurodegeneration in this murine model of AD.

Disclosures: **D.N. Gallardo:** None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 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

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.07/J8

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

Support: PA Department of State 4100087331
National Institutes of Health (P30AG066468-02)
National Institutes of Health R01 (NS120922)

Title: The amyloid precursor protein and DNA damage: a feed-forward cycle that drives the progression of Alzheimer's disease

Authors: *F. MA, J. XIE, G. GUTTA, R. KINGSTON, K. HERRUP;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease with no effective therapeutic options and unknown pathogenesis. The amyloid precursor protein (APP) has occupied a central position in the pathophysiological description of AD because its proteolytic product, the β-amyloid (Aβ) peptide, is the main constituent of the amyloid plaques that are hallmarks of AD. However, any Aβ-independent role for the full-length APP protein in AD is relatively understudied. We have approached this problem from the perspective that APP is recognized as a damage-response protein that is elevated after a variety of different stresses. Our current work explores the extent to which DNA damage is one such stress. It has been well documented by others that increased neuronal activity leads to DNA breaks that are normally quickly repaired. We have extended this finding by showing that neuronal excitation leads not only to DNA damage but also to a rapid increase in APP message and protein. DNA damage alone is sufficient to induce APP expression as shown in vitro and in the *Atm*$^{-/-}$ mouse where DNA repair is compromised, and DNA damage accumulated. Unexpectedly, in the other direction, we find that increase in APP is itself sufficient to cause DNA damage. We find in AD mouse models in which APP is overexpressed, DNA damage is significantly increased. To determine whether these effects were driven by Aβ rather than full-length APP, we applied oligomeric Aβ directly to neurons and found no DNA damage response. Similarly, a BACE1 inhibitor, which would block the production of Aβ had no effect on APP-induced DNA damage. We therefore propose a three-way relationship that has the potential to become a dangerous feed-forward loop. Our findings reveal a novel role for full-length APP in AD pathogenesis through its relationship with DNA damage.

Disclosures: F. Ma: None. **J. Xie:** None. **G. Gutta:** None. **R. Kingston:** None. **K. Herrup:** None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.08/J9

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

Support: NIH AG068215

Title: Treatment of app x ps1 mutant knock-in mice with the gamma-secretase inhibitor Semagacestat alters sex-related sleep differences

Authors: S. TURTON, S. PADGETT, *M. P. MURPHY, T. JESSUP, S. BARTH, V. BUZINOVA, H. WHITLOCK, K. KOHLER, E. MANAUIS, C. JOHNSON;
Univ. of Kentucky, Lexington, KY

Abstract: Loss of sleep or sleep fragmentation (SF) has long been associated with an elevated risk for Alzheimer's Disease (AD). Our lab has documented differences in sleep patterns in several AD-related mouse lines, including the observation that female mice sleep less than males and may be more vulnerable to the effects of SF. We observed that these differences were particularly striking in mutant APPxPS1 knock-in mice and were present from an age at which the amount of amyloid-β (Aβ) pathology was barely detectable. We tested the hypothesis that this effect may be related to the function of the γ-secretase enzyme (the final enzyme in the pathway that generates the Aβ peptide) by treating these mice with the γ-secretase inhibitor (GSI) Semagacestat (LY-450139). As expected, the GSI was a potent inhibitor in vitro, resulting in a ~99% inhibition of Aβ secretion and a striking increase in APP C-terminal fragments (CTFs). We treated male and female (n = 48) APPxPS1 knock-in (KI) mice once / day with 12.5 mg/kg of Semagacestat, suspended in 0.5% hypromellose, by gavage, for 28 days. Control mice received vehicle only. Sleep was recorded using the PiezoSleep system for the final 14 days of treatment, which included a 5 day period of manual SF (Duncan et al, Neuroscience, 481: 111-122, 2022). At the end of the study, brains were collected for analyses by ELISA and immunoblot. Female mice were significantly more wakeful than males, an effect that was observed in both the light and dark phases of their daily activity cycle. Treatment with the GSI in vivo resulted in a relatively modest decrease in Aβ, and a small increase in CTFs. Decreased Aβ was primarily observed in the more soluble forms of the peptide in the males, and in the less soluble forms in the females. In contrast to other studies performed in our lab, the SF protocol slightly increased overall sleep, an effect largely driven by the increase in dark phase sleep in the males. It is possible that these differences may be due to a shorter SF protocol, or to the timing of the drug administration, immediately prior to the onset of dark phase. These results also suggest that pharmacological inhibition of gamma-secretase may result in complex, sex-dependent changes in daily sleep. Funding provided by NIH (AG068215).

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Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.09/Web Only

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

Support: AMED JSPS KAKENHI

Title: Hypothalamic and hippocampal transcriptome changes in App^{NL-G-F}mice as a function of metabolic and inflammatory changes in the progression of Alzheimer's disease

Authors: P. JOSEPH[1], E. GUTIERREZ RICO[2], ***K. POON**[1];
[1]SUNY Old Westbury, Old Westbury, NY; [2]Tohoku Univ., Sendai, Japan

Abstract: The progression of Alzheimer's disease has a silent phase that predates cognitive decline and eventually gives rise to active cognitive deficits. Metabolism, diet, and obesity have been correlated to the development of Alzheimer's disease but is poorly understood. The hypothalamus is a brain region that exerts homeostatic control on food intake and metabolism, and has been noted to be impacted during the active phase of Alzheimer's disease. This study, in using a human amyloid overexpression mouse model, examines blood markers in young and old App^{NL-G-F} mice that corresponds to the silent and active phases of Alzheimer's disease, and global gene expression changes in the hypothalamus and the hippocampus. The results show a large panel of inflammatory mediators, leptin, and other proteins involved in weakening the blood brain barrier, to be increased in young App^{NL-G-F} mice but not in the old App^{NL-G-F} mice. There were also several differentially expressed genes in both the hypothalamus and the hippocampus in the young App^{NL-G-F} mice prior to amyloid plaque formation and cognitive decline that persisted in the old App^{NL-G-F} mice, including GABRa2, Wdfy1, and several pseudogenes with unknown function. These results show that a larger panel of inflammatory mediators may be used as biomarkers to detect silent AD, and that a change in leptin and hypothalamic gene expression exists prior to cognitive decline, suggesting a coupling of metabolism with amyloid plaque induced cognitive decline.

Disclosures: P. Joseph: None. **E. Gutierrez Rico:** None. **K. Poon:** None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.10/J10

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

Support: Cure Alzheimer's Fund 239769 (RB)

Title: Neuronal BIN1 regulates APP endocytosis in a RIN3 dependent pathway.

Authors: *R. BHATTACHARYYA[1], C. A. F. TEVES[2], J. C. ZELLMER[3], R. E. TANZI[4];
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Abstract: Introduction: The bridging integrator 1 (*BIN1*) is a major susceptibility gene for Alzheimer's disease (AD), only second to *APOE* [1]. Recent whole-exome sequencing (WES) analyses have identified genome-wide significance associated with AD risk at the *SLC24A4/RIN3* locus, providing strong evidence that *RIN3* is an AD-risk gene [2]. RIN3 (product of the *RIN3* gene) is a binding partner for BIN1 (product of the *BIN1* gene). The role of BIN1 in AD is largely unknown primarily because there are at least 10 BIN1 splice variants of BIN1 (BIN1V1- V10) with different tissue distributions and functions. The neuronal isoform BIN1V1 contains the clathrin and AP-2-binding sites (CLAP domain) which is absent in non-neuronal BIN1V9. The CLAP domain is essential for clathrin-mediated endocytosis, which is a key process of vesicle trafficking that transports numerous cargo proteins from the cell surface to endosomes. APP endocytosis is clathrin-mediated, but BACE1 is not.
Results: The study of RIN3 in AD is a nascent area of AD research. A recent report has shown a correlation between RIN3 upregulation and endosomal dysfunction in AD mice. RIN3 is primarily localized in intracellular membranes while BIN1 (all isoforms) is predominantly cytoplasmic. We found that the overexpression of RIN3 translocated BIN1V1, but not BIN1V9, to the RAB5+ endosomes in neuro 2A (N2A) cells. Interestingly, RIN3 binds to both BIN1V1 and BIN1V9 with equal affinity, likely via the N-terminal SH3 domain that binds to the internal proline-rich domain of RIN3 (RIN3 PRD). Moreover, overexpression studies in neuronal cells showed that BIN1V1 lowered the rate of APP endocytosis but BACE1 endocytosis was unaffected [3].
Conclusion: The SH3 domain and the clathrin-binding CLAP domain of BIN1V1 undergo an intramolecular interaction, which is predicted to form a "closed" BIN1V1 conformation. Based on our studies Our working hypothesis is that the RIN3 binding to the SH3 domain of BIN1V1 via the proline-rich domain (PRD) of RIN3 converts the "closed" BIN1V1 to an "open" BIN1V1 conformation which exposes the CLAP domain for clathrin-dependent endosomal recruitment of the "open" BIN1V1 outcompeting APP's clathrin-dependent endocytosis resulting in a reduced Aβ production. A recent report has also shown a correlation between RIN3 upregulation and endosomal dysfunction in AD mice.
References:1. Harold, D., et al., Nat Genet, 2009. **41**(10): p. 1088-93.2. Holstege, H., et al., Nat Genet, 2022.3. Bhattacharyya, R., et al., Sci Rep, 2022. **12**(1): p. 3486.

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Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.11/K1

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

Support: CIHR Grant PJT-166127

Title: Pathogenic presenilin 1 mutations affect amyloid-beta formation and neuronal cell death independently of beta-secretase in vitro

Authors: *I. BESTARD, K. STERLING, F. CAI, W. SONG;
Univ. of British Colombia, Vancouver, BC, Canada

Abstract: Alzheimer's (AD) is the leading cause of dementia, affecting millions of people worldwide. Presenilin 1 (PS1) mutations are the primary cause of familial AD. These mutations have been causally linked with increased amyloid-beta (Aβ) production and other AD hallmarks such as neurotoxicity and cell death. Studies have suggested that pathogenic PS1 mutations affect Aβ production by altering the amyloid precursor protein (APP) processing in the amyloidogenic pathway, but this mechanism is still unclear. This study aimed to investigate how PS1 mutations regulate the amyloidogenic pathway, Aβ production and neuronal cell death *in vitro*. To determine the effects of the PS1 mutations on APP processing, PS1 plasmids corresponding to the PS1 wild-type (control) and five AD-related PS1 mutations were transfected into human embryonic kidney cells that overexpress the $APP_{Wild\text{-}Type}$ gene called HAW cells. PS1 plasmids were also transfected into cells overexpressing both the $APP_{Wild\text{-}Type}$ and β-secretase, the beta site APP-cleaving enzyme 1 (BACE1), and cells that overexpress both BACE1 and the APP gene carrying the AD-linked Swedish mutation. To examine the effect of pathological PS1 mutations on neuronal cell death, we transfected those plasmids in a PS1-knockout neuro2A cell line called N2A-KO cells. This study consists of six experimental groups that differ based on the PS1 plasmid transfected, and we used a minimum of three replicates for each experiment. One-way ANOVAs followed by Dunnett's multiple comparison test were used for all statistical analyses. Various molecular techniques were performed to determine how each PS1 mutation affects APP processing, Aβ production, cell viability and neurotoxicity, including Western Blots, ELISA, and cell death assays. Overall, our findings indicate that these pathogenic PS1 mutations significantly affected APP cleavage and increased Aβ formation in HAW cells, but surprisingly this was not the case for the cells overexpressing BACE1, suggesting that the mutations' effect on APP processing might be independent of β-secretase. Furthermore, using N2A-KO cells, we found that the pathogenic PS1 mutations significantly decreased cell viability. Our findings provide novel insights into the pathological effects of the PS1 mutations on the amyloidogenic pathway and cell death which, unlike previous studies suggested, might not be mediated by its effect on β-secretase.

Disclosures: I. Bestard: None. **K. Sterling:** None. **F. Cai:** None. **W. Song:** None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.12/K2

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

Support: NIH grants NS085171
NIH Grant AG065290

Title: Investigation of Bach1 as a transcriptional regulator of disease susceptibility in Amyloid Precursor Protein transgenic mice

Authors: *P.-Y. CHUANG, C.-H. FU, J. CHIN;
Baylor Col. of Med., Houston, TX

Abstract: Amyloid-β (Aβ) and tau accumulation are pathological hallmarks of Alzheimer's disease (AD). However, in some cases there is a mismatch between the presence of these hallmarks and the occurrence of cognitive symptoms. It is not clear what factors drive the variable incidence of susceptibility or resilience to cognitive deficits in the presence of AD neuropathology. To begin to understand the underlying mechanisms, we investigated human amyloid precursor protein transgenic mice (APP mice) that also exhibit variable susceptibility or resilience to development of memory deficits, despite having similar levels of APP/Aβ. Both susceptible and resilient APP mice exhibit epileptiform activity and seizures early in disease progression, similar to some patients with AD; however, by 4 months of age, susceptible APP mice develop robust memory deficits whereas sibling resilient APP mice do not. At this age, resilient APP mice also exhibit a reduction in seizure activity, suggesting that the ongoing presence of seizures is one feature of susceptible APP mice. RNA-sequencing of the dentate gyrus identified genes that are specifically differentially expressed in susceptible APP mice, some of which may represent susceptibility genes. Of the genes we identified, we were particularly interested in the transcription factor Bach1 because it is not only increased in susceptible APP mice, but it is also bioinformatically predicted to be one of the key regulators of the differentially expressed genes (DEGs) between susceptible and resilient APP mice. Bach1 is a repressor of oxidative stress-related pathways that has been linked to neurological disorders: Bach1 is increased in Parkinson's disease, and excess Bach1 in Down Syndrome (DS) causes disruption of cellular response to oxidative stress and contributes to the development of AD in DS patients. We found that Bach1 mRNA increases in an age- and seizure-dependent manner in susceptible APP mice. Since Bach1 is ubiquitously expressed in neurons, glial cells, and endothelial cells in the brain, we used RNAScope to characterize the cellular localization of Bach1 mRNA in the hippocampus of APP mice. We found that Bach1 is expressed in very high abundance in dentate granule cells of APP mice, with little expression in other cell types in the hippocampus. Gene ontology network analyses on the DEGs predicted to be Bach1 target genes indicated they are primarily related to synaptic transmission, neuron death, and response to oxygen level, which are relevant to AD disease pathogenesis. These findings suggest that Bach1 may be a transcriptional regulator of susceptibility in AD.

Disclosures: P. Chuang: None. **C. Fu:** None. **J. Chin:** None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.13/K3

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

Support: PPG

Title: Bridging single-cell transcriptomic profiles with neural network function and locomotor behavior in an Alzheimer's Disease mouse model

Authors: *F. JIANG[1], S. MILLER[1], J. PAN[2], A. AGRAWAL[1], P. HONMA[1], J. SHIN[1], D. XIA[3], P. SANCHEZ[3], R. THOMAS[1], I. COBOS[2], J. J. PALOP[1];
[1]Gladstone Institutes, UCSF, San Francisco, CA; [2]Stanford Univ., Palo alto, CA; [3]Denali Therapeut., South San Francisco, CA

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disease characterized by abnormal protein deposit in the brain, including amyloid plaques and neurofibrillary tangles. AD patients show disease-associated transcriptomic alterations in specific cell types and also display aberrant brain network activities, which eventually leads to behavioral deficits. However, few studies have revealed the relationship between transcriptomic expression at single cell resolution, neural network dynamics, and behavioral activity. To systematically investigate how variation in transcriptomic profiles interact with neural network dynamics, we performed single-nucleus RNA sequencing (snRNA seq) on dissected hippocampi sections from AD knock-in (App KI) mice (n=39) following 14-day continuous telemetric electroencephalography recording which includes a readout of mouse locomotor activity. We identified and quantified disease alterations in theta and gamma band power and detected multiple types of epileptiform spike events in male and female KI mice, and we observed nocturnal locomotor hyperactivity specifically in female KI animals. Using the snRNA seq data, we found differentially expressed genes in major cell type including interneurons, oligodendrocytes, astrocytes, and microglia, corresponding to locomotive hyperactivity, gamma power alterations, and epileptiform spikes, and performed functional analysis to show potential proteomic pathways relevant to neural network dynamics. Our study provides new insights on the mechanistic relationships connecting behavioral alterations, network dysfunction and transcriptomic changes, and may support the development of treatments for AD.

Disclosures: F. Jiang: None. **S. Miller:** None. **J. Pan:** None. **A. Agrawal:** None. **P. Honma:** None. **J. Shin:** None. **D. Xia:** None. **P. Sanchez:** None. **R. Thomas:** None. **I. Cobos:** None. **J.J. Palop:** None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.14/K4

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

Support: BrightFocus ADR Postdoctoral Fellowship (A2022016F)
CAMH Discovery Fund

Title: Electrophysiological and sleep disturbances coincide with failed proteostasis and precede cognitive decline in Alzheimer's disease mouse models

Authors: *C. MORRONE[1], A. TSANG[1], A. SEREGIN[1], S. GIORSHEV[1], R. ALAM[1], F. AZHAR[1], D. WEAR[1], H. YU[2];
[1]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Ctr. for Addiction and Mental Hlth., Univ. of Toronto, Toronto, ON, Canada

Abstract: Alterations in sleep and proteostasis are implicated in Alzheimer's disease (AD), especially in early disease stages. Recent work has demonstrated a link between sleep and proteostasis, including reduced clearance, post-processing and degradation via autophagy, leading to increases in β-amyloid and tau, hallmark pathologies of AD. We posit the sleep-proteostasis positive-feedback-loop is critical to proteinopathy and AD progression, in which we aim to elucidate the contributions to cognitive impairment, and discern neuronal populations vulnerable to failed autophagy and rampant proteinopathy. Using the App^{NL-G-F}xMAPT double knock-in (dKI) mice, we tested mice at 3 ages (4-, 8-, 12-month), representing early to advanced β-amyloid plaques (6F3D) and tau hyperphosphorylation (PHF1) by immunohistochemistry, and compared to MAPT single knock-ins that have no major pathology. Mice were surgically implanted with headcaps for EEG and hippocampal depth electrode measurements, and tested for sleep and neuronal function. The Barnes maze task was utilized to probe spatial learning and memory, and executive function. Immunohistochemistry for β-amyloid and tau pathology, autophagy, and neuronal labelling was conducted on brain tissue. In dKI mice, sleep impairment, including circadian arrhythmicity and loss of rapid eye movement (REM) sleep, begins at 4-months, preceding development of cognitive deficits. Learning, memory and executive function were significantly impaired at 12-months only in dKI mice, and most notably in male mice. In dKI mice, cortical EEG and hippocampal field potential impairments including reduced total and beta power, and higher delta power during wake, precede cognitive decline. Neuronal integrity (NeuN) in the hippocampus and entorhinal cortex is preserved until the 12-month stage at which dKI mice exhibit loss compared to MAPT counterparts. Modalities disrupting sleep increase proteinopathy and disrupt proteostasis (p62) in mouse hippocampus and entorhinal cortex. Conversely, activating autophagy therapeutically with trehalose improves sleep and memory. Discerning mechanisms linking sleep loss with proteostasis failure will elucidate early electrophysiological, behavioral, and molecular signs of cognitive decline, aiding in therapeutic design for AD.

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Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.15/K5

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

Title: Endothelin-converting enzyme-2 (ECE-2) regulates endogenous synaptosomal and secreted β-amyloid in distinct brain regions

Authors: *D. CLAUSEN[1], J. PACHECO-QUINTO[2], H. PENG[2], K. D. BECK[1], E. ECKMAN[2]; [1]Pharmacology, Physiol. & Neurosci., Rutgers, The State Univ. of New Jersey, Newark, NJ; [2]Biomed. Res. Inst. of New Jersey, Cedar Knolls, NJ

Abstract: Levels of β-amyloid (Aβ), a peptide that abnormally aggregates in Alzheimer's disease (AD), are tightly regulated by the activity of proteases responsible for its production and degradation. Among known Aβ degrading enzymes, endothelin-converting enzymes (ECEs) have the unique trait of cleaving Aβ within the endosomal vesicles where it is produced, thus modulating Aβ secretion and preventing intraneuronal Aβ accumulation and aggregation. We have previously shown *in vitro* and *in vivo* that pharmacological inhibition of ECE family members causes rapid accumulation of Aβ in endosomal vesicles along with increased Aβ secretion. In brain, the expression patterns of ECE-1 and ECE-2 differ substantially, and knockout (KO) mouse studies show that their activities are non-redundant with respect to Aβ catabolism. Compared to ECE-1, ECE-2 expression is both spatially and neuronal cell-type restricted, with high expression in interneurons and enrichment in dentate gyrus, hypothalamus, midbrain, and cerebellum. A recent report (Liao et al 2020) implicates impaired ECE-2 activity as a risk factor for late-onset AD and demonstrates that ectopic overexpression of ECE-2 can prevent amyloid pathology in AD model mice. Understanding the spatial relationship between ECE-2 activity and endogenous Aβ metabolism, especially within neuronal synapses, may point toward early sites of Aβ aggregation and provide insights on neuronal vulnerability in AD. Using ECE-2 KO mice, we determined the regional impact of ECE-2 activity on endogenous synaptic and secreted Aβ. Whole brain (n=9-16/genotype) or microdissected regions (n=8/genotype) were homogenized to prepare crude synaptosomal vesicles and separate them from the extracellular (ISF-enriched) fraction. ECE-2 protein expression in synaptosomes was confirmed by western blot and Aβ was measured by ELISA. ECE-2 localizes to synapses and, globally, ECE-2 KO mice had significantly increased synaptosomal and secreted Aβ. Subcortical structures including diencephalon, midbrain and cerebellum had the highest ECE-2 protein expression and, in ECE-2 KO mice, the largest increases in synaptosomal Aβ. Secreted Aβ was significantly increased in all brain regions except cortex, with hippocampus showing the largest change and overall Aβ levels. Our results demonstrate that ECE-2 regulates endogenous synaptosomal and secreted Aβ within brain regions known to be important for cognition and impacted early in AD pathogenesis. Future research will determine how ECE-2 regulation may relate to the physiological function of Aβ and whether increased or decreased endogenous ECE-2 activity can alter the pathogenesis of AD.

Disclosures: D. Clausen: None. **J. Pacheco-Quinto:** None. **H. Peng:** None. **K.D. Beck:** None. **E. Eckman:** None.

Poster

PSTR529. APP/Abeta Pathway: Cellular and Animal Models II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR529.16/K6

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

Support: Singapore Ministry of Education AcRF Grant MOE2017-T3-1-002

Title: Unravelling the role of BACE2 in a human cerebral organoid model of Alzheimer's Disease

Authors: *Y. YEAP[1], M. LEE[1], T. TNG[1,2], A. ROVELET-LECRUX[3], D. NIZETIC[4], K. LIM[1]; [1]Lee Kong Chian Sch. of Medicine, Nanyang Technological Univ., Singapore, Singapore; [2]Interdisciplinary Grad. Programme (IGP-Neuroscience), Nanyang Technological Univ., Singapore, Singapore; [3]Dept. of Genet. and CNR-MAJ, Univ. de Rouen Normandie, Rouen, France; [4]Queen Mary Univ. of London, Barts & The London Sch. of Med. and Dent., London, United Kingdom

Abstract: Amid the controversies surrounding the etiology of Alzheimer's Disease (AD), new molecular players of the amyloid cascade hypothesis have emerged, and among these is β-site APP cleavage enzyme 2 (BACE2). Unlike BACE1, BACE2 cleaves APP within the Aβ domain that accordingly prevents Aβ generation, suggesting its neuroprotective potential, although some reports have implicated otherwise. In this study, we aim to clarify the role of BACE2 in Alzheimer's Disease (AD) by examining its effects on the extent of neuronal death, amyloid deposition, and tau pathology - the three hallmarks synonymous with AD, in the state-of-the-art cerebral organoid system. For this purpose, we have generated induced pluripotent stem cells (iPSCs) using non-integrational Sendai reprogramming from the fibroblasts of a male individual with Early Onset Alzheimer's Disease harboring a de novo 12kb deletion in intron 1 of BACE2. Alongside this, we also generated iPSCs from his asymptomatic parent (father) as a control. Cerebral organoids established from patient and control iPSCs were grown for >200 days in culture and 3 to 5 organoids were randomly selected for immunohistochemical analyses at periodic intervals. Interestingly, we observed an age-related increase in the concentration of amyloid plaques from day 100, where the patient organoids demonstrated a significant exacerbation in amyloid plaque deposition as compared to the control. Consistent with the amyloid hypothesis, the appearance of amyloid plaques preceded that of phosphorylated tau, with the latter appearing in patient organoids only at day 150 and beyond. At day 200, patient-derived organoids demonstrated a significant increase in phosphorylated tau and neuronal death as compared to the control. As the intronic deletion of BACE2 is associated with its deficient expression, our results support a neuroprotective role of BACE2 in AD. We believe that this is an important clarification that not only sheds light on the function of BACE2 but also positions it as a potentially druggable therapeutic target for AD.

Disclosures: Y. Yeap: None. **M. Lee:** None. **T. Tng:** None. **A. Rovelet-Lecrux:** None. **D. Nizetic:** None. **K. Lim:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.01/K7

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

Support: NIH, 1R01 AG059785

Title: Synaptic bioenergetic failure in a mouse model of tauopathy: implications for a direct role of tau

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Abstract: Synaptic bioenergetic failure in a mouse model of tauopathy: Implications for a direct role of tau
Disrupted synaptic mitochondrial function preceding synaptic failure, is a primary driver of neuronal loss in neurodegeneration and may result as consequence of toxic protein accumulation. Tauopathies, including Alzheimer's disease and frontotemporal dementia are characterized by the aberrant accumulation of toxic microtubule-associated protein tau (tau) species and concurrent oxidative stress, and mitochondrial dysfunction, which correlates with impaired cognition. Notably, tau mutations are associated with more aggressive disease progression and earlier onset. Despite this, studies mechanistically linking tau accumulation to mitochondrial damage and the significance of disease-associated tau mutations in this process are lacking. While indirect roles of tau are the subject of several reports, recent evidence from our lab suggests that overexpression of non-mutant human tau (htau) may directly associate with synaptic mitochondria, and thus we hypothesize that mutant tau accumulation similarly can directly alter synaptic bioenergetics. To investigate this, we isolated synaptosomes or synaptic and non-synaptic mitochondria from both sexes of transgenic wild-type htauwild-type and mutant tau (proline 301 to serine; tau^{P301S}) overexpressing (PS19) mice at age of three months of age and interrogated them using a combination of molecular biology, biochemistry, and metabolic flux assays. Here we demonstrate that PS19 mice exhibit accumulation of tau^{P301S} and phospho-tau^{P301S} in synapses by three months of age as well asthat coincides with impaired maximal and spare respiratory capacity in synaptosomes, that was not attributable to altered basal ATP synthesis, differences in synaptic mitochondrial content, or mitochondrial DNA damage. Using synaptic mitochondria isolated from tau knock-out mice we demonstrate that clarified brain homogenates from PS19 mice directly altered electron flow electron flow responses. Furthermore, these mitochondria captured soluble tau when co-incubated with clarified brain homogenates of PS19 or htau mice. Our results suggest that like non-mutant tau, tau^{P301S} associates with synaptic mitochondria and impairs synaptic bioenergetics. Future work will focus on characterizing the molecular determinants by which tau (non-mutant or mutant) associates with synaptic mitochondria and determine the mechanism by which tau directly alters mitochondrial function.

Disclosures: **A.J. Trease:** None. **N. Roland:** None. **H.S. Fox:** None. **K. Stauch:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.02/K8

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

Support: NIH Grant AG072458

Title: Resource network for protein polymorphisms in AD/ADRD: Materials for research on tau aggregates

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Abstract: Aims: Tauopathies including Alzheimer's disease (AD) are characterized by intracellular lesions composed of aggregated and post-translationally modified tau proteins. Although methods for preparation and biochemical characterization of stable filaments from various tauopathies are well established, soluble oligomeric tau aggregates has not been standardized to the same degree. Additionally, the structures of oligomers which associate most closely with bioactivity are not fully established. The AD/ADRD Resource Network aims to isolate structurally vetted tau filaments from human tauopathy cases and distribute them to the research community for experimentation. In addition, it seeks to develop protocols for standardizing the isolation and handling of bioactive oligomeric tau species. The overall mission of the network is to support impactful tauopathy research. Here we describe resource work flow for filamentous tau aggregates, the molecular characteristics of the preparations and potential utility for supporting tau-focused research. Methods: Human brain derived Tau aggregates were isolated from AD brain samples using biochemical methods. Filamentous aggregates were analyzed for morphology and length distribution by electron microscopy, content of tau, α-synuclein, Aβ, and TDP43 by immunoassay, seeding activity in biosensor cell and RT-QuIC formats, and post-translational modification signature through bottom-up proteomics analysis. Results: Purified filamentous tau preparations exhibited near-exponential length distributions with 85 ± 3% of the population (n = 3) adopting PHF morphology. Tau content averaged 25 ± 10% by weight with α-synuclein, Aβ, and TDP43 each contributing ≤ 0.1%. PTM signatures were dominated by phosphorylation with inconsistent detection of Lys modifications. Seeding activity was biphasic, with 5%-positive cells detected by flow cytometry at ~100 ng tau content and maximal 12% positivity at ~250 ng tau content. Resource Network members established cross-laboratory analytical benchmarks to ensure rigor and reproducibility of results. Conclusions: AD-derived filamentous tau polymorphs with consistent morphology, PTM

signatures and composition are available for biological experimentation in the research community. The materials are appropriate for supporting seeding experiments in animal, biosensor cell and RT-QuIC model formats, development and validation of aggregate-interacting probes including antibodies and small-molecules, and structural biology experiments leveraging CryoEM methods.

Disclosures: M. Orshoski: None. **T. Wagoner:** None. **J. Greenwald:** None. **D. Eisenberg:** None. **C. Keene:** None. **V. Wysocki:** None. **R. Kayed:** None. **J. Kuret:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.03/K9

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

Support: Grant-in-Aid for Transformative Research Areas (A) 21H05705
Grant-in-Aid for Scientific Research (C) 21K07580
JP23gm6510013h0002

Title: In vivo assessment of progressive astrogliosis in rTg4510 tauopathy animal brains

Authors: *M. SHIMOJO[1], T. MINAMIHISAMATSU[1], T. SHIMIZU[1], S. UCHIDA[1], M. TAKAHASHI[1], H. TAKUWA[1], Y. MATSUSHITA[1], R. YANAI[1], K. MINATOHARA[1], M.-R. ZHANG[2], N. SAHARA[1], M. HIGUCHI[1], *M. SHIMOJO[3];
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Abstract: Progressive inflammatory gliosis associated with neuronal deposition of hyperphosphorylated tau is currently hypothesized as a key component in the early stage of pathological cascade in neurodegenerative tauopathies including Alzheimer’s disease. Current evidence indicates that astrocyte activates in response to pathological tau accumulation and transforms their gene expression and cellular composition from a physiological state to a reactive state. However, it is still not fully clarified when and how this astrocytic transition occurs during the disease progress. To address this fundamental question, we investigated the time-course change of astrocytic pathophysiology in the forebrain of rTg4510 mouse model of tauopathy, which typically develops tau depositions and brain atrophy around 5-6 months of age. In the conventional biochemical and immunohistochemical assessment of postmortem brain tissues, we first demonstrate that alteration of several astrocytic components is initiated without obvious pathological tau deposition and brain atrophy in the neocortex of rTg4510 mice at 2-3 months of age. The RNAseq-based profiling of astrocytic gene expression reveals that specific gene signatures categorized to distinct types of reactive astrocytes (i.e. pan-reactive, neurotoxic, and neuroprotective astrocytes) individually alter in the brain of rTg4510 mice at 3 months of age, which is followed by uniform upregulation of gene expression observed in the most

classification of reactive astrocytes around 6 months of age. We also show that the alteration of these gene profiles may associate with intracellular calcium metabolism of cortical astrocytes assessed by two-photon microscope and the broad distribution of astrocytes across the whole brain captured by positron emission tomography. Our findings indicate that alteration of astrocyte status can be an initial trigger of the early stage of the tau-associated neurodegenerative process, implicating the restoration of the astrocytic gene profile may be a beneficial approach for ameliorating disease progress.

Disclosures: **M. Shimojo:** None. **T. Minamihisamatsu:** None. **T. Shimizu:** None. **S. Uchida:** None. **M. Takahashi:** None. **H. Takuwa:** None. **Y. Matsushita:** None. **R. Yanai:** None. **K. Minatohara:** None. **M. Zhang:** None. **N. Sahara:** None. **M. Higuchi:** None. **M. Shimojo:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.04/K10

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

Title: Building cell based assays to monitor aggregation and degradation of pathological tau

Authors: *A. KRASOWSKA-ZOLADEK[1], Y. KOVALYOVA[1], S. SUON[1], L. PRICE[2], B. MAGLIARO[2], X. HAN[3], R. BERGER[3], J. MARCUS[1], M. USENOVIC[1];
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Abstract: In Alzheimer’s disease (AD) levels of aggregated tau protein correlate with neuronal dysfunction and cognitive decline. Therefore, clearing tau aggregates could modify disease progression in AD and primary tauopathies. The growing field of proximity induced modalities that enable targeted degradation of proteins of interest prompted us to explore these approaches for clearance of pathological, aggregated tau. Here, our work describes the development of cell-based assays which would monitor the aggregation of pathological tau species and then subsequent degradation with proposed targeted protein degradation (TPD) modalities. To build the assays we utilized B35 rat neuroblastoma and ChoK1 hamster ovarian cell lines expressing human tau with a P301L mutation, a mutation associated with the primary tauopathy frontotemporal dementia. To induce aggregation and accumulation of pathological tau species we applied to the cells a sarkosyl insoluble protein fraction (SI) obtained from brains of Tg4510 mice, a mouse model of tauopathy with P301L tau mutation. Following treatment, the induction of tau pathology was confirmed by Western Blot, Immunohistochemistry (ICC) and alphaLISA detecting aggregated and hyperphosphorylated tau, suggesting the presence of disease relevant proteinopathy. Here we present our efforts in building these models and examining different TPD modalities targeting intracellular pathological tau species, highlighting the challenge in conclusively demonstrating reduction of tau pathology using proximity induced degradation.

Disclosures: **A. Krasowska-Zoladek:** None. **Y. Kovalyova:** None. **S. Suon:** None. **L. Price:** None. **B. Magliaro:** None. **X. Han:** None. **R. Berger:** None. **J. Marcus:** None. **M. Usenovic:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.05/L1

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

Support: Molecule AG/VITADAO (#282942)
Norwegian Cancer Society & Norwegian Breast Cancer Society (#207819)

Title: A novel mitophagy inducer that crosses the blood-brain barrier in mice and protects against tau pathology in mouse and human mini-brain models of Alzheimer’s disease

Authors: *S. CAO, E. F. FANG;
Epi-Gen, Univ. of Oslo and Akershus Univ. Hosp., Lorenskog, Norway

Abstract: Microtubule-associated protein Tau plays important roles in microtubule assembly and stabilization, neurite outgrowth, and axonal transport. However, hyper-phosphorylated Tau (p-Tau) forms abundant insoluble aggregates known as neurofibrillary tangles in the brains of patients with tauopathies such as Alzheimer’s disease (AD). Imbalance of production of soluble Tau and clearance of aggregated p-Tau with additional inactive autophagy (especially its subtype mitophagy) speed up AD progression. Our previous studies show turning up autophagy as a therapeutic strategy. Here, we find that a fruit-derives compound EFF-AA is able to induce autophagy/mitophagy in neurons and normalize p-Tau-related neuropathology in cell, worm & mouse models of AD. EFF-AA attenuates aggregation of heparin-assembled hTau P301S & promotes degradation of tau aggregates & suppresses the formation of p-Tau (Thr217). EFF-AA alleviates cognitive deficits in hTau[P301L] nematodes and hTau[P301S] mice&reduces neuroinflammation in 3D-cultured AD mini-brains. Mechanistically, EFF-AA enhances mitochondrial health via ULK1/UNC-51-dependent autophagy/mitophagy. EFF-AA has a high translational potential as it exhibits a favorable pharmacokinetic profile and crosses the blood-brain barrier in mice. Our study supports therapeutic strategies that targeting on autophagic/mitophagic clearance of intracellular pathological Tau with further clinical studies of EFF-AA on AD encouraged.

Disclosures: **S. Cao:** A. Employment/Salary (full or part-time):; University of Oslo and Akershus university Hospital. 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.; Molecule AG/VITADAO. C. Other Research Support (receipt of drugs, supplies, equipment or other in-

kind support); Akershus University Hospital. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Molecule AG/VITADAO. **E.F. Fang:** A. Employment/Salary (full or part-time):; University of Oslo and Akershus university Hospital. 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.; Molecule AG/VITADAO. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Akershus University Hospital. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Molecule AG/VITADAO.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.06/Web Only

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

Support: CIHR PJT-169197

Title: The effect of environmental factors in a rat pretangle tau model of alzheimer's disease: a single-nuclei transcriptomics study

Authors: *S. HASAN, S. TORRAVILLE, C. REINHARDT, T. BENOUKRAF, Q. YUAN; Biomed. Sci., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Alzheimer's disease (AD) is a widespread neurodegenerative disorder characterized by the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) in the brain. Tau pathology starts from abnormally phosphorylated pretangle tau, which occurs early in human life and originates in the locus coeruleus (LC). Recent research, including ours, has shown that pretangle tau is toxic and results in neuronal degeneration and cognitive impairment. The early onset of pretangle tau and the long prodromal period before AD diagnosis implies a potential interaction between environmental factors (EFs) and tau pathology during the preclinical stages of AD. Previous studies have shown that EFs, such as stress and enrichment, impact AD pathology. However, the precise relationship between EFs and tau pathology remains unclear. We developed a rat model that mimics the early onset of abnormally phosphorylated tau in the LC in humans. We investigate the interaction between EFs and pretangle tau using this model. We hypothesize that EFs induce cell-specific changes that alter plasticity markers in key brain structures involved in memory and affect tau pathology development. We infused AAVs carrying pseudo-phosphorylated human tau (AAV9-DIO-EGFP-htauE14) or control vector in the LC of 3-month-old TH-CRE rats. Early enrichment and stress were conducted by maternal separation of various durations at post-natal days 2-10. Late enrichment (social, physical, and environmental) and stress (chronic unpredictable stress) were carried out at 6-7 months. To identify critical molecular changes induced in AD, we conducted single-nuclei RNA sequencing

(snRNA-seq) to analyze cell-specific transcriptomic profiles of two distinct regions (LC and hippocampus) at 10-12 months post-infusion. This analysis aims to identify the involved pathways and affected cell types, addressing the tissue heterogeneity and transcriptome noise. We initially classified cells by examining the known cell marker genes, successfully characterizing all major neuronal and non-neuronal cell types. Differential expression analyses across the groups allow us to monitor artifacts associated with our model and accurately measure the stress and enrichment effects linked to AD. Importantly, our experimental design includes biological replicates, enabling statistically relevant assessments of cell-type-specific gene differential expression and the characterization of enriched ontologies and pathways. Overall, this study can provide valuable insights into transcriptional responses associated with tau pathology in relation to environmental effects at cellular resolution.

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Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.07/L2

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

Support: NIH Grant AG069112

Title: Variation in unsaturated fatty acid length and unsaturation impacts tau aggregation in vitro

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Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Toxic tau aggregation is a common hallmark in many diseases collectively known as tauopathies. Studying tau aggregation *in vitro* provides a quick way to screen for therapeutic drugs that target tau aggregation. However, for tau to aggregate *in vitro* a cofactor is needed to induce aggregation. Previous research has shown that polyunsaturated fatty acids (PUFA) can promote *in vitro* tau to aggregate and form filaments. The PUFA arachidonic acid (ARA) has been extensively used for *in vitro* tau aggregation studies, with an optimal concentration ratio of 75μM ARA to 2μM tau protein to produce the most amount of aggregation. While it is known that other PUFAs induce tau aggregation, it is not known how the structure of a PUFA can influence aggregation. In this study we aim to determine how the carbon chain length and number of double bonds of a PUFA can impact the optimal concentration ratio of inducer to protein for tau aggregation, and to determine if PUFAs potentially produce unique aggregate structures that can replicate the structures found in disease. This was done by utilizing seven different PUFAs, starting with linoleic acid (LA) with a carbon chain length of 18 and 2 double bonds. The amount of tau aggregation was measured for a range of LA concentrations from 25μM to 125μM. Tau aggregation was measured after a 24-hour incubation of tau with LA, and

measured with Thioflavin T fluorescence assay, and right-angle laser light scattering. Optimal concentration was determined based on the concentration of LA that produced the most aggregation. This process was repeated for the remaining PUFAs, which differed from LA by an increasing carbon chain length, number of double bonds, or both. The seven PUFAs were compared based on structure to determine if only changing chain length or double bonds would affect the optimal concentration and to determine any patterns. The results showed that changing either chain length or number of double bonds does result in changes in optimal concentration of PUFA to produce the maximum amount of aggregation. The results also show that PUFAs differing by both chain length and double bonds by two have the same optimal concentration patterns. These results suggest that the differences in PUFA optimal concentrations could potentially be an indication that PUFAs produce different molecular structures of tau aggregates. The structure of tau aggregation induced by various PUFAs will be examined in future studies with cryo-electron microscopy to compare to the structures of tau aggregates found in various tauopathies.

Disclosures: C.A. Garcia: None. **T.C. Gamblin:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.08/L3

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

Support: NIH AG064231

Title: Locus Coeruleus Noradrenaline is Required for Sleep Loss Cleavage of Tau and Amyloid Precursor Proteins

Authors: *S. VEASEY[1], Y. ZHU[1], P. V. FENIK[1], S. A. THOMAS[2];
[1]Med., Perelman Sch. of Med. Univ. of Pennsylvania, Philadelphia, PA; [2]Pharmacol., Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Chronic sleep disruption (CSD) commonly occurs, and yet research supports the concept that sleep disruption increases the risk of Alzheimer’s disease (AD). A major gap in our scientific knowledge is how specifically sleep disruption influences AD pathogenesis. CSD in wild-type mice results in early injury to and loss of noradrenergic (NA) locus coeruleus neurons (LCn). One year following CSD, neural injury in the hippocampus is evidenced as CA1 neuron loss, gliosis, increased phosphorylated tau and $A\beta_{42}$ peptide, and persistent spatial memory impairment. Across the lifespan, LCn are the first neurons to accumulate hyperphosphorylated tau, and across Braak stages of AD, LCn are progressively lost. Yet, whether the LCn are victims or victims turned culprit in AD is unclear. Sleep disruption increases NA production and metabolism in LCn. A metabolite of NA, DOPEGAL, was recently shown to activate asparagine endopeptidase (AEP), prompting cleavage of tau at N368 and amyloid precursor protein (APP) at

N373 and N585. These fragments increase tau aggregation, propagation, and promote APP cleavage to $A\beta_{42}$. We hypothesized that CSD would increase NA and activate AEP, increasing N373 tau and N373APP in LCn, while mice deficient in NA would confer resistance. We first confirmed that in the LC CSD increased the NA synthesizing enzymes tyrosine hydroxylase (TH, $p<0.001$) and dopamine beta-hydroxylase (DBH, $p<0.0001$). Adult male and female mice (n=6/genotype, sleep condition) homozygous for DBH deficiency (no NA) and mice heterozygous (normal NA) were randomized to CSD on a rotor platform for 16 weeks and then examined at ages 13-15 months. Analyzing mean gray values of fluorescence in confocal images of tau N368 immunolabeling in TH+ LCn, with scorers blinded to conditions, there were overall differences by two-way ANOVA, $p<0.01$. Using Tukey's post hoc analyses, Dbh+/- CSD mice showed greater mean gray values than all 3 of the other groups, and in Dbh-/- mice there was no effect from CSD on tau N368. In contrast, the mean gray values for total tau immunofluorescence within LCn of the same sections showed no genotype or sleep condition effects. A similar genotype/sleep condition response was observed for APP N373, with a CSD increase in LCn neurons only in the Dbh+/- mice. $A\beta_{42}$ puncta density in the hippocampus and increased ($p<0.05$) in Dbh+/- mice exposed to CSD but not in Dbh-/- mice exposed to CSD (N.S.). Collectively these results identify a novel mechanism whereby CSD activates AEP, which then incites cleavage of both tau and APP into toxic fragments associated with AD pathogenesis in the LCn, unveiling how sleep loss changes in LCn can contribute to AD pathogenesis.

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Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.09/L4

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

Title: The effects of sleepdysregulationon the progression of Alzheimer's disease in a fruit fly model.

Authors: *R. RUBIO[1], F. AMAYA REYES[2], A. BRAN[1], E. WOO[2], D. D. LENT[3];
[1]Biol., [2]Psychology, CSU, Fresno, Fresno, CA; [3]Biol., CSU Fresno, Fresno, CA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease connected to sleep dysregulation and insomnia. This is in addition to the well known cognitive and memory deficits. As the disease progresses the less sleep the individual gets, and the less sleep an individual gets the more the disease progresses. Sleep is a critical biological function and is known to among other things be critical for waking cognition. This study reveals a trend of sleep deprivation and tau pathology; a protein responsible for AD. *Drosophila melanogaster*, fruit flies, is the animal model system used to understand how sleep disruption affects tau pathology progression and subsequently impacts cognition. Sleep depriving fruit flies expressing tau in the

mushroom bodies reveals an accelerated progression of neurodegeneration compared to control groups. In a spatial learning task, fruit flies suffering from sleep deprivation perform more poorly compared to the fruit flies with no condition. This research would help further establish the fruit fly as a holistic model to study AD and would allow for the model to be used to draw correlations to sleep, AD, and the human circadian rhythm.

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Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

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Program #/Poster #: PSTR530.10/L5

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

Support: NIH grant AG077610
NIH grant AG079141

Title: Ad risk factor bin1 modulates tau pathology in a diabetes mouse model

Authors: *D. MOREIRA-SILVA, M. YUKSEL, M. DELOZIER, M. PONNUSAMI, S. PATEL, N. RAM, V. SKOROBOVENKO, S. WANG, L. COLLIER, G. THINAKARAN; Mol. Med., Univ. of South Florida, Tampa, FL

Abstract: Bridging Integrator 1 (*BIN1*) is the second most prevalent risk factor gene for late-onset Alzheimer's disease (LOAD). BIN1 modulates synaptic vesicle dynamics, can bind to cytosolic tau, influence tau spread, and contribute to neurodegeneration in AD. The accumulation of phosphorylated-tau (p-tau) induces microtubule destabilization, synaptic dysfunctions, and memory impairment in LOAD and diabetes. Diabetes shares many pathological features with AD, such as protein misfolding, insulin resistance, and cognitive deficits, increasing the risk of developing LOAD. Although BIN1 expression correlates with the memory performance of diabetic patients, the influence of BIN1 as a risk factor for individuals with diabetes to develop tau pathology is still unexplored. Hence, this study investigated whether the BIN1 expression influences tau pathology and behavioral responses under streptozotocin (STZ)-induced diabetes conditions. *Emx*-Cre:*Bin1*-cKO mice lacking forebrain BIN1 expression in the wildtype (*Bin1*-cKO) and the tau transgenic background (PS19:*Bin1*-cKO) were generated and injected for 5 days with STZ (40 mg/kg) at 2 months of age. *Emx*-Cre and *Emx*-Cre:*Bin1*-cKO mice were used as negative controls for tauopathy. All animals underwent behavioral tests at 4- and 8 months of age and were euthanized at 9 months. The brains and pancreas were harvested for immunofluorescence, advanced microscopy, and molecular analysis of markers related to diabetes, neurodegeneration, and tauopathy. The results showed that STZ-injected PS19 mice present fewer insulin-positive cells in the pancreatic islets than the vehicle group, mimicking important diabetes hallmarks. STZ-injected PS19 mice tend to accumulate more p-tau

in the brain, especially in the visual cortex. Interestingly, *Bin1*-cKO and PS19:*Bin1*-cKO mice presented behavior deficits, including a decrease in exploration in the novel object recognition and an increase of anxiety in the open field, and motor deficits in the rotarod and composite phenotype scoring in comparison with their respective *Emx*-Cre controls. PS19:*Bin1*-cKO mice also displayed mitigated tau pathology and neuroinflammation in the hippocampus and cortex, whereas STZ exacerbated the pathology in the same brain regions. Further histopathological and transcriptomic analyses are needed to understand the connection between diabetes and BIN1 function in tau pathophysiology. The successful outcome of this research would be a crucial step for the future use of BIN1 as a predictive factor for behavioral deficits and pathological extent in patients with AD, diabetes, or both diseases.

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Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.11/L6

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

Title: Neuroinflammatory response: is CASM (conjugation of LC3/ATG8 to single membranes) ready to perform?

Authors: *N. TRUONG;
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Abstract: Neuroinflammatory response: is CASM (conjugation of LC3/ATG8 to single membranes) ready to perform?
AuthorsNhi L.P. Truong [1, 2], Bradlee L. Heckmann [1, 2, *1] Department of Molecular Medicine, University of South Florida Morsani College of Medicine, Tampa, FL, USA.[2] University of South Florida Health Byrd Alzheimer's Center and Neuroscience Institute, Tampa, FL, USA.
DisclosuresNhi L. P. Truong: None. **Bradlee L. Heckmann:** None.
AbstractThe innate control of adaptive immunity is a well-established paradigm in which recycling concerning cross-presentation primes T-cell responses. LC3-associated phagocytosis (LAP), a receptor-mediated process for the engulfment of large particles, has been shown to be involved in antigen presentation. LC3-associated endocytosis (LANDO), an endocytic process ingesting and internalizing extracellular molecules to modulate degradation, can display a similar function depending on the size of the cargo. LANDO can also facilitate receptor recycling. LAP and LANDO are non-canonical autophagy processes, which have the umbrella term CASM (the conjugation of ATG8 family members to single membranes). There is a critical knowledge gap in understanding how CASM is involved in the process of immunology presentation and modulates the immune response, especially in the central nervous system (CNS). We report that

the inhibition of CASM leads to the sputtering of receptor recycling and extracellular beta-amyloid accumulation, causing neuroinflammation. We also observe that the recycling of several immune receptors, especially those of microglia, has a dependency on LANDO. Strikingly, new evidence suggests a potential role for T cells in adaptive immunology in neurodegenerative diseases. However, the
contribution of CASM to the adaptive immune response in the CNS is unknown. We aim to continue looking at the consequences of CASM deficiency in different neurodegenerative models, such as 5XFAD, PS-19, and h-Tau, thereby focusing on the primary inflammatory activation in innate immune cells and the adaptive immune responses in T cells. Using amyloidosis- and fibril-induced models *in vitro*, we examine if CASM regulates the recycling of T-cell receptors and plays crucial roles in the microglia-T cell crosstalk. This study will be the first to explore the potential roles of CASM in innate and adaptive immune responses with both *in vitro* and *in vivo* approaches, using a variety of different neurodegenerative mouse models. The findings from this study can contribute to a broader immune viewpoint of the CNS.

Disclosures: **N. Truong:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.12/L7

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

Support: NIH

Title: Analysis of spontaneous behaviors by machine learning reveals beneficial effects of tau reduction in models for Alzheimer's disease and Dravet syndrome

Authors: *P. NAMBIAR[1,2], R. A. GONÇALVES[1,2], S. R. MILLER[1,2], A. PICO[3], R. THOMAS[3], E. SHAO[1,2], G.-Q. YU[2], L. MUCKE[1,2], J. J. PALOP[1,2];
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Abstract: Many neurological and psychiatric disorders cause behavioral changes, but our knowledge of disease-induced spontaneous behavioral alterations is incomplete. To identify alterations in spontaneous behaviors of mouse models for Alzheimer's disease (5xFAD mice) and Dravet syndrome (*Scn1a*$^{RX/+}$ mice), we developed a highly sensitive machine learning imaging platform (ethoML) capable of deconstructing full sequences of mouse behavior into canonical behavioral units (motifs). Using this platform, we found robust alterations in spontaneous behaviors in 5xFAD and *Scn1a*$^{RX/+}$ mice, as compared to wildtype controls. The disease models differed from controls in their usage of 25-50% of the identified motifs, with 5xFAD and *Scn1a*$^{RX/+}$ mice showing distinct patterns of abnormalities. Genetic ablation of tau in 5xFAD mice and early postnatal knockdown of tau with an antisense oligonucleotide in

Scn1a$^{RX/+}$ mice markedly reduced these abnormalities. We conclude that the ethoML platform provides useful measures of neural dysfunction in mouse models of complex brain disorders as well as for the preclinical assessment of related therapeutic interventions.

Disclosures: P. Nambiar: None. **R.A. Gonçalves:** None. **S.R. Miller:** None. **A. Pico:** None. **R. Thomas:** None. **E. Shao:** None. **G. Yu:** None. **L. Mucke:** None. **J.J. Palop:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.13/L8

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

Support: Florida Department of Health Ed
Ethel Moore Alzheimer's Disease Research Program Award 21A11
McKnight Brain Research Foundation

Title: Effects of chronic cannabis smoke exposure on inflammatory markers and tau pathology in mice

Authors: *E. GAZAROV[1], B. MCCRACKEN[2], S. ZEQUEIRA[2], J. HOWARD[2], J. M. LEWIS[2], J. L. BIZON[2], B. SETLOW[3];
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Abstract: With the rise in cannabis use among older adults, as well as increasing cases of Alzheimer's disease (AD), there is a need to understand how cannabis impacts the aging brain and AD pathology. Aging is associated with an increase in chronic low-grade inflammation, which plays a role in the pathogenesis of Alzheimer's disease. Cannabinoids can reduce inflammatory markers, protect against oxidative stress, and reduce plaque burden in mouse models of AD-like pathology; however, studies of cannabinoids on aging and AD-like pathology have primarily used methods of cannabinoid administration that do not effectively model human use, and have focused on models of amyloidosis rather than tauopathy. To address these points, we are evaluating the effects of chronic cannabis smoke exposure on inflammatory markers in young and aged mice, and tau pathology in rTg4510 mutant tau transgenic mice.
To determine the effects of cannabis smoke on peripheral and brain markers of inflammation, we exposed young adult (4 months old) and aged (22 months old) C57BL/6J mice (n = 40, half female) to smoke from burning either cannabis (5.9% THC) or placebo (0% THC) cigarettes daily for 30 days. Serum and brain lysate were analyzed for 40 cytokines using Quantibody cytokine arrays (RayBiotech, Peachtree Corners, Georgia). Additionally, rTg4510 tau-overexpressing transgenic mice (n = 23, 4 months old) were exposed to cannabis or placebo smoke daily for 6 weeks. Immunohistochemical analyses for pathological tau using MC1, antibodies to a number of tau phosphoepitopes, as well as Iba1 and GFAP to measure gliosis will be conducted. Soluble and sarkosyl-insoluble tau levels and phosphorylation state will also be

assessed.
Results from the inflammation study revealed that aged mice had significantly higher IL-12p40 levels and significantly lower levels of galectin-3 in serum compared to young-adult mice. Additionally, aged female mice exposed to cannabis smoke had significantly higher levels of RANTES in serum compared to young adult female mice and aged female mice exposed to placebo smoke, which was not evident in aged male mice and suggests sex-specific effects of cannabis on aging in this model. Analyses of brain inflammatory markers and effects of cannabis on rTg4510 tau pathology are ongoing.

Disclosures: **E. Gazarov:** None. **B. McCracken:** None. **S. Zequeira:** None. **J. Howard:** None. **J.M. Lewis:** None. **J.L. Bizon:** None. **B. Setlow:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.14/M1

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

Support: NIH grant AG077610
NIH grant AG079141
NIH grant AG056061

Title: The m6A Reader YTHDF1 modulates inflammatory responsesin the P301S mouse model

Authors: *S. WANG[1], M. PONNUSAMY[1], G. THINAKARAN[2];
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Abstract: N6-methyladenosine (m^6A) is the most abundant epitranscriptional modification in eukaryotic mRNA and non-coding RNA. The dynamic and reversible modification has been documented to play essential roles in regulating gene expression, splicing, RNA editing, RNA stability, and controlling mRNA lifespan and degradation. Emerging evidence indicates mRNA methylation is involved in Alzheimer's disease (AD) through the mitochondrial pathway, inflammatory response, and oxidative stress. The aggregation of misfolded tau protein in tangles inside the cell is a hallmark of AD. Tau-mediated neurodegeneration also occurs through a mechanism mediated by RNA-binding proteins and the translational stress response. A recent study linked tau oligomers with stress granules, which recruit RNA-binding proteins and the m^6A transcripts and contribute to tau oligomer toxicity in the cytoplasm. The assembly of such complexes, which is increased in the brain tissue of individuals with and mouse models of AD, is part of a stress response that involves the formation of stress granules and reduced protein synthesis. How m^6A affects this process is largely unknown. To accomplish this, I generated *Ythdf1* (one of the cytoplasmic m^6A reader genes) knockout mice in the background of a transgenic tauopathy model (PS19:*Ythdf1*$^{-/-}$) to study the role of m^6A methylation as it relates to

tau pathogenesis. Comparing with PS19 mice, the 9-month-old PS19:*Ythdf1*$^{-/-}$ mice results show higher soluble phosphorylated tau in high salt RAB buffer but not in RIPA buffer. Insoluble phosphorylated tau extracted in 70% formic acid was decreased in PS19:*Ythdf1*$^{-/-}$. Loss of YTHDF1 reduced the hyperactivity in this tauopathy model and ameliorated the working memory deficits in PS19 mice in the novel object recognition test. As an unbiased approach to gaining molecular insights, we performed RNAseq analysis of transcriptome changes in PS19 and PS19:Ythdf1-/- mice brains. From bulk RNAseq results, we found that deletion of YTHDF1 in the tauopathy mouse model suppressed the inflammatory responses for both genders**.** The gene ontology analysis on the downregulated differentially expressed genes (such as *Ccl6*, *Cst7*, *Itgax*, *Ccl4*, and *Clec7a*) indicates that the YTHDF1 as an m^{6}A reader protein plays an essential role in promoting tau pathology-related immune response in the brain.

Disclosures: S. Wang: None. **M. Ponnusamy:** None. **G. Thinakaran:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.15/M2

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

Support: NIH/NIA R01AG071512
NIH/NIA R21AG073684
NIH/NIDA 90098173
AHA/Paul Allen Frontiers Grant 90094834
Johns Hopkins Catalyst Award to B.D.P.

Title: Cystathionine γ-lyase hydrogen sulfide axis ameliorates neurotoxicity-associated with tau in Alzheimer’s disease

Authors: *S. CHAKRABORTY[1], S. J. TRIPATHI[1], D. GIOVINAZZO[2], S. H. SNYDER[1,2,3], M. R. FILIPOVIC[4], B. D. PAUL[1,2,3,5];
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Abstract: Alzheimer’s disease (AD) is one of the leading causes of dementia and age-associated neurodegeneration, which is characterized by progressive cognitive decline, memory loss, and reduced executive functioning. AD-associated neuropathological hallmarks include neurofibrillary tangles (NFTs), paired helical filaments, and amyloid plaques. Tau, a microtubule-associated protein undergoes extensive hyperphosphorylation, misfolding, and is translocated to the somatodendritc compartment and forms NFTs. Our recent study and that of others have demonstrated a crucial role of the reverse transsulfuration pathway in AD. Notably,

the production of hydrogen sulfide (H_2S) by its neuronal biosynthetic enzyme, cystathionine γ-lyase (CSE) is diminished in AD. In addition, H_2S produced by CSE precludes the hyperphosphorylation of tau via sulfhydrating and inhibiting tau kinases. Furthermore, we demonstrated that CSE binds to wild-type tau and increases its catalytic activity. Although we showed an interaction between CSE and tau, it is currently unknown which isoforms of tau bind to CSE and how this differential binding could influence microtubule dynamics. Accordingly in the current study, we characterized the binding of different tau isoforms to CSE and evaluated its effect on the microtubule dynamics and sulfhydration of different kinases. We also studied the role of H_2S donors on microtubule dynamics. We found that CSE binds to tau isoforms and affects microtubule dynamics. Additionally, H_2S donors inhibit cyclin-dependent kinase 5 (CDK5) and GSK-3β to decrease tau hyperphosphorylation. Taken together, upregulation of CSE and enhanced H_2S signaling might reverse regressive plasticity and neurodegeneration.

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Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.16/M3

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

Support: NIH Grant AG0041274
NIH Grant AG062509
NIH Grant AG062509

Title: The monocyte-mediated effect of blood phosphorylated Tau on cognitive function

Authors: Y. DONG[1], S. GAN[2], H. LIU[3], F. LIANG[1], M. XU[1], A. SONG[4], E. HUANG[4], W. LI[1], Y. ZHANG[1], G. YANG[5], ***Z. XIE**[1];
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Abstract: Introduction: Perioperative neurocognitive disorder is associated with an increased risk of Alzheimer's disease (AD) and AD-related dementias (ADRD). Blood phosphorylated Tau (pTau) or the mitochondria permeability transition pore component Cyclophilin D (CypD) can regulate cognitive function. Thus, our study aimed to investigate the interaction between pTau and CypD in blood monocytes and its impact on inflammation and cognitive function in mice. **Methods**: Female 9-month-old wild-type, Tau knockout (KO), cyclophilin D KO, and aged (18-month-old) mice were assigned to either an anesthesia/surgery group or a control group. We assessed the effects of anesthesia/surgery on inflammation, Tau phosphorylation at threonine 217

(Tau-PT217), CypD, and NF-kB in blood monocytes, as well as cognitive function using various techniques including monocyte isolation, Co-immunoprecipitation, and fear conditioning system. Furthermore, we investigated the impact of Tau-PT217 peptide on TNF-α levels in harvested monocytes. Lastly, we examined Tau trafficking from blood to monocytes and its interaction with CypD using immunostaining and nano-beam technology. **Results**: Anesthesia/surgery increased the levels of Tau-PT217 and TNF-α in blood, as well as the levels of Tau-PT217, CypD, and NF-kB in monocytes, leading to cognitive impairment in mice. However, these effects were not observed in Tau KO or CypD KO mice. Tau and Tau-PT217 were found to bind to CypD within monocytes obtained from mice after anesthesia/surgery. Additionally, anesthesia/surgery seemed to facilitate the trafficking of Tau from blood to monocytes, resulting in the generation of TNF-α. Finally, treatment with Tau-PT217 peptide increased TNF-α levels in monocytes. **Conclusion**: Our findings indicate that anesthesia/surgery can promote the production of Tau-PT217 in blood and induce the trafficking of Tau and Tau-PT217 from blood to monocytes, ultimately leading to CypD-dependent inflammation and cognitive impairment in mice.

Disclosures: Y. Dong: None. **S. Gan:** None. **H. Liu:** None. **F. liang:** None. **M. xu:** None. **A. Song:** None. **E. huang:** None. **W. li:** None. **Y. zhang:** None. **G. Yang:** None. **Z. Xie:** None.

Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.17/M4

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

Support: NIH Grant R01AG067617

Title: Stimulating mitophagy reverses behavioural deficits in a novel Caenorhabditis elegans Alzheimer’s disease model

Authors: *U. GANGULY[1], T. CARROLL[2], G. JOHNSON[1], K. NEHRKE[2];
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Abstract: Accumulation of damaged mitochondria and misfolded tau (rich in post-translational modifications or PTMs, particularly phosphorylation) are important causative factors for Alzheimer’s disease (AD). Neurons are highly energy-demanding which makes mitochondria a critical regulator of aging and neurodegeneration. However, how these two parallel pathways of tau-phosphorylation and mitochondrial dysfunction converge in neurodegeneration is still not well understood. Previous studies from our lab report that phospho-mimetic mutations of tau PTM sites that appear early in AD can drive age-dependent neurodegeneration and also selectively inhibit oxidative-stress-induced mitophagy in a novel *C. elegans* AD model [Guha, 2020, 2022]. Here, we tested the hypothesis that restoring mitophagy by pharmacological

stimulation would improve the neurodegenerative phenotypes in mutant animals. Worms expressing wild-type human tau (0N4R) or mutant tau T231E in the mechanosensory neurons of *C. elegans* were treated with a variety of pharmacological molecules known to stimulate mitophagy- nicotinamide mononucleotide (NMN), urolithin A (UA), epigallocatechin 3-gallate (EGCG), and celastrol. The worms were assayed for sensation to light touch and mitophagy at different time points representing "young" and "old" animals (Day 3 and Day 10 of adulthood). Our results indicate that the pharmacological stimulators of mitophagy (NMN, UA, and EGCG) can induce mitophagy and thereby reverse the touch deficits in young worms (Day 3) but fail to stimulate mitophagy (and fail to suppress the touch deficit) in older animals (Day 10). This is consistent with many adaptive regiments becoming less effective with age. In contrast, celastrol is able to stimulate mitophagy and also to reverse touch deficits in both young and old worms, apparently overcoming this effect of aging – potentially through a novel mechanism of action that may be relevant to treating AD in aged individuals. Previous work suggests that celastrol acts through a non-canonical pathway involving liquid-liquid phase separation of Nurr77 and also by inhibiting mechanistic target of rapamycin (mTORC1) activation via transcription factor EB. We hypothesize that age-dependent hyperactivation of the mTORC1 together with the presence of phospho-tau may play critical roles in the clearance of damaged mitochondria. We are exploring whether mTORC1 mutant alleles in *raga-1* (Ras-related GTP binding A) and *rsks-1* (Ribosomal protein S6 kinase 1) can stimulate mitophagy and recover touch deficits elicited by phospho-tau.

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Poster

PSTR530. Tau: Pathology, Behavior, and Interventions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR530.18/M5

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

Support: NINDS Grant T32 NS007224
R01 AG061190-01
1R21AG079145-01

Title: Exploring the relationship between loss of calcium regulation and tau hyperphosphorylation in AD

Authors: *I. J. PERONE[1], D. WIJEGUNAWARDANA[3], D. BOLAT[1], J. ARELLANO[1], A. DUQUE[4], C. VAN DYCK[5], A. F. T. ARNSTEN[6], D. DATTA[2];
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Abstract: The primary pathological hallmarks of Alzheimer's disease (AD) consist of extracellular of amyloid beta (Aβ) plaques and intraneuronal neurofibrillary tangles comprised of hyperphosphorylated, fibrillated tau. While the rare, early onset, autosomal dominant forms of the disease are caused by genetic mutations, the etiology of more common, late-onset AD remains unknown. Decades of research suggest that dysregulated calcium signaling is an early toxic event associated with the rise of tau pathology, e.g. via calpain activation of GSK3b, which hyperphosphorylates tau at pT217Tau and other epitopes. For example, the pyramidal cells in dorsolateral prefrontal cortex (dlPFC) most vulnerable to tau pathology express the calcium-binding protein, calbindin, when young (Exp Neurol 111:293-301, 1991) but lose calbindin with age (Biol Psych 57:549-58, 2005; Alzh&Dem17:920-32), while the layer II cell islands in entorhinal cortex (ERC) most vulnerable to tau pathology never express calbindin (J Comp Neurol 321:241-66, 1992). In contrast, surrounding ERC pyramidal cells do express calbindin and develop tau pathology at a later age. The current study examined the relationship between calcium dysregulation and the accumulation of hyperphosphorylated tau with advancing age in rhesus macaques with naturally-occurring tau pathology (Alzh&Dem 14:680-91, 2018). We used multiple label immunofluorescence (MLIF) to determine the spatial and temporal pattern of calbindin vs. pT217Tau expression in aging macaque ERC (layer II) and dlPFC (layer III), two cortical areas with magnified calcium signaling that are vulnerable to tau pathology in AD (Mol Psych 26:3684-700, 2021), and focused on the pT217Tau epitope due to its potential as a CSF and plasma biomarker for AD. We examined tissue from three age groups, middle aged adults (~8-10y, n=3), "younger" aged (~18-20y, n=2), and old aged (~28-31y, n=3); we previously determined that earliest stage tau pathology first emerges in layer II ERC in middle age and at later ages in dlPFC. Emerging data indicate that there is a decrease in calbindin positive neurons and an increase in pT217Tau with age, and that the cells with lower levels of calbindin also had higher levels of pT217Tau positivity. These data suggest that in neurons with high levels of calcium signaling, calbindin is needed to reduce calcium's toxic effects on tau hyperphosphorylation.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.01/M7

Topic: C.03. Parkinson's Disease

Support: NIH Grant P50-NS123109
NIH Grant P30-NS076408

Title: Behavioral Outcomes in a Parkinson's Disease Patient during Evoked Interference Closed-Loop Deep Brain Stimulation

Authors: *B. PARKS[1], B. MOHANTY[1], K. J. O'NEILL, III[1], S. L. ALBERICO[1], M. HILL[1], D. BAUER[1], B. POBIEL[1], D. E. SANABRIA[1], M. C. PARK[2], L. A. JOHNSON[1], J. WANG[1], J. L. VITEK[1], J. E. AMAN[1];
[1]Dept. of Neurol., [2]Dept. of Neurosurg., Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Parkinson's disease is often associated with increased beta oscillations[1-2], but a causal relationship between beta power and motor sign severity has not been established. Previous studies have selectively modified beta power through phase-specific stimulation termed evoked interference deep brain stimulation (eiDBS) in the globus pallidus internus (GPi)[3]. In this study, we aim to investigate causal effects of modulating beta power during a movement task. **Methods:** eiDBS was accomplished via an "externalized" segmented DBS lead. Detailed methods and procedures are described by Sanabria et al.[3-4]. Briefly, LFP data was acquired and single stimulation pulses were delivered at specific phases of the oscillation to maximally *amplify* (-75°) their beta oscillations (peak beta ± 3 Hz). Based on simulations we also stimulated 180° off that phase (105°) to test peak beta *suppression* during a reach-to-target task[3]. Task cues were presented on a touchscreen; the task was performed under different medication (on/off) and phase stim conditions (off/-75°/105°). Hand peak acceleration / jerk, reaction time, and reach duration were calculated based on inertial monitoring unit sensors (Delsys Inc.). Band-specific analysis focused on beta band (12-30 Hz) and subject-specific peak beta band (20 ± 3 Hz). **Results:** Off medication, -75° phase stim led to a significant decrease in peak acceleration and jerk compared to no stim ($p < 0.05$). Unexpectedly, reaction time decreased and became less variable compared to other stimulation conditions ($p < 0.05$). On medication, -75° phase stim resulted in a slight, but significant, reduction in peak acceleration compared to no stim. All med/stim permutations, other than -75° phase stim / off med, showed increased variability during reaction time compared to no stim ($p < 0.05$). Spectral analysis showed increased beta power during -75° phase stim compared to no stim, which was then reduced during 105° phase stim. **Conclusions:** Here we show the ability to alter reach kinematics by selectively modulating beta oscillations in the GPi. However, during 105° phase stim, oscillatory activity in frequencies immediately adjacent to the target band were unintentionally increased in amplitude, increasing the overall power of beta relative to no stimulation. This may explain the decreases in peak acceleration and jerk relative to no stimulation. Faster and less variable reaction times during -75° phase stim potentially represents a change in nearby non-motor pathway activation resulting in faster reaction. Work is ongoing to reduce adjacent beta increases, and to determine the clinical significance of these findings.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.02/M8

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01-NS081118

Title: Neural Pathways Underlying Gait Changes During VIM DBS in Essential Tremor Patients

Authors: *M. BLUMENFELD, R. BUTLER, A. BRINDA, J. KRIEG, D. MARTICORENA, C. SPENCER, D. SULLIVAN, S. PANDEY, T. PALNITKAR, R. PATRIAT, J. VITEK, L. SCHROCK, K. DOMINGO, T. ORCUTT, N. HAREL, S. COOPER, J. MATSUMOTO, M. JOHNSON;
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Abstract: Objective: Relate neural pathway activation predicted by subject-specific computational models ofthalamic deep brain stimulation (DBS)to kinematic changes in tandem gait during DBS for EssentialTremor (ET).**Introduction:** While the hallmark symptoms of ET include kinetic and postural tremor, other non-tremorsymptoms including gait dysfunction are common. DBS therapy targeting the ventral intermediatenucleus (VIM)of the thalamus can effectively reduce tremor, and has been shown to reduce gaitsymptoms in some cases. However, VIM DBS can also induce gait side effects including gait ataxia andbalance disturbances, though gait outcomes are unpredictable and the pathways responsible for thesechanges are unknown. In this study, we developed subject-specific computational pathway activationmodels and used quantitative gait analysis to assess the relationship between neural pathway activationduring VIM DBS and the corresponding changes in tandem gait.**Methods:** Pre-operative magnetic resonance (7T)images and post-operative CT images were used toconstruct subject-specific computational models predicting neural pathway activation during VIM DBS.Models were constructed for 12 directional DBS leads implanted across 7 subjects with ET, and includedprojections from the motor cortex and dentate nucleus of the cerebellum to motor thalamus and zonaincerta. Subjects completed a tandem gait task with DBS off and at 10 monopolar stimulationconfigurations. Gait trials were scored based on the Brief Ataxia Rating Scale (BARS)and markerlesspose estimation was used to track 3D joint positions across video data from each trial. Joint positionswere used to calculate outcome metrics including step cycle time and wrist-torso distance. Linear mixedeffects and logistic regression models were used to identify the relationship between activation ofmodeled neural pathways and change in BARS score and tandem gait metrics relative to baseline.**Results:** Activation of axons projecting from the dentate nucleus to the external VIM (VimE)corresponded to increased BARS score ($p<0.005$)and mean and variance of wrist-torso deviation($p<0.05$), indicative of gait worsening. Activation of axons projecting between motor cortex and VimEcorresponded to decreased mean step cycle time ($p<0.05$)and mean wrist-torso deviation ($p<0.005$),indicating gait improvement.**Discussion:** Activation of axons projecting from deep cerebellar nuclei to the lateral motor thalamus maybe associated with DBS-induced gait side effects, while activation of axons projecting from motor cortexto motor thalamus may have beneficial effects on disease-related gait symptoms in ET.

Disclosures: M. Blumenfeld: None. **R. Butler:** None. **A. Brinda:** None. **J. Krieg:** None. **D. Marticorena:** None. **C. Spencer:** None. **D. Sullivan:** None. **S. Pandey:** None. **T. Palnitkar:**

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.03/M9

Topic: C.03. Parkinson's Disease

Support: P50-NS123109
P30-NS076408

Title: High frequency oscillations in the basal ganglia of Parkinson's disease and dystonia: A causality of synchronized neuronal bursting?

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Abstract: Background: Previous studies show high frequency oscillations (HFOs) in local field potentials (LFPs) in patients with Parkinson's disease (PD) and dystonia. Neuronal recordings in these patients have also demonstrated irregular firing patterns and bursting. In this study we explored the power and prevalence of HFO activity in the subthalamic nucleus (STN) and globus pallidus internus (GPi) of patients with PD and those with dystonia using microelectrode recordings collected during DBS lead placement surgery.
Methods: Rest recordings were collected from 41 awake patients; 36 GPi surgeries from 25 PD patients (1,592 channels recorded), 15 STN surgeries from 12 PD patients (496 channels) and 7 GPi surgeries from 4 dystonia patients (306 channels). During microelectrode mapping, neural activity was collected every 1-2 millimeters or upon single-unit isolation from the STN or GPi using three simultaneous microelectrodes each containing a micro (tip) and macro (ring) contact separated by 3.5 mm (Neuro Omega, Alpha Omega). To isolate HFO activity each recorded channel was lowpass filtered to 500 Hz and downsampled to 1000 Hz. Power spectral density (PSD) estimates were computed using Welch's method. HFO power (150-350 Hz) was identified as the height above the 1/f curve based on a gaussian best fit using the FOOOF toolbox. Significance was determined with the Kruskal-Wallis test controlling for multiple comparisons with the Tukey's HSD.
Results/Discussion: Significant HFO activity was identified in 900 channels (57%) in PD patients targeting GPi, 68 channels (14%) in PD patients targeting STN, and 108 channels (35%) in dystonia patients targeting GPi. HFO peak power in GPi of PD patients was significantly higher compared to peak power outside of GPi in PD patients ($p<0.001$). HFO peak power in GPi of PD patients was also significantly higher compared to HFO peak power in STN of PD

patients and GPi of dystonia patients ($p<0.001$). Preliminary analysis of 112 GPi neurons (12 PD patients) showed GPi spike-LFP coupling was not evident when aligned to individual spikes but rather when aligned to bursts of spike activity and showed a predominance of ~200-250 Hz instantaneous intra-burst firing rates, similar to predominating frequencies observed in LFPs. **Conclusion:** These data suggest the peak power of HFOs is distinct to GPi in PD patients, indicating a location and potentially disease-specific biomarker of PD. Irregular bursting activity in STN of PD and GPi of PD and dystonia patients could underlie the development of these HFO's. Further analysis to explore the role of neuronal firing patterns in the etiology of HFOs is underway.

Disclosures: K.J. O'Neill: None. **S.L. Alberico:** None. **M. Hill:** None. **B. Parks:** None. **B. Pobiel:** None. **D.L. Bauer:** None. **L.A. Johnson:** None. **J. Wang:** None. **L.E. Schrock:** None. **S.E. Cooper:** None. **L.B. de Almeida:** None. **D.P. Darrow:** None. **R.A. McGovern:** None. **M.C. Park:** None. **J.E. Aman:** F. Consulting Fees (e.g., advisory boards); Surgical Information Sciences. **J.L. Vitek:** F. Consulting Fees (e.g., advisory boards); Medtronic, Boston Scientific, Abbott, Surgical Information Sciences.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

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Topic: C.03. Parkinson's Disease

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R01 NS110613
R01NS131371
MnDRIVE Brain Conditions Program
Engdahl Family Foundation

Title: Basal ganglia beta oscillations during phasic and tonic REM sleep in a Parkinson's disease patient with REM sleep behavior disorder

Authors: *D. L. BAUER[1], A. K. VERMA[2], J. E. AMAN[2], S. L. ALBERICO[2], K. O'NEILL III[2], B. MOHANTY[2], B. PARKS[2], M. E. HILL[2], B. T. POBIEL[2], C. D. MACKINNON[2], M. J. HOWELL[2], J. L. VITEK[2], L. A. JOHNSON[2];
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Abstract: Excessive beta oscillations (13-35 Hz) in the basal ganglia (BG) are associated with Parkinson's disease (PD) motor signs and increasingly used to inform closed-loop deep brain stimulation (DBS) approaches. Recent evidence suggests that beta oscillations in the BG may also play a role in sleep-wake disturbances such as rapid eye movement (REM) sleep behavior disorder (RBD). Approximately 40% of people with PD are impacted by RBD, a parasomnia characterized by dream enactment during REM sleep. Sleep studies in PD patients with RBD

have shown that the power of beta oscillations in the BG during REM sleep can be similar to the wake state as opposed to the typical suppression seen in PD patients without RBD. Although REM sleep is generally treated as a single sleep stage, growing evidence suggests that REM sleep consists of two microstates: phasic and tonic. The dynamics of BG beta oscillations across these two states of REM sleep remain unclear, however. The goal of this study was to improve our understanding of BG neurophysiology during REM sleep by characterizing the dynamics of beta oscillations in the BG during phasic vs tonic REM sleep and in comparison to beta oscillations during wakefulness. This study was approved by the University of Minnesota Institutional Review Board and informed consent was obtained prior to data recording. Local field potentials from an externalized DBS lead in the internal globus pallidus (GPi) in a PD patient with a clinical diagnosis of RBD were recorded using an Atlas workstation (Neuralynx, Inc.). REM sleep was identified from video-polysomnography using American Academy of Sleep Medicine guidelines. Periods of bursts noted on electrooculogram (EOG) were identified as phasic REM while the silence on EOG between phasic events was identified as tonic REM. Power spectral density was computed to obtain beta power during wake, phasic and tonic REM sleep. The power across the three states was compared using a Kruskal-Wallis test followed by Tukey-Kramer post-hoc correction. We observed that GPi beta power was higher during phasic compared to tonic REM sleep and wake. We also observed that complex movements characteristic of RBD occurred primarily during phasic REM. These findings suggest that elevated GPi beta power during phasic REM sleep may contribute to the generation of complex movements in RBD patients. Further studies comparing BG neurophysiology during phasic and tonic REM sleep in PD patients with and without RBD are warranted and have the potential to inform the development of sleep-stage specific DBS approaches.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

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MnDRIVE Brain Conditions Program
Engdahl Family Foundation

Title: Effect of parkinsonism on thalamic low threshold spiking activity in the thalamus

Authors: E. BHARTI[1], *A. DENICOLA[2], B. NANDAKUMAR[1], A. VERMA[1], J. WANG[1], L. A. JOHNSON[1], J. L. VITEK, MD, PHD[1];
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Abstract: Neurophysiological studies exploring the effects of Parkinson's disease (PD) on the motor thalamus have demonstrated that there is an increase in burstiness in the firing patterns of thalamic neurons. In a prior report, the increased bursting activity of thalamic cells in the awake parkinsonian condition were suggestive of patterns that are seen predominantly during sleep. In the normal condition thalamocortical neurons burst through intrinsic Ca^{2+} mediated spikes, known as low threshold spiking (LTS) bursts, which are largely increased during sleep compared to awake states. The goal of this study was to understand how thalamic LTS burst physiology changes in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) non-human primate (NHP) model of PD during awake resting states. Two female rhesus macaques were chronically implanted with recording electrodes in the motor thalamus and motor cortex. Eye video data, spiking and local field potential (LFP) activity were collected while animals were at rest in the normal and PD conditions. Eye open periods were identified using eye video data to determine awake periods. Thalamic neurons were sorted offline, followed by LTS burst detection, and motor cortex LFPs were normalized and bandpass filtered in the delta frequency range (0.5-4Hz) to determine the presence of delta slow wave activity. LTS bursting characteristics were compared between normal and PD conditions in the awake state. Preliminary data suggest that thalamic cells exhibit a significant increase in LTS bursting in the awake parkinsonian condition compared to the normal awake state. We also observed that these PD awake-state LTS bursts are potentially associated with a transient increase in delta power in the motor cortex. This finding suggests that the LTS behavior in the awake PD state may contribute to intermittent delta slow wave activity in the cortex and therefore induce a "sleep-like" thalamic state. This "sleeping thalamus" could be a contributing factor to the development of excessive daytime sleepiness that is observed in the parkinsonian condition.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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NIH R01 NS131371
MnDRIVE (Minnesota's Discovery, Research and Innovation Economy)
Brain Conditions Program
Engdahl Family Foundation

Title: Role of basal ganglia-cortical network synchrony in excessive daytime sleepiness in parkinsonism

Authors: *K. ACEDILLO, A. K. VERMA, B. NANDAKUMAR, E. BHARTI, J. L. VITEK, L. A. JOHNSON;
Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Exaggerated beta oscillations (8-35 Hz) in the basal ganglia-cortical (BGC) network are commonly associated with Parkinson's disease (PD) motor signs. There is also emerging interest in their potential role in the development of sleep-wake disturbances (e.g., insomnia, sleep fragmentation, excessive daytime sleepiness (EDS)) that are prevalent in PD. We have previously shown that alterations in PD-related basal ganglia beta power were associated with an increase in daytime sleepiness in nonhuman primates (NHPs). Although EDS in PD patients is often associated with chronic use of antiparkinsonian medications, studies have suggested that dopamine loss itself is a significant contributor to EDS. The goal of this study was to further understand how dopaminergic tone impacts BGC beta oscillations and their relationship to daytime sleepiness. Local field potentials were recorded from the subthalamic nucleus (STN; using deep brain stimulation (DBS) leads) and the primary motor cortex (MC; using GrayMatter microdrive) in an NHP (female rhesus macaque, age 24) rendered parkinsonian with low-dose injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Video of eyes and neural data were simultaneously recorded using Tucker-Davis Technologies (TDT) acquisition system at 24 kHz sampling rate. Percentage of recording time the NHP was found sleeping was determined via a custom MATLAB script used to identify wake (eyes-open) and sleep (eyes-closed) epochs. STN↔MC synchrony was calculated using cross-power spectrum (1-s epochs) during the awake state. The neural and behavioral data from the off- and on-medication (levodopa, intramuscular route: 10 mg/kg) conditions were compared and reported here. Significant reduction in daytime sleepiness was noted in the on- vs off-medication condition. Reduction in daytime sleepiness was associated with a decrease in low-beta (8-20 Hz) but an increase in high-beta (20-35 Hz) STN↔MC synchrony. Furthermore, STN↔MC synchrony in delta (0.5-4 Hz) band was suppressed on medication. Our data suggest that alterations of the beta band in the BGC network is not only relevant in the PD-related motor signs, but also in PD-related sleep-wake dysfunctions, i.e., in EDS. Future studies with higher sample size are required to generalize these findings. An improved understanding of the neural oscillations associated with daytime sleepiness will inform the development of targeted therapies (e.g., DBS) for selective amplification or suppression of BGC network oscillations to impact EDS.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

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Topic: C.03. Parkinson's Disease

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NIH NINDS R01 NS110613
NIH NINDS P50 NS098573

Title: Effects of parkinsonism on neuronal activity in the thalamus during a center-out touch screen reaching task.

Authors: *E. KING[1], A. L. DENICOLA[2], J. WANG[2], L. A. JOHNSON[2], J. L. VITEK, MD, PHD[2];
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Abstract: The thalamus forms an important nodal point in the basal ganglia-thalamocortical (BGTC) circuit and likely plays an integral role in mediating the motor signs of Parkinson's disease (PD). In the healthy motor thalamus, neurons have receptive fields for specific joint movements and modulate their firing rate to encode the initiation, execution, and direction of active movements. In non-human primate (NHP) models of PD, studies examining thalamic neuronal activity find decreases in firing rate and elevated bursting activity at rest, as well as broadened receptive fields during passive manipulation. How PD affects encoding of active movement and direction selectivity in motor thalamic neurons during goal-directed behavior, however, remains unexplored. This study investigated how parkinsonism impacts the encoding of goal-directed reaching movement in the motor thalamus. Neural data were recorded from two female NHPs trained to perform a center out touch screen task (COT). Spiking activity was collected using a Gray Matter microdrive with 12-14 individually moveable electrodes targeting the motor thalamus and sorted offline. In order to determine the relationship of thalamic neurons to movement-related events, peri-event time histograms aligned to reach onset were created and firing rates during the reaction period (from target appear to movement onset) and the reaching period (from reach onset to target touch) were compared to a pre-movement baseline period. Recordings were performed before and after induction of a moderate parkinsonian condition using the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Preliminary analysis demonstrated a decrease in the proportion of neurons that were modulated during the reaction or reach periods in the parkinsonian condition. These findings suggest that movement encoding is disrupted in the parkinsonian motor thalamus, which likely contributes to difficulty in production and scaling of goal-directed movement seen in PD. Further studies will investigate the changes in directional selectivity and somatosensory responsiveness of neurons in the PD state and their relationship to the motor dysfunction in PD.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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MnDRIVE Brain Conditions Program
Engdahl Family Foundation

Title: Developing a non-human primate model to investigate the neural mechanism(s) underlying impaired gait in Parkinson's disease

Authors: *T. HUBBARD, N. HJELLE, B. MOHANTY, L. A. JOHNSON, J. WANG, J. L. VITEK;
Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Gait impairment is a common motor dysfunction in Parkinson’s disease (PD) that can be resistant to dopaminergic therapy. While PD has been associated with alterations in neuronal activity in the basal ganglia-thalamocortical (BGTC) network, the neuronal mechanisms contributing to the development of gait disorders is not well understood. To date, there exist few animal model methodologies to explore the neural underpinnings of impaired gait in PD or the mechanisms of action of subthalamic deep brain stimulation (STN DBS) on gait. At the same time, a better understanding of the BGTC network at the neuronal level is needed in order to optimize neuromodulation therapies like STN DBS for gait dysfunction in PD.One female non-human primate (NHP; Rhesus macaque, age 20) was implanted with three 96 channel Utah arrays (Blackrock) targeting the primary motor, premotor, and dorsolateral prefrontal cortices, as well as a DBS lead targeting the STN. Wireless recordings of neural activity and stance force data were collected simultaneously while the NHP walked from one end to the other of a custom-built walkway. Using stance force data, various spatiotemporal parameters of the gait cycle were calculated. Data were recorded in the naive state and after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration with and without therapeutic STN DBS. Gait impairment in PD is often reflected by reduced gait speed and step length. Compared to the naive condition, in the PD state stride and swing times were prolonged while overall gait speed and cadence decreased. STN DBS decreased swing and stride times while increasing cadence and gait speed.Gait dysfunction in the NHP model is similar to that observed in PD patients. Our

experimental preparation provides the ability to examine the neuronal changes underlying gait dysfunction in PD and changes associated with gait improvement during STN DBS. Future studies will allow more detailed examination of these mechanisms and provide the rationale for the development of novel DBS approaches for the treatment of gait disorders in PD.

Disclosures: T. Hubbard: None. **N. Hjelle:** None. **B. Mohanty:** None. **L.A. Johnson:** None. **J. Wang:** None. **J.L. Vitek:** F. Consulting Fees (e.g., advisory boards); Dr. Vitek serves as a consultant for Medtronic, Boston Scientific and Abbott., He also serves on the Executive Advisory Board for Abbott and is a member of the scientific advisory board for Surgical Information Sciences..

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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Title: Effect of Intermittent Burst Deep Brain Stimulation on Neuronal Firing in the Globus Pallidus Externus and Parkinsonian Motor Signs

Authors: *S. AMOOZEGAR, Z. LUO, K. ACEDILLO, A. DENICOLA, L. JOHNSON, J. VITEK, J. WANG;
Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Deep brain stimulation (DBS) is an effective treatment for advanced Parkinson’s disease (PD), however, it can be associated with current-spread related side effects. A novel intermittent burst DBS (ibDBS) pattern was developed from an optogenetic study in Parkinsonian mice. This study demonstrated that differentially modulating globus pallidus externus (GPe) neuronal activity induced sustained motor benefits and ibDBS in the globus pallidus internus (GPi) produced a similar effect. By using significantly less stimulation, ibDBS has the potential to reduce current-spread related side effects and lower battery consumption. Although ibDBS is promising, the optimal stimulation location is still unclear due to the anatomical and physiological differences between the mouse model and patients.
Objectives: To explore the effect of ibDBS on GPe neuronal activities and parkinsonian motor signs in the nonhuman primate (NHP) model of PD.
Methods: In NHP “Be”, a DBS lead was implanted that spans the subthalamic nucleus (STN)

and GPi regions. GPe neuronal data were collected before, during, and after ibDBS delivery through different DBS contacts. In NHP “Pa” and “Bu”, a DBS lead was implanted in the STN region and both animals were rendered mildly Parkinsonian. Each animal received ibDBS delivered to different DBS contacts within/close to the STN in a pseudorandomized order and each contact was assessed multiple times. Blinded clinical rating scale (mUPDRS) scores were obtained off and on ibDBS.
Results: In NHP “Be”, ibDBS delivered to dorsal STN and medial to GPi induced more balanced excitation and inhibition in the responses of GPe neurons during stimulation. Immediately following stimulation cessation, these balanced responses were observed after ibDBS, however, more excitatory responses in GPe neurons were observed after tDBS. In the other two NHPs, ibDBS delivered in the ventral and dorsal STN produced significant acute motor benefits.
Conclusion: These preliminary results show that ibDBS delivered to dorsal STN and medial to GPi can induce GPe neuronal changes in parkinsonian monkeys similar to that observed in the optogenetic study and STN ibDBS can improve motor function. Additional studies in more NHPs are needed to confirm these findings.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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Topic: C.03. Parkinson’s Disease

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MnDRIVE (Minnesota’s Discovery, Research and Innovation Economy)
Brain Conditions Program
Engdahl Family Foundation

Title: Spontaneous eye behavior, an objective biomarker of dopaminergic state

Authors: *B. NANDAKUMAR[1], K. ACEDILLO[2], A. VERMA[3], E. BHARTI[3], Y. YU[4], L. JOHNSON[5], J. L. VITEK[6];
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by akinesia, bradykinesia, tremor, rigidity, and postural instability. These motor signs are typically quantified using clinical rating scales (mUPDRS) that can be subjective and prone to inter-rater variability. There is a need to develop additional objective quantifiable biomarkers of PD. Prior studies have observed changes in eye behavior in PD patients, but its relationship to changes in motor signs, therapeutic interventions, and pathological beta oscillations (8-35 Hz) in the basal ganglia in PD remains unclear. The goal of this study was to investigate changes in spontaneous saccades in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) non-human primate (NHP) model of PD and characterize their relationship to motor signs and beta oscillations in basal ganglia as well as their response to therapeutic intervention (Levodopa). A NHP was implanted with a DBS lead targeting the subthalamic nucleus (STN). Simultaneous eye video and local field potential (LFP) recordings were obtained at rest before and after rendering the animal progressively parkinsonian (Mild, Moderate) with repeated low-dose administration of MPTP as well as following the administration of Levodopa. DeepLabCut was used to identify spontaneous saccades and LFP spectral power was used to quantify the dynamics of STN beta oscillations. Spontaneous saccade and blink rates decreased in the PD state and were restored by levodopa, accompanied by a concomitant reduction in pathological beta oscillation in the STN and improvement in motor signs. Acute administration of Levodopa modulated eye behavior and motor signs as early as 10 minutes upto 90 minutes post injection. Saccade rate modulation was better correlated to changes in beta power than mUPDRS score. Overall, these data suggest spontaneous eye behavior in PD may be a valuable biomarker to characterize the parkinsonian condition and a useful objective measure to quantify the effects of therapies in PD.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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Title: Reduced Task-Related modulation of DLPFC Neurons in Parkinsonism

Authors: *N. HJELLE, T. HUBBARD, B. MOHANTY, L. A. JOHNSON, J. WANG, J. L. VITEK;
Neurol., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Parkinson's disease (PD) has beenassociated with alterations inneuronal activity inthebasal ganglia-thalamocortical (BGTC) network. The dorsolateral prefrontal cortex (DLPFC) is acritical node inthe BGTCthat has beenimplicated inexecutive control functions that canbeimpaired inPD. There are limited electrophysiological data, however, describing how theDLPFCis impacted inPD. Here we investigate the effects of parkinsonism onDLPFCneuronalactivity during ago/nogo(GNG) reaching task.One female nonhumanprimate (NHP; Rhesus macaque, age 20) was implanted with a96channel Utah array (Blackrock) targeting DLPFC. The NHP was trained toperform aGNGtouchscreentask and was rendered mildly parkinsonianby administering1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). DLPFCsingle unit activities were sortedinOffline Sorter (Plexon) and thenanalyzed inMatlab. Neuronal responses were aligned tothetime the target appeared, reach onset, and returnonset for each trial and averaged acrosstrials. Neurons with asignificant change infiring rate relative toapre-target baseline periodwere classified as modulated. Those with anincrease infiring rate were further classified asactivated, and those with adecrease as suppressed.Seventy five cells were recorded inthe naive state with 46% of neurons modulated inresponse togotrial target appearance, 28% tonogotarget appearance, 66% toreach onset,and 51% toreturnonset. Inthe PDstate there was adecrease intask success rate and anincrease inreactiontime. There was alsoamarked reductioninthe cell modulationduring thetask. Fifty four cells were recorded inPDwith 20% of neurons modulated inresponse togotrialtarget appearance, 9% tonogotarget appearance, and 51% toreach onset. There was nosignificant change inreturnonset modulationpercentage. The ratioof suppressed toactivatedcells increased significantly inthe PDstate during gotarget appearance, reach onset, andreturnonset compared tothe naive state, drivenby adecrease inthe number of activated cells.Although preliminary, these results suggest that DLPFCmodulationis significantlydecreased inthe parkinsonianconditionduring movement planning and execution. Furthermore,there is adisruptioninthe balance of modulationtype, with agreater loss tocellular activationcompared tosuppression. While further analysis is needed, this loss of DLPFCmodulationislikely asignificant factor contributing toimpaired executive functioninPD.

Disclosures: N. Hjelle: None. **T. Hubbard:** None. **B. Mohanty:** None. **L.A. Johnson:** None. **J. Wang:** None. **J.L. Vitek:** F. Consulting Fees (e.g., advisory boards); Dr. Vitek serves as a consultant for Medtronic, Boston Scientific and Abbott. He also serves on the Executive Advisory Board for Abbott and is a member of the scientific advisory board for Surgical I.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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MnDRIVE Brain Conditions Program

Title: Reduced network connectivity in the beta band associated with therapeutic coordinated reset deep brain stimulation

Authors: *L. ZHENG, Z. LUO, S. FERGUS, L. A. JOHNSON, J. L. VITEK, J. WANG; Univ. of Minnesota, MINNEAPOLIS, MN

Abstract: Coordinated reset deep brain stimulation (CR DBS) has shown promising therapeutic effects for Parkinson's disease (PD) with an additional beneficial carryover effect compared with traditional DBS. CR DBS is hypothesized to desynchronize neuronal activity by delivering burst stimulation through multiple contacts of the DBS lead. However, this hypothesis remains to be validated in vivo. In this study, we investigated the changes in connectivity between the subthalamic nucleus (STN), primary motor cortex (M1) and premotor cortex (PM) associated with the therapeutic effect of STN CR DBS in the non-human primate (NHP) model of PD.An 8-contact DBS lead was implanted into the STN of an adult female NHP (Macaca mulatta), and 2 ECoG arrays were placed over M1 and PM, respectively. The animal was rendered parkinsonian by administering the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). STN CR DBS using 4 different burst frequencies were evaluated. In each evaluation session, CR DBS was delivered over five consecutive days for 2 hours per day. A clinical rating scale (mUPDRS) was assessed and resting state local field potentials (LFPs) from STN, M1 and PM were recorded before and after stimulation on CR DBS days, as well as on the carryover days. Coherence and transfer function (TF) analysis were performed using LFPs from STN, M1 and PM in both the low (10-20 Hz) and high (21-35 Hz) beta bands. The relationship between the connectivity measurements and mUPDRS was investigated.In each evaluation session, mUPDRS decreased with the delivery of STN CR DBS and gradually returned to the baseline level 7-12 days after stimulation cessation. Similarly, the M1-STN and PM-STN coherences were reduced with CR DBS and slowly recovered along with the change in the mUPDRS. The mUPDRS was linearly and positively correlated with the M1-STN and PM-STN coherences in both the low and high beta bands, but not with the M1-PM coherence. Moreover, TF gain from STN to M1, STN to PM and M1 to STN in the low beta band as well as that from M1 to STN in the high beta band were all positively correlated with the mUPDRS. Although the M1-PM coherence was not correlated with the motor improvement, the TF gain from M1 to PM in both the low and high beta bands were positively correlated with the mUPDRS.These results indicate that the therapeutic effect of STN CR DBS is associated with reduced network connectivity. Specifically, the motor improvement induced by STN CR DBS might be associated with reduced information flow from STN to cortical areas and that from M1 to PM and STN. This study reveals the possible action mechanism of CR DBS which can potentially facilitate further development of this novel DBS approach.

Disclosures: L. Zheng: None. **Z. Luo:** None. **S. Fergus:** None. **L.A. Johnson:** None. **J.L. Vitek:** F. Consulting Fees (e.g., advisory boards); Medtronic, Boston Scientific, Abbott, Surgical Information Sciences. **J. Wang:** None.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.13/O1

Topic: C.03. Parkinson’s Disease

Support: ANR-20-CE37-0023-02
NR-21-CE16-0025-01
FRM EQU202203014688
Maison de Creveaux N: 2021-01

Title: Synaptic changes in pallidostriatal observed in parkinsonian model triggers abnormal beta synchrony with accurate spatio-temporal properties across basal ganglia

Authors: *S. AZIZPOURLINDI[1], N. M. MALLET[2], A. LEBLOIS[3];
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Abstract: Excessive oscillatory activity across basal ganglia (BG) nuclei in the beta frequencies (12-30Hz) is a hallmark of Parkinson’s disease (PD). While the link between oscillations and symptoms remains debated, exaggerated beta oscillations constitute an important biomarker for therapeutic effectiveness in PD. The neuronal mechanisms of beta-oscillation generation however remain unknown. Many existing models rely on a central role of the subthalamic nucleus (STN) or cortical inputs to BG. Contrarily, neural recordings and optogenetic manipulations in normal and parkinsonian rats recently highlighted the central role of the external pallidum (GPe) in abnormal beta oscillations, while showing that the integrity of STN or motor cortex is not required. Here, we evaluate the mechanisms for the generation of abnormal beta oscillations in a BG network model where neuronal and synaptic time constants, connectivity, and firing rate distributions are strongly constrained by experimental data. Guided by a mean-field approach, we show in a spiking neural network that several BG sub-circuits can drive oscillations. Our results show that strong recurrent STN-GPe connections or collateral intra-GPe connections drive gamma oscillations (&gt 40Hz), whereas strong pallidostriatal loops drive low-beta (10-15Hz) oscillations. We show that pathophysiological strengthening of striatal and pallidal synapses following dopamine depletion provide the required strong connections in these loops that leads to the emergence of synchronized oscillatory activity. Our detailed model simulations of a network including all the loops shows that a healthy network (i.e. with no synchronized activity) can exhibit pathophysiological oscillations through implementation of the structural synaptic changes and firing rate modifications due to dopamine depletion. Moreover, we show that in this network, the interplay between the loops with low-beta and gamma frequencies gives rise to the experimentally-observed mid-beta range oscillations. This network not only produces spike-phase relationships between BG neuronal populations that are fully in-line with experiments up to now, but it also provides predictions for the activity of striatal population not yet recorded. Furthermore, according to our model, similar to experimental

findings the inhibition of GPe, contrary to STN, abolishes pathophysiological oscillations. Our modeling study uncovers the neural mechanisms underlying PD beta oscillations and may thereby guide the future development of therapeutic strategies.

Disclosures: S. Azizpourlindi: None. **N.M. Mallet:** None. **A. Leblois:** None.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.14/O2

Topic: C.03. Parkinson's Disease

Support: NIDA R0107418
JPB Foundation

Title: Refinement of striatum direct pathway neuronal activity is required for learning

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Abstract: It has been suggested that the dorsomedial striatum (DMS) is engaged in the early stages of motor learning for goal-directed actions, whereas at later stages, control is transferred to the dorsolateral striatum (DLS), a process that enables learned motor actions to become a skill or habit. In previous work, by evaluating improvements in motor coordination during training with simultaneous neuronal calcium activity in the striatum, we found that DMS direct pathway neurons exhibited decreased activity as the mouse gained proficiency in a treadmill running task. In contrast, direct pathway activity in the DLS was similar throughout training. Pharmacological blockade of D1 dopamine receptors in these subregions during task performance demonstrated that dopamine neurotransmission in the direct pathway activity is necessary for efficient motor coordination learning. Preliminary data show that direct pathway neurons have increase excitability after training. These data combine with a general decrease in calcium signal suggest a reorganization of high activity cells increasing the signal-to-noise ratio and contributing to better transmission of information. In this regard, we note that calcium signals detected by fiber photometry describe the bulk activity of a multitude of neurons, and an overall reduction in amplitude but not in the number of events could indicate that fewer, "specialized" neurons fire at the same rate once the skill is acquired. On going experiments are exploring calcium signals at a single cell resolution using 2-photon imaging. The data discussed here provides a means to evaluate motor ability in healthy animals and disease models and is designed to be useful for the evaluation of motor disorders and motor learning deficits, and the development of more effective therapies.

Disclosures: S. Cataldi: None. **C. Lacefield:** None. **N. Shashaank:** None. **D.L. Sulzer:** None.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.15/O3

Topic: C.03. Parkinson's Disease

Support: DoD IIRA Grant W81XWH2110943

Title: Progressive cortico-amygdala circuit dysfunction associated with alpha-synuclein aggregation

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects both motor function and cognition/emotion. While most research has focused on motor symptoms, the biology underlying the development of cognitive impairment and neuropsychiatric deficits remains poorly understood. The limbic system is essential for cognitive function and emotion regulation, and it also develops heavy Lewy pathology in PD and other neurodegenerative disorders. This study aims to understand how the development of Lewy-like pathology affects the anatomy and function of the limbic system and its relevance to neuropsychiatric deficits in PD. To achieve this goal, we injected α-synuclein (αSyn) preformed fibrils (PFFs) into the dorsomedial striatum of mice to induce the development of synucleinopathies in the basolateral amygdala (BLA). We conducted a battery of behavior, electrophysiology, optogenetics, and immunohistochemistry studies to systematically analyze the impact of αSyn aggregation on cellular and synaptic properties of BLA neurons, as well as potential impairments of motor and emotion function of mice, at 1- to 12-months after the αSyn PFFs injection. Our data suggest that intrastriatal injection of PFFs induced αSyn aggregates in both the BLA and the medial prefrontal cortex (mPFC) with dynamic changes in both their quantity and morphology. We also showed that αSyn aggregation gradually impairs the functional connectivity between the mPFC and the BLA, starting as early as 1-month post-injection. However, the amount of αSyn aggregation within the BLA declined significantly, which also associates with normalized mPFC-BLA connectivity. Our ongoing work focuses on the correlation between pathology, circuit dysfunction, and behavioral impairment at different times post PFFs injection.

Disclosures: W. Zhou: None. **S. Daniels:** None. **H. Chu:** None.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.16/O4

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R21NS108068
Parkinson’s Foundation

Title: Substantia nigra pars reticulata projections to the pedunculopontine nucleus modulates dyskinesia

Authors: Y. HU[1], T. C. MA[1], S. ALBERICO[2], Y. DING[1], ***U. J. KANG**[1];
[1]Neurol., New York Univ. Grossman Sch. of Med., New York, NY; [2]Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: *Background:* Long-term use of levodopa for Parkinson's disease (PD) treatment is often hindered by development of motor complications including levodopa-induced dyskinesia (LID). The substantia nigra pars reticulata (SNr) and globus pallidus internal segment (GPi) are the output nuclei of the basal ganglia. Dysregulation of SNr and GPi activity contributes to PD pathophysiology and LID. *Objectives:* Determine whether direct modulation of SNr GABAergic neurons and SNr projections to the pedunculopontine nucleus (PPN) regulates PD symptoms and LID in a mouse model. *Methods:* We expressed Cre-recombinase activated channelrhodopsin-2 (ChR2) or halorhodopsin (NpHR) AAV2 vectors selectively in SNr GABAergic neurons of Vgat-IRES-Cre mice in a 6-hydroxydopamine model of PD to investigate whether direct optogenetic modulation of SNr neurons or their projections to the PPN regulates PD symptoms and LID expression. The forepaw stepping task, mouse LID rating scale, and open field locomotion were used to assess akinesia and LID, respectively, to test the effect of SNr modulation. *Results:* Akinesia was improved by suppressing SNr neuron activity with NpHR. LID was significantly reduced by increasing SNr neuronal activity with ChR2, which did not interfere with the anti-akinetic effect of levodopa. Optical stimulation of ChR2 in SNr projections to the PPN recapitulated direct SNr stimulation. *Conclusions:* Modulation of SNr GABAergic neurons alters akinesia and LID expression in a manner consistent with the rate model of basal ganglia circuitry. Moreover, the projections from SNr to PPN likely mediate the antidyskinetic effect of increasing SNr neuronal activity, identifying a potential novel role for the PPN in LID.

Disclosures: Y. Hu: None. **T.C. Ma:** None. **S. Alberico:** None. **Y. Ding:** None. **U.J. Kang:** None.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.17/O5

Topic: C.03. Parkinson’s Disease

Title: Cortical connectivity profile of effective subthalamotomy in Parkinson's Disease

Authors: R. RODRIGUEZ-ROJAS[1], J. MÁÑEZ-MIRÓ[2], J. PINEDA-PARDO[1], M. LOPEZ-AGUIRRE[1], R. MARTINEZ-FERNANDEZ[1], *J. BLESA[1], J. OBESO[1];
[1]Fundacion de Investigacion HM Hospitales, Madrid, Spain; [2]Univ. Autónoma de Madrid, Madrid, Spain

Abstract: Introduction: Thermal ablation of the subthalamic nucleus using magnetic resonance guided focused ultrasound (FUS-subthalamotomy) is a novel therapeutic approach that can efficiently control motor features in patients with Parkinson's disease (PD). However, effective treatment involves optimal targeting to modulate the motor network. This study aimed to map-out the cortical connectivity fingerprint of FUS lesions and to identify the impact on white matter fiber pathways associated with maximum improvement in bradykinesia, rigidity and tremor. **Methods**: Motor scores of the Movement Disorders Society- Unified Parkinson's Disease Rating Scale were evaluated in 39 PD patients at baseline and 1 year after FUS-subthalamotomy. Voxel-based statistical analysis of therapeutic lesions, segmented on post-treatment multimodal MRI, was used to identify effective clusters for each motor feature, lastly defined as seeds for tractography to cortical targets within the motor circuitry. A public high-resolution human connectome data was used to identify structural connections reliably associated with clinical improvement. **Results:** The predictive connectivity model showed different cortical patterns for each cardinal feature. Thus, the focal ablation within the STN related with the optimal improvement in bradykinesia was mostly connected to the SMA with mild projections to the premotor and primary motor cortices respectively. Conversely the best effect against parkinsonian tremor was related to the impact within an STN area with a major projection to the primary motor cortex (M1). The effective cluster for rigidity showed connectivity to both SMA and M1, and interestingly additional and relevant projections to the ventral and particularly dorsal premotor cortex (PMC) were unraveled. **Conclusions**: Effective subthalamotomy for PD is associated with a specific connectivity profile that can predict clinical outcome. Distinct circuits need to be targeted to improve bradykinesia, rigidity and tremor and those networks could be identified based on functional zones in motor cortices and STN.

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Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.18/O6

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01NS121371
Aligning Science Across Parkinson’s ASAP-020572

Title: Reduced thalamic excitation to motor cortical pyramidal tract neurons in parkinsonism

Authors: *L. CHEN, S. DANIELS, R. DVORAK, H.-Y. CHU;
Van Andel Res. Inst., Grand Rapids, MI

Abstract: Degeneration of midbrain dopaminergic (DA) neurons alters the connectivity and functionality of the basal ganglia-thalamocortical circuits in Parkinson's disease (PD). Particularly, the aberrant outputs of the primary motor cortex (M1) contribute to parkinsonian motor deficits. However, cortical adaptations at cellular and synaptic levels in parkinsonism remain poorly understood. Using multidisciplinary approaches, we found that DA degeneration induces cell-subtype- and input-specific reduction of thalamic excitation to M1 pyramidal tract (PT) neurons. At molecular level, we identified that NMDA receptors play a key role in mediating the reduced thalamocortical excitation to PT neurons. At circuit level, we showed that the reduced thalamocortical transmission in parkinsonian mice can be rescued by chemogenetically suppressing basal ganglia outputs. Together, our data suggest that cell subtype- and synapse-specific adaptations in M1 contribute to altered cortical outputs in parkinsonism and are important aspects of PD pathophysiology.

Disclosures: L. Chen: None. **S. Daniels:** None. **R. Dvorak:** None. **H. Chu:** None.

Poster

PSTR531. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR531.19/O7

Topic: C.03. Parkinson's Disease

Support: MOST 108-2321-B-009-007-MY2
MOST 110-2321-B-009-004

Title: A practical guide to implant the deep brain electrode in the subthalamic nucleus of the minipig

Authors: *H. LIN, Y. WU, M. KER;
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Abstract: The development of implantable neuromodulation devices, such as deep brain stimulators, for clinical use, requires validation of their safety and efficacy in large animal models before clinical trials. Minipigs are a suitable alternative to large animals such as non-human primates, but the complexity of the electrode implantation procedure in the brain and the uncommon devices required hinders the use of minipigs. We propose a simple and reproducible guide to implant deep brain electrodes into the minipig brain, focusing specifically on the subthalamic nucleus. The electrode implantation trajectory can be pre-planned using T1-weighted brain MR images of individual minipig, which by the software downloaded on the internet, and the pig brain atlas as reference. Stereotaxic surgery was performed using a commercial stereotaxic apparatus and bregma as a skull landmark to orientate the implantation procedure. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonian minipigs (n

= 8) were used to validate the implantation procedure. The microelectrode recording was used to detect the single-unit activities and local field potentials. The beta band oscillations of the local field potentials served as a pathological biomarker for anatomical verification. Our results demonstrated the T1-weighted brain MR images provide sufficient contrast of the subcortical structures. In addition, the T1-weighted MR images also reveal the bregma position that can be used to measure the relative distance to the electrode insertion point. Through the adjustment of the MR images and the head position of the minipig in the same plane, the deep brain electrodes can be easily implanted into the subthalamic nucleus according to the pre-planned trajectory. Beta band oscillations were recorded in six out of eight animals. Our method provides a practical guide to electrophysiological experiments using minipigs and may promote the use of minipigs for brain research.

Disclosures: H. Lin: None. **Y. Wu:** None. **M. Ker:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.01/O8

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant : R01NS123557
Hereditary Disease Foundation
Brigham Research Institute

Title: Focused Ultrasound for Improved Delivery of AAV Vectors to the Brain for the Treatment of Huntington's Disease

Authors: *B. S. OWUSU-YAW[1], Y. ZHANG[1], L. GARRETT[2], A. YAO[3], K. SHING[4], R. BATISTA[5], M. SENA-ESTEVES[5], K. KEGEL-GLEASON[4], N. TODD[1];
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Abstract: Huntington’s disease (HD) is a fatal, neurodegenerative disorder caused by an expanded CAG repeat sequence in exon 1 of the huntingtin gene (*HTT*). There are currently no cures for HD and lowering mutant huntingtin (mHTT) via gene therapy represents a promising strategy for this disorder. Adeno-associated viral vectors (AAV) can be used to deliver DNA sequences encoding genes, small interfering RNAs (siRNAs) or microRNAs (miRNAs) to the central nervous system (CNS) through direct injection or intravenous administration. However, the blood brain barrier (BBB) is a significant obstacle for AAV delivery to the brain. Direct injection methods are invasive and systemic delivery requires BBB permeable capsids which have variable transduction efficiencies across different species. Focused ultrasound (FUS) with microbubbles (MB) has emerged as a therapeutic modality that can be used to temporarily

disrupt the BBB in non-invasive manner to deliver drugs to specific brain regions. A series of phase one clinical trials in patients with Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis have demonstrated the feasibility and safety of using this technique in humans. To date, no clinical trials have been set up to assess the safety of using FUS+MB to deliver gene therapy. Here, we tested the delivery of ssAAV9 using FUS in combination with MB to the right striatum of Q175 HD mice. Our results demonstrate that ss-AAV9-CBA-GFP infected astrocytes and neurons in the right striatum of WT 2-month, Q175 2- and 6-month-old mice following FUS treatment and tail vein injection of ss-AAV9-CBA-GFP at a dose of 2.2×10^{10} vg/g. The permeability of the BBB was assessed using gadolinium-enhanced T1-weighted images and results demonstrate increased BBB permeability in the sonicated hemisphere across the experimental groups. In addition to this, strong GFP expression was detected in the FUS-treated hemisphere compared to the untreated hemisphere. These findings show that FUS+MB can be used to enhance AAV-based gene delivery to specific brain regions in a mouse model of HD. The BBB is a huge obstacle in treating CNS diseases. This strategy can be used to overcome this barrier by enabling the non-invasive delivery of therapeutic genes to customized brain regions to treat a wide range of neurodegenerative diseases.

Disclosures: **B.S. Owusu-Yaw:** None. **Y. Zhang:** None. **L. Garrett:** None. **A. Yao:** None. **K. Shing:** None. **R. Batista:** None. **M. Sena-Esteves:** None. **K. Kegel-Gleason:** None. **N. Todd:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.02/P1

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Huntington's Disease Society of America Donald A. King Fellowship
Office of Undergraduate Research University of Central Florida Grant

Title: Applying artificial intelligence to neuropathological assessment in a mouse model of Huntington disease

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Abstract: As our populations' life expectancy increases, neurodegenerative diseases, such as Huntington disease (HD), become more prevalent. Neurodegeneration leads to regional brain atrophy, typically prior to symptom onset. As medical treatments designed to combat neurodegeneration advance, effects on atrophy are quantified in animal models to test validity. This is important because treatments designed to combat neuropathology are more likely to modify the disease itself, per contra to treatments designed to mask or treat symptoms. One brain region size quantification method uses MRI, which while accurate, is prohibitively expensive.

Conversely, stereological volume assessment, the process of estimating the volume of individual 3D brain regions from 2D brain sections, is more commonly used. This method involves manually tracing cross sections of a brain region of interest, followed by application of the Cavalieri principle to calculate the volume. The pertinent caveats of this approach are lack of efficiency, resulting from the labor-intensive manual tracing process, and potential inaccuracies that arise due to individual differences in perception of boundaries within the brain, requiring that a single investigator evaluate all brains for a particular study. For this reason, we are employing the latest advancements in artificial intelligence (AI) to not only automate, but also to improve precision of stereological volumetric assessments. Recent advancements on self-attention have been used for AI models such as Chat-GPT. We are leveraging our novel down sample and patch self-attention techniques to create the same level of accuracy with images. The way AI learns is by giving the program an input, and then the desired output to commence self-correction. Allowance of the program to complete a wide range of tasks depends on a large data set of varying inputs and desired outputs. For our project, we are using HD model mice to compile a large data set of images of brain regions affected in HD, including the striatum, frontal cortex, and corpus callosum, which are traced by hand to train our AI. We are using a second set of brain sections to test the program and compare to manual assessment using genotypic differences between HD and wildtype mice, Inter-group variability, and assessment time as output measures. The success of our program, meaning our trained AI is able to more accurately and rapidly evaluate regional brain atrophy than manual stereology, will increase efficiency of preclinical evaluation of neuropathology, allowing for a greater number of experimental therapies to be tested and facilitating drug discovery for intractable neurodegenerative diseases.

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Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.03/P2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Characterization of conditional Q175 (cQ175) and Q20 (cQ20) knockout models

Authors: L. RAUHALA[1], ***K. PALDANIUS**[1], A.-M. PENTTINEN[1], K. KUHLBRODT[3], B. BALDO[3], E. KRITIKOU[2], S. DERAKHSHAN[2], M. GRIMBERGEN[2], M. PLIEGO CABALLERO[2], D. HOWLAND[4], R. CHEN[4], D. MARCHIONINI[4];
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Abstract: Developing therapeutics for Huntington’s disease (HD) requires extensive preclinical research and the establishment and maintenance of well-characterized mouse models. Mouse strains which enable conditional lowering of WT or mutant Huntingtin (mHtt) will be important

tools to follow the effects and develop biomarkers after mHtt lowering strategies. For this purpose, LoxP sites were inserted at 1357 bp 5' from the start of exon 1 and 3663 bp 3' from the end of exon 1 in the Q175 knock-in (KI) mouse to generate conditional Q175 mice. LoxP sites were inserted at 1356 bp 5' from the start of exon 1 and 2150 bp 3' from the end of exon 1 in the Q20 KI mouse to generate conditional cQ20 mice. These cHtt-Q175_3'LoxP or cHtt-Q20_3'LoxP mice were crossed to UBC-Cre-ERT2 to generate cQ175 and cQ20 progeny. Mice were treated with vehicle or tamoxifen (TM) at 2 months of age to induce Cre recombination and global excision of total WT or mHtt. Animals were sacrificed at either 3 or 9 months of age to assess efficacy of the Cre excision and the leakiness of the flox-Cre system. Vehicle or TM treated cQ175 mice were investigated for the expression of the KI human CAG repeat transcript (KI HTT) by QuantiGene in the brain and liver. Similarly, protein levels of the expanded mHTT or total HTT were assessed by the Meso Scale Discovery (MSD) assays. Expression of the KI HTT transcript was significantly decreased in all the tested tissues of the TM-treated mice) at both 3 and 9 months. Lowering of up to 60% was seen in the liver, whereas the KI HTT transcript was reduced by up to 40-50% in the striatum, cerebellum and cortex in the cQ175. There was no apparent lowering of KI HTT in any tissue in the absence of TM. The MSD data also indicated considerable suppression of the mHTT protein. The mHTT lowering was approx. 60-70% in the cerebellum, cortex, and liver in 9-month-old mice. Higher knockdown efficiencies in total HTT (> 70%) were observed in the cQ20 mice. The data demonstrates successful generation of the cQ175 and cQ20 mice, which may be useful models to develop mHtt lowering or safety biomarkers.

Disclosures: **L. Rauhala:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **K. Paldanius:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **A. Penttinen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **K. Kuhlbrodt:** A. Employment/Salary (full or part-time):; Evotec SE. **B. Baldo:** A. Employment/Salary (full or part-time):; Evotec SE. **E. Kritikou:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **S. Derakhshan:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **M. Grimbergen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **M. Pliego Caballero:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **D. Howland:** None. **R. Chen:** None. **D. Marchionini:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.04/P3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Natural Science foundation of Jiangsu Province (BK20221155)

Title: B6-hHTT130-N: An Improved Mouse Model for Drug Efficiency Screening and Therapies for HD

Authors: H. QI[1], F. LIU[1], ***Z. LI**[2], Z. LAI[1], C. JU[1], J. ZHAO[1], X. GAO[1], M. W. MOORE[2]; [1]GemPharmatech Co., LTD., Nanjing, China; [2]GemPharmatech LLC, La Jolla, CA

Abstract: Huntington's disease (HD) arises from the expansion of CAG repeats in the huntingtin gene (HTT), resulting in a neurodegenerative disorder. Transgenic mouse models of HD are utilized to unravel the pathophysiological mechanisms underlying the HD phenotype. The R6/2 mouse, which exhibits rapid disease onset (as early as 9 to 11 weeks of age) and a brief lifespan (between 13 to 16 weeks), is an appropriate model for mimicking HD features. However, its swift and early disease onset limits therapeutic interventions. In order to broaden the applicability of HD mouse models, we developed the B6-hHTT130-N model utilizing gene-editing technology. In brief, the new strain was transformed with a human fragment that contains HTT exon1 and 130 CAG repeats, which was then integrated into the C57BL/6J mouse genome. At 2 months of age, significant neuropathological phenotypes such as an increase in aggregated mutant HTT inclusions, reduction in brain weight and volume, and neuron loss are evident. Furthermore, decreased motor function is observed before 2 months of age. B6-hHTT130-N mice are also observed to develop hyperglycemia by 3 months of age, presenting with impaired glucose tolerance. Importantly, the B6-hHTT130-N model exhibits a longer lifespan, averaging over 24 weeks, which enables the study of age-related effects and facilitates the attainment of rapid and definitive outcomes. In conclusion, the B6-hHTT130-N model presents a moderate to rapid disease onset and progression, well-defined behavioral abnormalities that can be quantified, and neuropathological features that make it ideal for drug efficacy screening and potential therapy development for HD.

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Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.05/P4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Characterization of the R61 Mouse Model of Huntington’s Disease

Authors: C. TORTURO, J. GRESACK, K. COX, *S. RAMBOZ;
PsychoGenics, Paramus, NJ

Abstract: The B6.Cg-Tg(HDexon1)61Gpb/J mouse line (commonly referred to as the R6/1 line) is a transgenic model of Huntington’s disease that displays progressive neurological degeneration. These mice are transgenic for the 5' end of the human huntingtin gene and exhibit motor deficits, seizures, as well as decreased body weight and muscle mass. We sought to build on previous research by characterizing behavioral and pathological endpoints in gender mixed R61 mice starting at 15 weeks of age. Animals were tested in a battery of behavioral tests,

including open field and elevated plus maze. Additionally, gait and cognition were assessed using our proprietary cube technologies. The data demonstrated that R6/1 hemizygous animals exhibited decreased movement in the open field, decreased performance in the elevated plus maze, and abnormal gait. Once the animals reached 6 months of age, tissues and fluids were collected for biochemical and immunohistochemical analyses. Concluding remarks are pending additional behavioral data analysis and histological readouts. The ultimate goal of this work is to identify robust readouts that can be used to determine the efficacy of disease modifying therapies for Huntington's disease.

Disclosures: **C. Torturo:** A. Employment/Salary (full or part-time):; PsychoGenics. **J. Gresack:** A. Employment/Salary (full or part-time):; PsychoGenics. **K. Cox:** A. Employment/Salary (full or part-time):; PsychoGenics. **S. Ramboz:** A. Employment/Salary (full or part-time):; PsychoGenics.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.06/P5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Evaluating obsessive compulsive behaviors in Huntington disease model mice

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Abstract: Obsessive compulsive symptoms (OCS) are more frequent in individuals with Huntington disease (HD) than in the general population. OCS are experienced by 15-50% of HD gene carriers, making them an important consideration for patient quality of life. OCS have been linked to dysfunction of neural circuits connecting the striatum, thalamus, and frontal cortex. The frequent occurrence of OCS in HD patients is unsurprising given that the earliest and most aggressive neurodegenerative hallmark of HD is progressive striatal degeneration, which is accompanied by degeneration to the cortex and thalamus. Recent studies have indicated the presence of the psychiatric symptoms of HD decades prior to motor symptom onset or clinical diagnosis. Pre-manifest HD individuals report higher levels of OCS, with symptoms increasing as disease onset approaches. Multiple mouse models of HD display progressive HD-like behavioral and cognitive abnormalities beginning prior to onset of motor deficits. However, until now these evaluations have primarily focused on traits analogous to anxiety, depression, and disrupted learning and memory. This study aims to evaluate the presence of obsessive and compulsive behaviors in young and aged HD mice. The Q175FDN mouse model of HD contains human huntingtin (*HTT*) exon 1, with approximately 200 CAG repeats, knocked in to the WT mouse Huntington disease homolog (*Hdh*) gene. Q175FDN heterozygous mice exhibit motor and cognitive deficits by 6 months of age but show neuropathological signs of degeneration, gene expression changes, and depressive and anxiety-like behaviors as early as 3 months of age. We

assessed these mice for obsessive and compulsive behaviors either at 12 months of age when disease is advanced or prior to 3 months of age to determine whether the Q175FDN line can serve as an effective model of OCS in pre-manifest HD.

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Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

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Program #/Poster #: PSTR532.07/P7

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Instituto de Salud Carlos III, which is an agency of the MINECO, co-funded by FEDER funds/European Regional Development Fund (ERDF) – a way to Build Europe (CP13/00225 and PI14/00834, to EC). Agencia Estatal de Investigación (AEI), an agency of MINECO, and co-funded by FEDER funds/European Regional Development Fund (ERDF) – a Way to Build Europe (PID2020-114518RB-I00 to EC, BFU2017-87109-P to RdD).

Title: Investigating altered limbic reward system in Huntington's Disease: Implications for apathy

Authors: V. BIKOU[1], I. VICENTE[1], A. DE PAEPE[2], C. GARCIA-GORRO[1], N. RODRIGUEZ-DECHICHA[3], I. VAQUER[3], M. CALOPA[4], R. DE DIEGO-BALAGUER[1,5,6], ***E. CAMARA**[2]; [1]Cognition and Brain Plasticity Unit, IDIBELL, L'Hospitalet de Llobregat (Barcelona), Spain; [2]IDIBELL, Barcelona (Hospitalet de Llobregat), Spain; [3]Hestia Alliance, L'Hospitalet de Llobregat (Barcelona), Spain; [4]Movement Disorders Unit, Neurol. Service, Hosp. Universitari de Bellvitge, L'Hospitalet de Llobregat (Barcelona), Spain; [5]Develop. and Educ. Psychology, Barcelona Univ., Barcelona, Spain; [6]ICREA (Catalan Inst. for Res. and Advanced Studies), Barcelona, Spain

Abstract: Apathy, the most prevalent psychiatric symptom in Huntington’s disease (HD), has a significant impact on the function and quality of life of patients. Although apathy is linked to the loss of goal-directed behavior and motivation, the relationship between apathy and reward valuation, as well as compromised limbic territories in HD, remains poorly understood. This study aimed to dissociate the functional correlates that are altered in reward processing and underlie apathy in HD. We aimed to tease apart whether apathy is associated with insensitivity to processing rewards, hypersensitivity to losses, or both, leading to the observed lack of motivation in apathetic individuals. Thirty-nine HD individuals (apathetic (N=15) and non-apathetic (N=24)) and 26 healthy participants, according to the short-Lille Apathy Rating Scale, underwent functional magnetic resonance imaging during a gambling task. The goal was to identify disrupted reward-related regions in patients with HD and their association with apathetic

symptoms. Whole brain analysis of gains and losses separately showed a significant reduction in activity within the left ventral striatum (VS), which includes the nucleus Accumbens, in the HD group compared to controls. The effect observed in the VS remained when clinically apathetic patients were compared with controls. Conversely, non-apathetic patients did not show any significant differences compared to controls. Interestingly, these group differences appeared exclusively during the processing of the reward. Additionally, higher levels of apathy were associated with decreased activity related with the processing of gains in this region. Our findings highlight the vulnerability of the left VS in HD and its association with the altered function of gains, particularly in relation to apathy, while preserving the valuation of losses. This suggests that reward insensitivity associated with VS dysfunction may be an important component of apathy in HD. Understanding the underlying mechanisms of reward processing and apathy in HD may help to explore the implications of limbic regions rather than frontal executive dysfunction in apathy.

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Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.08/P8

Topic: E.04. Voluntary Movements

Support: CIHR Fdn-143210
CIHR Fdn-143209

Title: High-throughput Characterization of Prolonged Motor Learning in a Huntington Disease Mouse Model: Insights from an Automated Homecage System

Authors: *D. RAMANDI[1], T. H. MURPHY[1], L. A. RAYMOND[2];
[2]Dept Psychiatry and Ctr. for Brain Hlth., [1]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Probing the neuronal alterations that underlie motor learning is a key step in developing novel treatment strategies for motor disorders such as Huntington’s Disease (HD). While prior studies have analyzed motor skill learning, there has been a limited focus on long-term behavioral changes. In this research, we devised a novel skilled lever-pulling task within an automated home-cage testing framework. This allowed us to evaluate the learning progress of both male and female wild-type (WT) and zQ175 HD mice at 6 months of age over several weeks. The 24/7 measurement system allowed us to gauge participation and performance adjustments.In this self-directed behavioral task, animals were taught to hold a lever for progressively longer intervals, up to 1 second. The hold-time for successful trials was personalized daily based on each animal's previous day's performance (75th percentile of all

trials' hold-times). Our results showed a consistent increment in the average hold-time for WT animals, whereas the zQ175 mice struggled to adapt to the task's increasing demands. It is noteworthy that the daily trials' number and time spent in the behavior chamber did not differ between genotypes.Additionally, we found a distinct pattern in zQ175 mice, with a heightened jerkiness in their pull trajectories, marked by an increase in high-frequency un-coordinated movement amplitude. The zQ175 mice also showed different patterns of engagement with the task and modulation of movement variability following trial outcomes. The iterative adjustment in required hold-time throughout the learning process mitigates the habituation process, which is corroborated by a consistent trial-to-trial correlation, suggesting stable motor variability.These findings point to early-stage HD mice displaying deficits in fine motor learning and performance. Furthermore, our platform's vast data collection capacity hints at the potential for reinforcement learning modeling, contributing to a deeper understanding of motor learning dynamics.This research is funded by the Canadian Institutes of Health Research Fdn-143210 to LAR and Fdn-143209 to THM.

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Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.09/P9

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH/NINDS- 5R01NS089750-08
NIH/NINDS-R25 NS089463
Dixon Family Foundation

Title: Striatal Somatostatin Interneuron Involvement in the Pathogenesis of Huntington's Disease

Authors: *M. GRAY, **J. A. R. FOWLER**;
Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Huntington’s disease (HD) is an autosomal dominant disease caused by an expansion of a CAG repeat in the gene encoding the Huntingtin protein. The resulting abnormal polyglutamine-containing protein is expressed throughout neuronal and non-neuronal cell types. HD patients exhibit progressive motor dysfunction. This disease is characterized by significant degeneration of medium spiny neurons (MSNs) in the striatum. These cells are very important members of the basal ganglia circuit and are critically involved in controlling motor coordination. The function of striatal MSNs is regulated by extrastriatal glutamatergic input from cortex and thalamus as well as intrastriatal and extrastriatal GABAergic input. One population of GABAergic interneurons express the neuropeptide somatostatin (SST) and are defined as persistent low-threshold spiking interneurons. In multiple mutant Huntingtin (mHTT) expressing mice, including the conditional human mHTT expressing BACHD model, the SST+ interneurons

have increased spontaneous firing that likely impinges on the activity of MSNs that displayed an increased in inhibitory post-synaptic current. The increase in SST firing in these mice could be contributing critically to the abnormal function of MSNs in HD. Thus, understanding the role mHTT expression plays in these cells is important and can provide insight into the dysfunction observed in the striatum of HD patients. In this study, we will use a genetic approach to knockdown mHTT expression in SST cells, by crossing BACHD mice to SST-Cre mice, to determine if mHTT expression in SST cells contributes to the behavioral, neuropathological, and electrophysiological changes observed in BACHD mice. We hypothesize that expression of mHTT in SST cells influences the increased GABAergic changes observed in MSNs. Here, we report that knockdown of mHTT in SST cells by 6 months of age does not improve motor abnormalities in BACHD mice at 6 months of age. Further analysis of these behaviors, as disease progresses, is being performed. Preliminarily, these studies indicate no significant contribution of mHTT in these cells to motor dysfunction, however we will perform additional studies of non-motor phenotypes in the mice.

Disclosures: **M. Gray:** None. **J.A.R. Fowler:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

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Program #/Poster #: PSTR532.10/P10

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS119255
NIH Grant R01NS125260
NIH Grant R01NS096144

Title: Genetic rescue of interneurons prevents motoric deficits, striatal degeneration, and myelination deficits in a mouse model of Huntington's disease

Authors: A. E. MOLERO, ***Y. M. ALTUN**, G. S. DEVAKANMALAI, M. F. MEHLER; Neurol., Albert Einstein Col. of Med., BRONX, NY

Abstract: Huntington’s disease (HD) is a relentlessly progressive neurological disorder whose primary hallmarks include striatal degeneration and motor deficits. To date, we still have a poor understanding of the pathogenic mechanisms underlying HD. Previous studies have shown that selective impairments in cortical interneurons (INs) are differentially associated with disease symptomatology in HD. To better define the pathogenic roles of INs in HD, we rescued this lineage by driving the excisional recombination of a floxed mutant gene in the BACHD model, with CRE recombinase driven by Nkx2.1 (BACHD-N), a patterning gene selectively expressed by a major IN neurogenic domain within the ventromedial telencephalon. Motoric testing (open field, balance beam, and Rotarod tests) of 12-month-old mice showed this genetic rescue greatly prevented the emergence of motor deficits. Electron microscopic analyses showed that the

number of striatal neurons with degenerative morphology was comparable between control mice and mice with rescued INs - in contrast to observations in HD mice, in which 47% of striatal cells displayed degenerative features. Moreover, there was correction of myelination deficits in deep white matter tracts, likely resulting from non-cell autonomous mechanisms also mediated by interneurons. Overall, the genetic rescue of the Nkx2.1 progenitor domain of IN subtypes dampened the progression of two of the most prominent hallmarks of HD - motor deficits and striatal neuronal degeneration, strongly suggesting this neural lineage plays critical roles in HD pathogenesis.

Disclosures: **A.E. Molero:** None. **Y.M. Altun:** None. **G.S. Devakanmalai:** None. **M.F. Mehler:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

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Program #/Poster #: PSTR532.11/Q1

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH R01124084
NIH R01127344

Title: Impaired blood-brain barrier precedes motor dysfunction and striatal atrophy in the zQ175 Huntington’s disease mouse model

Authors: ***H. LIU**[1], Z. WEI[2], C. LIU[3], Q. WU[3], C. H. VALENCIA[5], Y. OUYANG[6], F. YANG[4], Y. LI[8], J. XU[8], H. LU[7], W. DUAN[7];
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Abstract: The current clinical diagnosis of the onset of Huntington’s disease (HD) relies on the detection of movement symptoms. However, by the time clinical symptoms occur, significant striatal atrophy has already been detected through structural MRI. Multiple recent clinical trials which are targeted at manifest HD have failed. Because almost 50% of the striatal volume has been lost at the time of clinical onset, it would be preferable to begin treatment in the premanifest period before the massive loss of striatum. An unmet challenge is how to reliably evaluate therapeutic efficacy in the absence of clinical symptoms as outcome measures. Therefore, there is a growing demand for functional measures that are noninvasive and can be used in premanifest therapeutic trials. It has been reported that the integrity of the blood-brain barrier (BBB) was disrupted in human HD based on histology analysis of postmortem tissue. In this study, a non-contrast arterial-spin-labeling (ASL) based MRI technique called water-extraction-with-phase-

contrast-arterial-spin-tagging (WEPCAST) MRI was used to assess BBB permeability to water in the zQ175 HD mouse model. This technique measures the relative fraction of magnetically labeled water spins exchanged into the brain tissue compared to those remaining in the cerebral veins, providing indices of global BBB permeability to water, including the water extraction fraction (E) and permeability surface-area product (PS). Quantitative analysis of E and PS values in wild-type mice revealed E to be 59.9±3.2% and PS to be 260.9±18.9 ml/100g/min. In contrast, 5 months old zQ175 HD mice exhibited significantly higher E (69.7±2.4%, P=0.026) and PS (318.1±17.1 ml/100g/min, P=0.040), indicating increased BBB permeability to water in zQ175 HD mice, while no motor symptoms and striatal atrophy were evident at this age. Reproducibility studies demonstrated a coefficient-of-variation (CoV) of 4.9±1.7% and 6.1±1.2% for E and PS, respectively. Western blotting results indicated decreased levels of tight junction protein (Claudin-5, ~ 36% reduction; ZO-1, ~ 27% reduction) in the striatum of zQ175 HD mice. Altogether, these results suggest that altered BBB permeability to water is an early event prior to motor deficient and striatal atrophy in HD mice, further validation of these results in other HD models and human HD will be important to determine whether WEPCAST MRI measure of BBB can serve as a noninvasive biomarker to monitoring disease progression and evaluate treatment efficacy in HD.

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Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.12/Q2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS116099
HDSA HBP grant

Title: Cerebrospinal fluid mutant huntingtin: origins and modulators

Authors: R. J. HARDING[1], Y. XIE[2], A. G. THOMAS[3], N. POTLURI[2], D. LANGBEHN[4], B. S. SLUSHER[5], ***A. L. SOUTHWELL**[2];
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Abstract: Quantification of mutant huntingtin (mtHTT) in cerebrospinal fluid (CSF) is currently being used as a pharmacodynamic biomarker in HTT lowering clinical trials. To better understand this measure, we are using immunoprecipitation and flow cytometry (IP-FCM) in

combination with multiple model systems to investigate the origins of HTT protein in the cerebrospinal fluid, its mechanisms of entry, and factors that modulate signal intensity other than protein concentration. We have found that in addition to passive release from injured or dying cells, HTT is secreted by neurons and that both mutant and wild-type HTT are present in CSF. Additionally, we have found that there is a progressive bias toward striatal contribution to CSF mtHTT, likely due to neurodegeneration and the elevated HTT secretion activity that we have observed in the striatum. Using cell-type specific cre driver lines to inactivate mtHTT in astrocytes or neurons from Hu97/18 mice, we have determined that CSF mutant HTT is largely neuronal in origin, though astrocytes appear to modulate neuronal release of HTT possibly through better neuronal support, a function influenced by whether astrocytes are also expressing mtHTT. We have also determined that in addition to protein concentration, huntingtin conformation, fragmentation, protein interaction, affinity tag positioning, oligomerization and polyglutamine tract length affect assay signal intensity. Considering the unknown milieu of HTT proteoforms in CSF, this precludes absolute quantification of HTT concentration in heterogenous biosamples. Thus, we support reporting of relative CSF HTT protein levels in normalized arbitrary units of signal intensity rather than the common practice of reporting concentrations in fM. Understanding the origin of CSF mtHTT and the factors that modulate its release and detection are providing critical information about which HD interventions can be accurately assessed by changes in CSF mHTT as well as better interpretation of what treatment-induced changes in CSF mtHTT mean about the brain.

Disclosures: R.J. Harding: None. **Y. Xie:** None. **A.G. Thomas:** None. **N. Potluri:** None. **D. Langbehn:** None. **B.S. Slusher:** None. **A.L. Southwell:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.13/Q3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: PID2020-113953RB-I00 Spanish Ministry of Science and Innovation/Spanish State Research Agency (10.13039/501100011033)
2021 Human Biology Project. Huntington’s Disease Society of America (HDSA)
PhD fellowship from the Spanish Ministry of Science and Innovation.

Title: Extracellular small RNAs from biofluids define biomarkers of premanifest changes in Huntington's disease

Authors: *M. HERRERO-LORENZO[1], J. PÉREZ-PÉREZ[2,3,4], G. ESCARAMÍS[1,5], S. MARTÍNEZ-HORTA[2,3,4], R. PÉREZ-GONZÁLEZ[2,3,4,6], E. RIVAS-ASENSIO[2,3,4], J. KULISEVSKY[2,3,4], A. GÁMEZ-VALERO[1,5], E. MARTÍ[1,5];
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Abstract: Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the Huntingtin gene. Molecular biomarkers categorizing mutation-carriers during the preclinical stage (P-HD) preceding the functional decline (manifest stage, M-HD), can assist in optimizing patient management and potentially enable targeted interventions. Extracellular small RNAs (exRNAs), which can be found in body fluids as freely circulating or encapsulated in extracellular vesicles (EVs), are a promising source of biomarkers since their expression levels are highly sensitive to pathobiological processes. Herein, we aimed to define exRNA-based biomarkers to monitor changes that occur in the pre-symptomatic stage, by exploring plasma and cerebrospinal fluid (CSF) sRNA levels. Using an optimized method for plasma EVs purification by Size-exclusion chromatography (SEC) and Ultrafiltration (UF), we explored EVs-exRNA transcriptome through an exhaustive analysis pipeline of sRNA sequencing data from P-HD and M-HD mutation carriers and control subjects (n=10/group). Differentially expressed candidate sRNAs were validated by qRT-PCR in additional plasma-EVs and paired CSF samples (n=20/group). Longitudinal analyses were also conducted at two different time-points. We also evaluated the association between sRNAs expression and diverse HD patients´ clinical features. The characterization of plasma-EVs revealed no differences in their size and morphology between groups. We show that most of plasma EV-sRNAs are early downregulated in mutation-carriers, and that this deregulation is associated with premanifest cognitive performance. The expression of seven candidate sRNAs (tRF-Glu-CTC, tRF-Gly-GCC, miR-451a, miR-21-5p, miR-26a-5p, miR-27a-3p, and let7a-5p) was validated in additional samples, showing a significant diagnostic accuracy at premanifest stage. Of these, miR-451a and miR-21-5p were significantly decreased over time; and miR-21-5p and miR-26a-5p levels correlated with cognitive changes in the premanifest group, suggesting these sRNAs as novel prognostic biomarkers. The plasma validated sRNAs were found to be significantly altered in the opposite direction in CSF samples of the same patients. In summary, the present results suggest that exRNAs, both in plasma and CSF, are early deregulated in HD, with a pattern of deregulation dependent on the type of biofluid. In addition, early deregulated plasma EV-sRNAs senses the progression and cognitive changes occurring at the premanifest stage. Overall, our results define a novel early RNA-based biosignature in HD with potential to improve mutation-carriers classification.

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Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

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Program #/Poster #: PSTR532.14/Q4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: P01NS092525

Title: Hierarchical changes of tau and MAP2 in the BACHD mouse model of Huntington's disease

Authors: *X.-Q. CHEN, W. C. MOBLEY;
UCSD/Neuroscience, La Jolla, CA

Abstract: Motivation: Huntington's disease (HD) is an inherited neurodegenerative disease. Dysregulated tau and MAP2 proteins have been implicated in the pathogenesis of HD. However, conflicting evidence exists regarding the involvement of tau in HD, especially tau phosphorylation status. Therefore, the exact nature of these manifestations and their temporal progression remains unclear. Methods: In this study, we investigated the progression of tau and MAP2 pathologies in a well-established HD mouse model known as BACHD mouse. We analyzed the protein levels of tau and MAP2, as well as the phosphorylation status of tau at multiple epitopes, in the brains of BACHD mice at different developmental stages: newborn (P3), young (2 months), mid-aged (5-6 months), and elderly (12 months). Additionally, we examined the activity of potential kinases/phosphatases involved in tau regulation and assessed tau's binding capacity to microtubules in BACHD brains. Results: Our findings showed that while the high molecular weight isoforms of MAP2 were reduced around 5 months, the overall level of tau remained largely unchanged across all stages examined. Interestingly, we observed that tau was hypophosphorylated starting from the young adult stage (2 months), both in the striatum and cortex, and this hypophosphorylation persisted throughout the lifespan of BACHD mice. Both reduced GSK3β activity and reduced levels of p25 favored the hypophosphorylation of tau. Consistently, the hypophosphorylation of tau was associated with an increased binding affinity to microtubules. Conclusions: Our study provides new insights into the developmental changes in tau and MAP2 pathology in BACHD mouse brains, shedding light on the potential role of tau in HD.

Disclosures: X. Chen: None. **W.C. Mobley:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

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Program #/Poster #: PSTR532.15/Q5

Topic: C.04. Movement Disorders other than Parkinson's Disease

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College of Medicine

E. Malcolm Field and Gary Leo Dunbar Endowed Chair of Neuroscience
John G. Kulhavi Professorship in Neuroscience at CMU

Title: Analysis of cell surface markers along with donor age and passage number of mesenchymal stem cells on efficacy in treating motor deficits in R6/2 mouse model of Huntington's disease

Authors: *B. SRINAGESHWAR[1], G. L. DUNBAR[2], J. ROSSIGNOL[1];
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Abstract: Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by CAG repeat expansions in the Huntingtin gene (HTT), leading to production of mutant huntingtin protein (mHTT), which culminates in severe loss of neurons in the striatum and other areas of the brain. Currently, only palliative treatments are available and none of these effectively slow down the progression of this devastating disease. Previous reports have shown the therapeutic effects mesenchymal stem cells (MSC), which were derived bone marrow and umbilical cord reduced motor deficits in rodent models of HD. However, our previous study has indicated that the therapeutic effect of MSCs for HD depends on how many times the cells have been passaged prior to transplantation. It was shown that higher passaged MSCs (P40 to P50) delayed the onset of motor, cognitive, and neuropathological loss in HD mouse models, most likely through the release of neurotrophic factors, specifically, brain derived neurotrophic factor (BDNF). The present study examined these two critical aspects - the donor age and the passage number of the bone marrow derived MSCs in the context of alleviating motor deficits in R6/2 mice. Our findings indicate higher passaged MSCs, derived from a young donor (5 week old mouse), alleviates motor deficits in R6/2 mice. However, MSCs derived from an old donor (10 mo-old mouse) worsens the symptoms. There is also evidence showing that the higher passaged MSCs may lead to a compromised safety profile, due to accumulation of chromosomal abnormalities that may occur over several passages. Hence, higher passage MSCs may not confer optimal clinical utility. Therefore, we further, analyzed the cell surface markers (Sca-1, CD90, CD105, CD45, SSEA and MHCII) of low passage and high passaged MSCs using fluorescence-activated cell sorting (FACS) to arrive at a specific population of MSCs having the maximum therapeutic efficacy in alleviating the HD symptoms. Our findings indicate that the sub-population of BM-MSCs that are $CD90^+$ alone and $CD105^+$ alone show reduced survival and have increased morphological changes over time in culture when compared to the BM-MSCs containing mixed populations of $CD90^+$ $CD105^+$ and $Sca1^+$ cells. Moreover, the Sca1+ cells were found to be a dominating sub-population of MSCs at both lower and higher cell passages. Therefore, identifying a specific sub-population of MSCs with specific cell surface markers is an important factor to consider when designing MSCs-based therapies for HD.

Disclosures: B. Srinageshwar: None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.16/Q6

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Evaluation of a small molecule splicing modulator in a humanized HTT intron 49 mouse

Authors: *D. MAGNANI;
Charles River, Saffron Walden, United Kingdom

Abstract: ***Evaluation of a small molecule splicing modulator in a humanized HTT intron 49 mouse*** Magnani, Dario[1*]; Kritikou, Eva[1]; Mota, Daniel[1]; Macabuag, Natsuko[1], Herva Moyano, Maria[1]; Mitchell, Phil[1]; Fischer, David F. [1]; Liu,Longbin[2] , Kheterpal, Vinod[2] ; McAllister, George[2]; Chen, Richard[21] *Charles River, [2] CHDI Management Inc./CHDI Foundation, *presenting author*Lowering the expression of the pathogenic mutant huntingtin (mHTT) mRNA and protein is a leading therapeutic approach for the treatment of Huntington's Disease (HD).Branaplam (LMI070) and PTC-518 are small molecule drugs that target splicing of the exon junction 49-50 of HTT pre-mRNA, promoting the retention of a pseudo-exon within intron 49, causing the degradation of HTT mRNA by nonsense-mediated decay, and consequential decrease in protein levels.Both drugs recently proceeded to Phase 2 clinical evaluation. However, the branaplam VIBRANT-HD trial was terminated due to emergent signs of neurotoxicity and peripheral neuropathy in some patients, and will not progress further. While underway in Europe and Australia, further enrolment in the PTC- 518 PIVOT trial in the US has been paused after the FDA requested additional data to allow the study to proceed.Branaplam, PTC-518 and other compounds targeting this mechanism do not modulate endogenous rodent, dog or primate Htt splicing due to species-specific *HTT* intron 49 sequence differences. This makes it difficult to investigate pre-clinical efficacy and safety in standard rodent or large animal preclinical models prior to clinical trials. To evaluate potential on-mechanism, on-target (ie HTT lowering) in vivo efficacy and safety liabilities for this class of compounds, we used a newly generated Q175-iExon49 /Q7-iExon49 mouse, in which the mouse *Htt* intron 49 in both the mutant (Q175) and endogenous (Q7) Htt alleles are replaced with the human *HTT* intron 49 sequence , enabling the splice modulation of both the expanded and non-expanded *HTT* alleles, as would occur in treated HD patients. Q175 knock-in mice, which carry a human HTT Exon 1 with approximately 190 CAGs knocked into the murine Htt gene, were selected because they have been extensively characterized previously, making them a suitable model for preclinical safety and efficacy testing. Upon treatment with Branaplam, we show the expected human intron 49 retention and disruption of the endogenous 49-50 junction, in both peripheral and CNS tissues of Q175-iExon49 /Q7-iExon49, with lowering of both normal and polyglutamine expanded HTT protein in a variety of tissues.

Disclosures: D. Magnani: None.

Poster

PSTR532. Huntington's Disease Mouse Models: Behavior, Pathology, and Therapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR532.17/Q7

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: John G. Kulhavi Professorship in Neuroscience
E. Malcolm Field and Gary Leo Dunbar Chair of Neuroscience
Office of Research and Sponsored Programs at Central Michigan University
Shepherd's Gift
Central Michigan Neuroscience Program

Title: Systemic treatment of cognitive and motor effects using bovine-sourced GM1 ganglioside in the R6/2 mouse model of Huntington's Disease mice

Authors: *D. DOYLE[1], O. SMITH[2], K. ADAMS[2], L. GARMO[3], A. UPRETY[2], N. DAY[2], B. SRINAGESHWAR[4], J. ROSSIGNOL[5], G. L. DUNBAR[6];
[1]Central Michigan Univ. Grad. Program In Neurosci., Mt Pleasant, MI; [2]Neurosci., [3]Neurosci. Grad. Program, Central Michigan Univ., Mt Pleasant, MI; [4]Neurosci. Grad. Program, Central Michigan Univ., CHENNAI, India; [5]Neurosci. Grad. Program, Central Michigan Univ., Mount Pleasant, MI; [6]Psychology/Program in Neurosci., CENTRAL MICHIGAN UNIVERSITY, MOUNT PLEASANT, MI

Abstract: Huntington's disease (HD) is a genetic, autosomal dominant, neurodegenerative disease that leads to the overproduction and aggregation of mutant huntingtin protein (mHTT), resulting in cognitive, motor, psychological, and physical decline, ultimately culminating in death. There is no cure for HD, but the use of GM1 ganglioside, a potential disease-modifying therapy, has shown promise in decreasing mHTT accumulation, while increasing facilitating the efficacy of neuroprotective compounds, such as brain-derived neurotrophic factor (BDNF), slowing disease progression. GM1 treatment has been shown to be efficacious within HD models via direct injections within the brain. However, only limited studies employing clinically translatable, less invasive injection strategies, such as intraperitoneal injections, have been conducted. The purpose of this study was to analyze the effects of systemically injected GM1in the R6/2 mouse model of HD . A total of 47, five-week-old, R 6/2 transgenic mice and their wild-tyep (WT) counterparts were given daily IP-injections of either 30 mg/kg of bovine GM1 or control saline solution . Behavioral testing, using the rotarod-, water-T-maze-, and tape-tests, wereperformed to assess the therapeutic efficacy of bovine-sourced GM1 on cognitive and motor function following systemic injections. Our data revealed a robust a genotype effect with R6/2 mice showing significant deficits. However,t the GM1 treatments did not significantly reduce these deficits in either the rotarod or the water-T-maze tasks, but did demonstrate a significance reduction in time to remove the tape wrapped around the wrists of the mice.. Further work is underway to explore the extent to which systemically administered bovine-sourced GM1 confers therapeutic efficacy in the R6/2 mouse model of HD and to compare this with systemically administered ovine-source GM1 to gain a more comprehensive understanding of the potential of this promising treatment for HD.

Disclosures: D. Doyle: None. **O. Smith:** None. **K. Adams:** None. **L. Garmo:** None. **A. Uprety:** None. **N. Day:** None. **B. Srinageshwar:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.01/Q8

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Heterogeneity in sporadic amyotrophic lateral sclerosis: mechanistic insights

Authors: *A. FROLOV, M. A. GUZMAN, G. HAYAT, J. R. MARTIN, III;
St. Louis Univ., St. Louis, MO

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disorder with fatal outcome. It is most common in Caucasians but has been seen with diverse racial and ethnic backgrounds. The onset of the symptoms is commonly seen in the 5th- 6th decades. It leads to weakness of limb or bulbar muscles and later affects the respiratory muscles. There is currently no cure for ALS and the number of efficient disease-modifying drugs for ALS is limited to a few despite a large number of clinical trials conducted during recent years. The latter could be explained by a significant heterogeneity of ALS clinical phenotypes where even well-characterized familial (inherited) form of ALS (fALS), accounting for about 15% of ALS cases and associated with a single and highly potent mutations, display variable clinical phenotypes even within the group of patients sharing similar mutations. The ALS clinical heterogeneity is even more complex in patients with non-inherited (sporadic) form of ALS (sALS) which accounts for the vast majority (~ 85%) of ALS cases. Therefore, the current practice treating very diverse ALS cases as a single disease calls strongly for a shift to personalized ALS diagnoses and demonstrates urgent need in understanding the biological mechanisms responsible for ALS clinical diversity. To address this issue, we conducted genetic and neuropathological postmortem examination of two female patients without a family history of ALS but having a different onset (early vs late), different onset affected areas (bulbar vs limb), as well as the disease progression (fast vs slow), thereby presenting different clinical phenotypes. The very stringent bioinformatics analysis of the respective whole exome sequencing data identified rare pathologic mutations in five common genes with the genetic variants in three of those genes being identical between two patients. A set of additional rare and pathological genetic variants specific for each patient was also detected and the respective variants were grouped, based on their functional annotations, into the following categories: 1) Variants Linked to ALS; 2) Variants Linked to Other Neurological Disorders, and 3) Variants Linked to ALS Related Pathology. These data let us hypothesize that ALS could develop in an individual who is primed for ALS through a set of specific mutations that are common, or identical, in ALS patients. The disease could then be triggered by the mutations linked to ALS through a previously suggested multistep process (PMID: 25300936), and the respective clinical phenotype will be defined by mutations associated with other neurologic disorders as well as by mutations linked to ALS related pathology.

Disclosures: **A. Frolov:** None. **M.A. Guzman:** None. **G. Hayat:** None. **J.R. Martin:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.02/R1

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Effect of various charge neutralizing modifications on TDP 43 aggregation

Authors: *K. BHAVSAR, S. GUPTA;
Biol. engineering, Indian Inst. of Technology, Gandhinagar, Gandhinagar, India

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobardegeneration (FTLD) are two deleterious neurodegenerative disease, sharingdisease mechanism and causative protein TDP 43. TDP 43 is 414 amino acid long protein, which consist of 4domain: N-terminal domain (1-98), RNA Recognition domain (101 - 176 aa), RNArecognition domain 2 (191 - 239 aa) and C terminal domain (250 -414 aa). Theprotein has 2 nuclear sequence; nuclear localization sequence and nuclearexport sequence, which helps the protein to transport from nucleus to cytoplasm.Physiologically TDP-43 is a regulator of gene expression, RNA processing, etc.However, the mutation in TDP-43, leads to the excess deposition and formationof misfolded toxic protein aggregates in the cytoplasm of the cell, leading toneuronal degeneration.Aim: Effect of such Lysine-based PTMs on the aggregationpropensity of TDP 43 derived peptides. We synthesized and chemically modified;Core aggregation-prone sequences from N-terminal of the protein, the lysinerich sequence of RRM1 and C-terminal aggregation-prone serine rich sequence,which have not been studied much for their aggregation potential. Study isconcentrated to explore the lysine-rich peptide regions that could lead toaggregation upon charge neutralization via Acetylation and CarbamylationPost-translational Modification. Methods: Solid-phase peptide synthesis, RP-HPLC, LC-MS,MALDI/TOF, and Used an array of biophysical and microscopic analyses, such asThT kinetic assay, fluorescence microscopy, Congo red staining, scanning electronmicroscopy, and Bio-AFM. Moreover, we have studied the cellular toxicity of thederived-peptides in HEK 293 cells. Result and Conclusion: Through various chemical,bio-physical and imaging techniques, we confirmed the fibrillary amyloid natureof peptides aggregates after the charge-neutralising modifications, and theseaggregates were cytotoxic as well to HEK 293cells, with only 55% of cellssurviving after 24-h exposure. We have shown that Acetylation and Carbamylationcan induce aggregation *in vitro* in various unsuspecting lysine-richpeptide sequences in length dependent manner.

Disclosures: K. Bhavsar: A. Employment/Salary (full or part-time):; IIT Gandhinagar. **S. Gupta:** A. Employment/Salary (full or part-time):; IIT Gandhinagar.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.03/R2

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Mutation and loss of the WW domain-containing oxidoreductase worsen mitochondrial dysfunction in amyotrophic lateral sclerosis

Authors: *A. CASTILLO-TORRES[1], T. PETROZZIELLO[1], S. HUNTRESS[1], S. M. FARHAN[2], K. VAKILI[3], M. CUDKOWICZ[1], J. BERRY[1], G. SADRI-VAKILI[1]; [1]Massachusetts Gen. Brigham, Boston, MA; [2]McGill Univ., Montreal, QC, Canada; [3]Boston Childrens Hosp., Boston, MA

Abstract: Mitochondrial dysfunction plays a key and early pathogenic role in neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). However, the exact mechanisms leading to alteration in mitochondrial function are not completely elucidated. Here, we investigated the potential role of the WW domain-containing oxidoreductase (WWOX), a protein already implicated in neurodegeneration. WWOX plays a critical role in several cellular functions, including the regulation of the mitochondrial electron transport chain (mtETC). First, we identified rare and ALS-specific variants in *WWOX* by assessing the Project MinE dataset. Of interest, among these variants, the 261E stop codon mutation localizes in the mitochondrial binding region of WWOX short alcohol dehydrogenase/reductase domain (SDR). Importantly, treating SH-SY5Y cells with a human WWOX recombinant protein carrying the 261E stop codon mutation ($rWWOX^{STOP261E}$) decreased cell viability, reduced ATP levels, and increased reactive oxygen species (ROS). Consistently, there was a significant decrease in the mitochondrial ATP synthase of complex V and the cytochrome c oxidase of complex IV in ALS post-mortem motor cortex (mCTX). Additionally, there was an overall decrease in WWOX levels in ALS mCTX, further supporting a pathogenic role for loss of WWOX. Notably, knocking down WWOX using a small interference RNA (siRNA) decreased cytosolic and mitochondrial ROS levels as measured by aconitase activity. Taken together, our findings suggest that the 261E stop codon mutation and loss of WWOX contribute to oxidative stress in ALS.

Disclosures: A. Castillo-Torres: None. **T. Petrozziello:** None. **S. Huntress:** None. **S.M. Farhan:** None. **K. Vakili:** None. **M. Cudkowicz:** None. **J. Berry:** None. **G. Sadri-Vakili:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.04/R3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH/NINDS RO1-NS101895
NIH/NINDS RO1-NS118145

Title: Aberrant protein citrullination and PAD2 dysregulation characterize reactive astrogliosis, microglia activation, and myelin protein aggregation in ALS patients.

Authors: *I. YUSUF[1], S. PARSI[2], P. THOMPSON[3], Z. XU[3];
[1]Univ. of Massachusetts Chan Med. School, Dept. of Biochem. and Mol. Biotech., Worcester, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Biochem. and molecular biotechnology, Univ. of Massachusetts Chan Med. Sch., Worchester, MA

Abstract: Protein citrullination is a posttranslational modification that involves the conversion of peptidyl-arginine to peptidyl-citrulline and is catalyzed by a family of enzymes known as protein arginine deiminases (PADs). Of the five PADs, PAD2 is the most dominant isoform in the CNS. Abnormal protein citrullination and PAD2 dysregulation have been shown in several neurodegenerative diseases. Also, previously, we reported PAD2 dysregulation and abnormal protein citrullination in mouse models of amyotrophic lateral sclerosis (ALS), a deadly neurodegenerative disease characterized by loss of motor neurons, paralysis, and eventual death, however, these observations are yet to be confirmed in human ALS. Therefore, we investigated protein citrullination and PAD2 in ALS patient's post-mortem samples, using Immunoblotting, Immunohistochemistry, Immunofluorescent and filter trap assays. We report that protein citrullination and PAD2 expression are altered in the spinal cord of ALS patients increasing in astrocytes with reactive astrogliosis. Also, while PAD2 and protein citrullination are largely not altered in Iba1+ microglia, some microglia populations show increased PAD2 and protein citrullination, with the former correlating positively with HLA-DR, a marker of activated microglia. Furthermore, in the spinal cord and motor cortex subcortical white matter, citrullinated proteins forms aggregates that contain PLP and MBP in patients compared to non-neurological controls. These results suggest that increased protein citrullination and PAD2 dysregulation are critical characteristics of reactive astrogliosis, microglia activation and myelin protein aggregation in ALS. Further studies are needed to dissect the specific roles that PAD2 and protein citrullination play in the pathogenesis of ALS.

Disclosures: I. Yusuf: None. **S. Parsi:** None. **P. Thompson:** None. **Z. Xu:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.05/R4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: A MEK1/2 inhibitor trametinib for the treatment of amyotrophic lateral sclerosis - Interim analysis of a Phase 1/2a clinical trial

Authors: *T. HONG[1], M.-Y. KIM[2], H. SHIN[3], B.-J. KIM[4], K.-K. KIM[5], S. OH[6,7], S. KIM[3], Y.-M. LIM[5], S.-Y. LEE[2], **J. CHOIH**[8,9], J.-Y. KIM[2], S. HAN[2,9], B. KIM[10];
[1]Res. Ctr., [2]Genuv Inc., Seoul, Korea, Republic of; [3]Dept. of Neurol., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [4]Dept. of Neurol., Korea Univ. Col. of Medicine, Korea Univ. Anam Hosp., Seoul, Korea, Republic of; [5]Dept. of Neurol., Asan Med. Center, Univ. of Ulsan Col. of Med., Seoul, Korea, Republic of; [6]Dept. of Neurol., Busan Paik Hospital, Inje Univ. Col. of Med., Seoul, Korea, Republic of; [7]Dept. of Neurol., Kyung Hee Univ. Hosp., Seoul, Korea, Republic of; [8]Genuv Inc., Seoul, Republic of Korea, Seoul, Korea, Republic of; [9]Genuv Inc., US Subsidiary, Cambridge, MA; [10]Dept. of Neurol., Samsung Med. Center, Sungkyunkwan Univ. Sch. of Med., Seoul, Korea, Republic of

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease with a huge unmet need for disease-modifying therapies. As the pathogenesis and pathophysiology of ALS is largely unknown, various therapies with unique mechanisms are under development to find clues for ALS treatment. By utilizing a neural stem cell screening platform (ATRIVIEW®), trametinib (SNR1611, Mekinist®) was identified as the most neuroprotective and neurogenerative drug from an FDA-approved drug library. Trametinib inhibits MEK1/2 in the MEK/ERK pathway and efficiently decreases phospho-ERK level. Several studies demonstrated that phospho-ERK level is elevated in *in vitro*, *in vivo* ALS models and the spinal cords of sporadic ALS patients, which may trigger motor neuron death. Based on its efficacy in nonclinical studies and known safety profiles in its original cancer indications, a Phase 1/2a proof-of-concept clinical trial for ALS was conducted with commercially available trametinib as drug repurposing (CT1SNR1611ALS1, NCT04326283). This clinical trial was originally designed to sequentially escalate dose of trametinib according to recommendation of Independent Data Monitoring Committee (IDMC), up to the recommended dose for cancer indications (2 mg daily). IDMC reviewed the minimum 4-week safety data of SNR1611 0.5 mg group and recommended initiation of SNR1611 1 mg group, which demonstrates favorable safety and tolerability of the SNR1611 0.5 mg daily dose. IDMC further recommended additional recruitment of SNR1611 1 mg group, instead of initiation of SNR1611 2 mg group, to supplement the safety data of SNR1611 1 mg group due to drop-outs. Adverse drug reactions (ADRs, ≥10%) of SNR1611 0.5 mg, SNR1611 1 mg, and Mekinist 2 mg (melanoma study) showed no significant difference in the interim analysis; some ADRs seem ALS indication-related (i.e. blood creatine phosphokinase increased, lethargy, etc.). Incidence of Grade 3/4 ADRs of each group showed dose dependency (0, 25, and 52%, respectively), demonstrating predictable and manageable safety profile of trametinib in ALS. Mean changes from baseline of the Korean version of Amyotrophic Lateral Sclerosis Rating Scale-Revised (K-ALSFRS-R) scores of SNR1611 groups (SNR1611 0.5 mg, SNR1611 1 mg) seemed similar to those of Riluzole group despite the large age differences between the SNR1611 groups and Riluzole group of over 13 years (SNR1611 0.5 mg: 63.38±6.55, SNR1611 1 mg: 63.25±6.30, Riluzole: 50.00±11.80 years). As older age at onset is related to faster progression, trametinib may show significant efficacy in age-stratified, well-powered clinical trials, warranting its further development for ALS.

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

property rights/patent holder, excluding diversified mutual funds); Genuv Inc.. **H. Shin:** None. **B. Kim:** None. **K. Kim:** None. **S. Oh:** None. **S. Kim:** None. **Y. Lim:** None. **S. Lee:** A. Employment/Salary (full or part-time):; Genuv Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genuv Inc. **J. Choih:** A. Employment/Salary (full or part-time):; Genuv Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genuv Inc. **J. Kim:** A. Employment/Salary (full or part-time):; Genuv Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genuv Inc. **S. Han:** A. Employment/Salary (full or part-time):; Genuv Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genuv Inc.. **B. Kim:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.06/R5

Topic: C.06. Neuromuscular Diseases

Support: Philanthropic Donations

Title: Evaluating TDP-43 Dysregulated RNA Targets in Blood for Potential Biomarker Identification

Authors: *N. G. KINNEY, R. K. PRADHAN, B. K. JENSEN, H. S. ILIEVA;
Weinberg ALS Ctr., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating progressive neuromuscular disease. Current therapies aim to slow progression with limited efficacy and there are no curative drugs. Disease biomarkers are of critical importance for diagnostic (accurate diagnosis of disease), prognostic (determining disease progression) and therapeutic purposes (measuring effect of disease modifying drugs). Elevations in cerebrospinal fluid (CSF) and blood neurofilaments (NF) have begun to be used as a diagnostic biomarker for pre-clinical ALS. Reductions in NFs are also used in clinical trials as a therapeutic outcome measure. However, increases in NF levels are not ALS specific, as they are associated with several other neurodegenerative conditions. Thus, a disease specific biomarker is needed. Cytoplasmic aggregation and subsequent dysfunction of TDP-43, a DNA/RNA binding protein involved in RNA splicing, is a hallmark of ALS pathophysiology. TDP-43 dysregulation can cause non-canonical splicing of various RNA transcripts that can reduce protein expression levels and/or disrupt function. One such abnormal variant which has been detected across animal and cell models is a deletion of exon 3 in the POLDIP3 gene. POLDIP3 encodes for an RNA binding protein which is ubiquitously expressed. In this study, we aimed to determine if POLDIP3 RNA for both the normal and non-canonical variant is detectable in either human plasma or blood. To

do this, we collected blood samples from volunteers diagnosed with ALS, a non-ALS neurological disease, and healthy controls. We quantified levels of both POLDIP3 RNA transcripts using quantitative RT-PCR. In blood, no significant difference in the expression pattern of the two POLDIP3 variants could be detected between groups, however a trend towards higher non-canonical variant expression was seen in both the ALS and non-ALS disease groups. In plasma, RNA concentrations were too low for analysis. We theorize that the variability in detection is due to a low percentage of cells in peripheral blood harboring significant TDP-43 pathology, diluting any effect. We plan to specifically enrich for cells with TDP-43 pathology to investigate if TDP-43 dysregulated targets can be more sensitively measured in these cells than in whole blood.

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Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.07/R6

Topic: C.06. Neuromuscular Diseases

Support: NeuroSense Therapeutics

Title: Shifting the Paradigm to a Biomarker Focused Approach for Developing an Amyotrophic Lateral Sclerosis (ALS) Therapy

Authors: *S. ZIMRI[1], **N. KEREM**[1], N. RUSSEK-BLUM[1], A. THOMPSON[2], J. HARRIS[2], J. XU[2], H. WONG[2], C. LAWLESS[2], M. BACHMAN[2], E. SIRKA[2], F. TRACIK[1]; [1]NeuroSense Therapeut., Herzelia, Israel; [2]Inoviv, London, United Kingdom

Abstract: ALS has a complex underlying pathophysiology, necessitating a multi factorial therapeutic strategy targeting multiple pathways. PrimeC is a novel formulation composed of ciprofloxacin and celecoxib, aiming to synergistically inhibit the progression of ALS by targeting key pathologies. Synergistic effects between ciprofloxacin, a miRNA regulator, and celecoxib, a neuroinflammation regulator, have been demonstrated in ALS preclinical models. In a 12 month, open label, phase IIa study, 15 patients with ALS treated with PrimeC were compared to propensity matched, non treated ALS patients. Changes were obtained in ALS related biomarkers following treatment (TDP43 $p = .002$, PGJ2 $p < .001$, CATD $p = .015$, LC3 $p = .05$), suggesting biological activity of PrimeC. Results corresponded to clinical outcomes, displaying an 18% and 30% difference measured by ALSFRS-R and forced vital capacity, respectively. In light of these results, NeuroSense commenced PARADIGM, a 6 month, randomized, double blind, placebo controlled, phase IIb study evaluating PrimeC. The primary objectives are the differences between PrimeC and placebo in plasma concentrations of 2 key biomarkers: TDP 43 and Prostaglandin 2. In this joint initiative, we aim to broaden the

knowledge regarding indicative biomarkers for ALS drug development, its biological activity and target engagement. A cassette of biomarkers will be established by using an unbiased mass spectrometry based Proteomics Discovery screen. Biomarkers showing changes between treatment timepoints in the Discovery screen will be monitored as potential indicators of treatment response. Stage I- a pilot study will include cross sectional comparisons of 15 ALS patient samples and 15 matched controls. Stage II- the effect of PrimeC on the predefined panel of markers will be assessed utilizing samples from PARADIGM. This will be evaluated at two time points: at initiation, and after 6 months of treatment. In Stage I, an unbiased Data Independent Analysis proteomics discovery method will be applied to plasma samples depleted of the 14 most abundant proteins. Significant tryptic peptides will be taken forward into a Targeted Proteomics Assay using a triple quadrupole LC MS/MS system. Synthetic peptide Stable Isotope Internal Standards corresponding to the peptides selected from the Discovery study will be spiked into the patient samples from Stage II to support absolute quantification of the selected biomarker proteins using scheduled Multiple Reaction Monitoring mode. All 69 PARADIGM patients from Israel, Italy, and Canada have been recruited. Results from the pilot study will set the stage for the extensive proteomics cassette to be used.

Disclosures: **S. Zimri:** None. **N. Kerem:** None. **N. Russek-Blum:** None. **A. Thompson:** None. **J. Harris:** None. **J. Xu:** None. **H. Wong:** None. **C. Lawless:** None. **M. Bachman:** None. **E. Sirka:** None. **F. Tracik:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.08/R7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Edaravone Mitigates Neurodegeneration in Induced Pluripotent Stem Cell-Derived Neurons from an Amyotrophic Lateral Sclerosis Patient

Authors: *S. MIKURIYA, F. SALINAS, T. SHIRAKAWA, M. TAMURA; NeuroDiscovery Lab., Mitsubishi Tanabe Pharma America, Cambridge, MA

Abstract: Amyotrophic Lateral Sclerosis (ALS) represents a devastating neurodegenerative disorder characterized by progressive motor neuron degeneration and subsequent muscular atrophy. The lack of reliable clinical predictive models has hindered the development of disease-modifying therapies for ALS. This scarcity can be attributed to the complex nature and clinical heterogeneity of the disease. Despite the heterogeneity, the consistent observation of mislocalization of TDP43 in over 95% of sporadic ALS patients has provided valuable insight into the pathophysiology of the disease. Here, we utilized induced pluripotent stem (iPS) cell-derived neurons from a patient with ALS harboring a TARDBP mutation to establish a robust assay system for drug screening. iPS-derived neurons from a patient with ALS and age-matched healthy controls were cultured over weeks. The ALS patient-derived neurons presented

significant reductions in neurite length and neuronal populations over the culture period compared to healthy controls, in conjunction with ALS-specific abnormal phenotypes of TDP-43, including mis-localization of TDP-43 into cytoplasm and elevated STMN2 cryptic exon resulting from the loss of function of TDP-43. RNA-sequence analysis of cells harvested after a week in culture indicated alterations in molecular pathways relevant to ALS in the diseased cells. Subsequently, these iPS neurons were employed to evaluate the effectiveness of currently approved therapeutics for ALS. Following one week of culture, neurons were subjected to ALS drug treatment over a 24-hour duration. Results suggest that edaravone, a free radical scavenger, demonstrated significant neuroprotective effects exclusively in ALS cells, as evidenced by neurite length and cell death assay. Taken together, we developed a clinically-predictive *in vitro* assay system that may be capable of capturing the effect of commercial drugs. This assay system holds potential to significantly accelerate the discovery of novel ALS therapies and the optimization of chemical screens.

Disclosures: **S. Mikuriya:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma America. **F. Salinas:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma America. **T. Shirakawa:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma America. **M. Tamura:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma America.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.09/R8

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: The protective effect of edaravone on TDP-43-induced neurotoxicity in neuronal cells and neuroprotective mechanisms of edravone with transcriptome analysis

Authors: *A. KUSUNOKI[1], R. SAITO[1], K. WATABE[2];
[1]Mitsubishi Tanabe Pharma Corp., Yokohama, Japan; [2]Fac. of Hlth. Sci., Kyorin Univ., Tokyo, Japan

Abstract: Edaravone is a free-radical scavenger of peroxyl radicals potentially inducing oxidative stress in amyotrophic lateral sclerosis (ALS). A pathological hallmark of ALS is the accumulation of ubiquitinated or phosphorylated aggregates of the 43 kDa transactive response DNA-binding protein (TDP-43) in the cytoplasm of motor neurons. To identify the neuroprotective mechanism of edaravone, transcriptome analysis was conducted in 1464R-differentiated neuronal cells transduced with wild type and fragmented TDP-43. Oxidative stress by ethacrynic acid reduced the viability of the rat neural stem cell-derived neurons expressing TDP-43. Edaravone, at ≥10 μmol/L, concentration-dependently suppressed the neurotoxicity induced by oxidative stress in neuronal cells expressing TDP-43. In addition, edaravone prevented the reduction in viability of cells expressing TDP-43 under standard conditions

without oxidative stress Differential gene expression analysis revealed changes in gene expression of pathways related to nuclear erythroid 2-related-factor (Nrf2)-mediated oxidative stress response in cells expressing TDP-43. In edaravone-treated TDP-43 expressed cells, significant changes in gene expression were identified in pathways of Nrf2-oxidative response, unfolded protein response, and autophagy. Furthermore, the expression of genes related to phosphatidylinositol metabolism pathways was upregulated. These findings suggest that the neuroprotective effect of edaravone involves not only the regulation of redox reaction but also the prevention of TDP-43 misfolding and enhanced clearance of pathological TDP-43 in TDP-43 proteinopathy. (Reference: published in Pharmaceuticals 2022, 15(7), 842)

Disclosures: **A. Kusunoki:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Corporation. **R. Saito:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma Corporation. **K. Watabe:** A. Employment/Salary (full or part-time):; Kyorin University Faculty of Health Sciences.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.10/S1

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Hyperactivity and Altered Physiological Responses in iPSC Derived Motor Neurons from an ALS Patient with a TARDBP Mutation

Authors: *A. TOYCHIEV, T. SHIRAKAWA, M. TAMURA;
NeuroDiscovery Lab., Mitsubishi Tanabe Pharma America, Cambridge, MA

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that leads to the loss of motor neurons and death. The common pathological feature of familial and sporadic ALS is the mislocalization of TDP43, with a noted decrease in the nucleus and an accumulation of insoluble forms in the cytoplasm. Furthermore, aberrant neuronal activity and hyperexcitability in the ALS motor neurons have been observed preceding molecular or anatomical features of neurodegeneration. However, the link between TDP43 mislocalization and aberrant neuronal activity is not fully understood. Here, we aimed to investigate physiological alterations in induced pluripotent stem cells (iPSC)-derived motor neurons from an ALS patient with a TARDBP mutation. Motor neurons from healthy control and an ALS patient were plated in high-density microelectrode array (HD-MEA) plates or on cover glasses with astrocytes. The motor neurons plated in HD-MEA plates were recorded every week for neuronal and network activity. In addition, iPSCs were assessed with the single-cell patch clamp to further elucidate the electrophysiological properties of these cells. HD-MEA recordings indicated that ALS-derived iPSCs exhibited fewer active electrodes compared to their healthy counterparts, despite equal seeding densities. ALS motor neurons demonstrated higher firing rates, more frequent bursts, and increased vulnerability to stressors, such as tunicamycin and ethacrynic acid.

Furthermore, these neurons responded differently to synaptic blockers, showing alterations in network activity indicative of a role for excitatory and inhibitory receptors. A similar bursting pattern with higher spontaneous activity in ALS motor neurons was observed in single-cell electrophysiology. Additionally, electrical stimulation evoked an earlier onset response in ALS iPSCs, with a delayed latency and shorter response time. Notably, despite a higher number of evoked responses, the signal-to-noise ratio in ALS iPSCs was lower than in healthy cells. In summary, the results of this study suggest that ALS motor neurons with the TARDBP mutation showed hyperactivity and hyperconnectivity as well as vulnerability to stressors than healthy control. These neurons also displayed altered network activity modulated by synaptic blockers or electrical stimulation. These results may provide additional insights into the early onset and impaired evoked response characteristic of ALS motor neurons, representing a step towards additional understanding of the pathological mechanisms underlying ALS.

Disclosures: **A. Toychiev:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma America. **T. Shirakawa:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma America. **M. Tamura:** A. Employment/Salary (full or part-time):; Mitsubishi Tanabe Pharma America.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.11/S2

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Development of robust IPSC-based α-synuclein, TAU and TDP-43 aggregation pathology in vitro models for drug discovery

Authors: J. KOEPKE, I. ONOFRE, C. VAN BERKEL, T. WUST, L. SMIT, B. SAMSON-COUTERIE, ***S. JAIN**;
Ncardia, Leiden, Netherlands

Abstract: Ever since Alois Alzheimer noticed what we now call amyloid plaques in the brains of deceased dementia patients over a hundred years ago, many advances in neuroscience has been conveyed to link these plaques with the disease pathology. The same holds true for other neurodegenerative disorders such as Parkinson's (PD) or Amyotrophic lateral sclerosis (ALS) where the disease pathology is linked to misfolding and self-aggregation of proteins leading to the presence of large aggregates. We have developed *in vitro* disease pathology models for synucleinopathies (e.g Parkinson's Disease), Tauopathies (TAU aggregation) and ALS (TDP-43 aggregation) using iPSC-derived neuronal cell models. These robust *in vitro* assays in physiologically relevant human models are amenable to various therapeutic to support all stages of the drug discovery process (i.e. hit ID, hit-2-lead and lead optimization) . We used iPSC-derived cortical neurons (iPSC-CN) seeded to quantify disease relevant phenotypes after treatment with SNCA and Tau recombinant pre-formed fibrils (PFFs). Using high content

imaging, aggregate localization, size, counts and intensity as well as co-localization with phosphorylated forms (pS129 α-syn and pTau (AT8)) were calculated. Modulators of protein degradation, both inhibitors and activators, were able to significantly increase or decrease (respectively) the counts and size of SNCA and counts of pS129 α-syn in a concentration-dependent manner.In this study, we used mutant iPSC-derived motor neurons (iPSC-MN) to quantify mis-localization and aggregation of TDP-43 and were able to quantify the human specific mis-splicing of STMN2. Additionally, we determined the electrophysiological deficits of the iPSC-derived motor neurons in healthy and disease conditions. This platform enabled the development and testing of efficacy of therapeutics for ALS targeting TDP-43 aggregation and the evaluation the potential to rescue key disease-phenotypes as TDP-43 aggregation, mis-splicing of STMN2 and electrophysiology deficits.In conclusion, we developed three custom assays using human stem cell-derived neuronal cell models to support drug developers, providing clinically relevant readouts, at various stages of drug development while reducing the use of laboratory animals.

Disclosures: **J. Koepke:** None. **I. Onofre:** None. **C. Van Berkel:** None. **T. Wust:** None. **L. Smit:** None. **B. Samson-Couterie:** None. **S. Jain:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.12/S3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH-NINDs (R01NS116143)
ALS Association (18-11A-418)

Title: Nucleocytoplasmic shuttling of RNA-binding proteins in iPSC-derived ALS neurons is altered under conditions of stress

Authors: *A. COLLINS[1], C. FALLINI[2], M. GREGOIRE[2], R. SIRTORI[2], E. GASTON[2]; [1]Interdisciplinary Neurosci. Program, [2]Cell and Mol. Biol., Univ. of Rhode Island, Kingston, RI

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that leads to loss of motor function, paralysis, and death within a few years of onset. Nucleocytoplasmic transport (NCT) is an essential function in eukaryotic cells, regulating the dynamic distribution of transcription factors and other regulatory proteins between the nucleus and cytoplasm, particularly in response to cellular stimulation and stress. Interestingly, defects to the nuclear pore complex (NPC) and impaired NCT have been identified as a major disease mechanism in neurodegenerative disorders, including ALS. It is still not known how NCT disruption alters the nuclear and cytoplasmic proteome in neurons, particularly after undergoing cellular stress. To answer this question, we are testing how NPC injury affects the nucleocytoplasmic distribution of different shuttling proteins in ALS neurons compared to

isogenic wild type controls under conditions of oxidative stress. Treating induced pluripotent stem cell-derived neurons with sodium arsenite, a well-established oxidative agent, resulted in similar stress granule formation in both *C9ORF72* mutant neurons and isogenic wild type controls. However, the dissolution of granules occurred with different dynamics in mutant cells, and the overall cellular levels of many stress granule components in response to stress was altered in *C9ORF72* mutant neurons. Interestingly, these changes occurred at an age before neurons developed obvious NPC alterations, suggesting that specific alterations of protein shuttling in ALS neurons may occur at early time points during disease development. Overall, our study suggests that specific downstream cellular pathways that depends on the efficient shuttling of these proteins may be key players in the pathogenic cascade that ultimately leads to neuronal death in ALS.

Disclosures: **A. Collins:** None. **C. Fallini:** None. **M. Gregoire:** None. **R. Sirtori:** None. **E. Gaston:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.13/S4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Characterization of models of TDP43-related stress granule formation

Authors: *N. HEINSINGER, Y. WANG, S. NIROOMAND, Y. TIAN;
Biol. Discovery, Neurosci., Merck, West Point, PA

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that results in TAR DNA-binding protein 43 (TDP43) pathology and progressive motor decline. There currently is no cure and few treatments are available for patients. One of the pathological hallmarks of ALS is TDP43 mislocalization from the nucleus to the cytoplasm, where it can aggregate and become phosphorylated. Mislocalized TDP43 has been shown to colocalize with stress granules in both cell models of ALS as well as human postmortem samples of ALS patients; therefore, targeting stress granules could be a potential therapeutic strategy. We aimed to establish induced pluripotent stem cell (IPSC) and cell models of TDP43 pathology and stress granule formation as well as test tool compounds to evaluate if modulating stress granules can attenuate TDP43 pathology. We developed a TDP43 overexpression model using iGABA IPSCs transduced with AAV-TDP43, which results in increased cytoplasmic TDP43 and TIAR+ stress granule formation. When we use sodium arsenite to stress this model, we observe increased phosphorylated TDP43 and G3BP+ stress granules. We also developed a dNLS-TDP43 SHSY5Y cell line. Upon addition of sodium arsenite stress, we observed an increase in G3BP+ stress granules that colocalize with FLAG-TDP43 and increased aggregated TDP43. We tested pharmacological tools in both models to determine if targeting stress granules could reverse the observed phenotypes and found that we are able to reduce stress granules in these models.

Therefore, we present here two distinct neuronal models of TDP-43 pathology amenable to high throughput screening of compound libraries targeting the Integrated Stress Response and TDP-43 pathology in ALS.

Disclosures: **N. Heinsinger:** A. Employment/Salary (full or part-time):; Merck. **Y. Wang:** A. Employment/Salary (full or part-time):; Merck. **S. Niroomand:** A. Employment/Salary (full or part-time):; Merck. **Y. Tian:** A. Employment/Salary (full or part-time):; Merck.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.14/S5

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Japan Agency for Medical Research and Development (AMED) JP23ek0109497

Title: Oligonucleotide-based approaches for modulating FUS phase separation in ALS/FTLS intervention

Authors: *M. MONIRUZZAMAN[1], Y. FUJIOKA[1], A. TAKEUCHI[2], A. MASUDA[3], M. NEYA[4], Y. OKADA[5], S. ISHIGAKI[1];
[1]Shiga Univ. of Med. Sci., Otsu, Japan; [2]Developmental Biol. and Functional Genomics, Ehime Univ. Grad. Sch. of Med., Matsuyama, Japan; [3]Ctr. for Neurolog. Dis. and Cancer, Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; [4]4KNC Labs. Co., Ltd., Kobe, Japan; [5]Neurol., Aichi Med. Univ., Nagakute, Japan

Abstract: FUS (fused in sarcoma) is an RNA-binding protein that mainly localizes to the nucleus and participates in various aspects of RNA metabolism such as transcription, alternative splicing, and RNA transport. It has been identified as a causative gene for amyotrophic lateral sclerosis (ALS) and a pathological molecule involved in ALS and frontotemporal lobar degeneration (FTLD). FUS undergoes liquid-liquid phase separation (LLPS), a phase transition phenomenon associated with membraneless organelle formation. Abnormal LLPS can result in irreversible aggregation of FUS. This study aims to develop oligonucleotides that regulate the LLPS behaviors of FUS. Candidate oligonucleotides were designed based on FUS-CLIP clusters. They were first screened in vitro using turbidity assays followed by the secondary screening in which subcellular localization and aggregation formation of mutant FUS using a motor neuronal line. Through screening, we identified oligonucleotide #3 as a potential candidate which can ameliorate droplet formation of mutant FUS. It also reduced the cytoplasmic localization of mutant FUS as well as its stress granule formation. The FALS iPSC model successfully recapitulated key ALS characteristics including cytoplasmic mislocalization of FUS, which was normalized by oligonucleotide #3.Our findings conclusively demonstrate the ability of

oligonucleotides to target LLPS dynamics, thereby underscoring their therapeutic potential in ALS/FTLD.

Disclosures: M. Moniruzzaman: None. **Y. Fujioka:** None. **A. Takeuchi:** None. **A. Masuda:** None. **M. Neya:** None. **Y. Okada:** None. **S. Ishigaki:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.15/S7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Functional Phenotypic Excitation Forms of Human iPSC-Derived Spinal Motor Neuron Cultures from Amyotrophic Lateral Sclerosis Patients

Authors: *O. H.-U. SCHROEDER, L. SCHULTZ, A.-M. KNOSPE, M. WINKLER, K. JÜGELT;
NeuroProof Systems GmbH, Rostock, Germany

Abstract: Amyotrophic lateral sclerosis, ALS, is a fatal disease with not fully understood disease mechanisms. Therefore, phenotypic disease models are needed for the development of new therapies. The disease occurs in familial and sporadic forms, fALS and sALS. Although only about 10% of cases are familial with a known hereditary origin, they are important for disease modeling. Known fALS forms have mutations in the SOD1, C9orf72, TDP-43, FUS, and other genes. Human-induced pluripotent stem cells with fALS mutations can be differentiated toward spinal motor neurons. They are canonical disease models that reflect phenotypic disease symptoms. In our hands, iPSC-derived spinal motor neurons with a SOD1 D90A, a SOD1 A4V mutation, a C9orf72 mutation, a TDP-43 G298S, and a TDP43 A384V mutation could be cultivated on microelectrode array plates for 14 to 28 days. No difference in survivability between diseased and wild-type cells was observed. First electrophysiological activity can be observed after 7 days and lasts more than 5 weeks. After 14 days in vitro, a reliable hyperexcitation of the disease motor neurons compared to the wild-type neurons can be observed for C9orf72 and SOD1 mutated forms but not for the TDP43 mutated forms. Riluzole 2 µM applied on days 8 and 11 in vitro with medium change caused a reliable reduction of hyperexcitation after 14 days in vitro in three different models: with C9orf72 and SOD1 motor neurons and in TDP43 mutations, we saw any effect of riluzole. The excitation state of diseased ALS motor neurons can deliver new insights for potential treatment strategies.

Disclosures: O.H. Schroeder: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof Systems GmbH. **L. Schultz:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **A. Knospe:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **M.**

Winkler: A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **K. Jügelt:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.16/S8

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant AG073734

Title: Monitoring wildtype human TDP-43 self-interactions using cellular fluorescence lifetime-based FRET biosensors

Authors: *N. NATHAN KOCHEN[1], E. E. LIAO[2], A. BRAUN[3], J. SACHS[1];
[1]Biomed. Engin., Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Minnesota TC, MINNEAPOLIS, MN

Abstract: TDP-43 pathological aggregates are found in ~97% of ALS patients spanning both familial and sporadic presentations of the disease. TDP-43 aggregates induce cellular dysfunction and motor neuron death due to both loss of its native RNA-processing function and gain of toxic function due to the formation of higher-order assemblies and aberrant stress granule inclusions. Thus, finding small molecules capable of modulating the aggregation cascade of TDP-43 in cells offers a novel strategy to find new ALS therapeutics.
To address this need, we have developed a fluorescence lifetime-based FRET (FLT-FRET) biosensor that monitors TDP-43 self-interactions in living cells. We performed high-throughput screens of the FDA approved Selleck library with TDP-43 and control biosensors. Reproducible and non-overlapping hits in the TDP-43 biosensor will be tested in FLT-FRET dose response curves, TDP-43-induced toxicity and RNA-processing dysfunction models in human cells.

Disclosures: N. Nathan Kochen: None. **E.E. Liao:** None. **A. Braun:** None. **J. Sachs:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.17/S9

Topic: C.06. Neuromuscular Diseases

Support: JSPS KAKENHI, grant number 22K07500

Title: Ropinirole hydrochloride suppresses cholesterol synthetic pathway in ALS spinal motor neurons.

Authors: *S. TAKAHASHI[1,2,3], S. MORIMOTO[2], C. KATO[2], S. NAKAMURA[2], F. OZAWA[2], D. ITO[2], Y. DATÉ[3], K. OKADA[3], J. NAKAHARA[3], H. OKANO[2];
[1]Saitama Med. Univ. Intl. Med. Ctr,, Hidaka-City, Japan; [2]Dept. of Physiol., [3]Dept. of Neurol., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Purpose: The purpose of this study is to investigate the mechanism by which ropinirole hydrochloride (ROPI) attenuates the pathological progression of amyotrophic lateral sclerosis (ALS) patients' spinal cord motor neurons (MNs).
Methods: The ROPALS study, an investigator-initiated clinical trial based on existing drug screening and drug repositioning for disease-specific induced pluripotent stem cell (iPSC)-derived MNs (iPSC-MNs), confirmed the safety and tolerability of ROPI administration in ALS patients. The safety and tolerability of ROPI in ALS patients was confirmed. In comparison with a control group extracted from a large database (Pooled Resource Open-Access ALS Clinical Trials: PRO-ACT), one year of continuous ROPI administration was shown to attenuate disease progression in revised ALS Functional Rating Scale (ALSFRS-R). To explore the mechanism of this effect, we analyzed the effects of ROPI exposure using iPSC-MNs from 20 participants with sporadic ALS enrolled in the ROPALS study and 3 healthy controls, examining changes in neurite outgrowth and mRNA expression over time in vitro (Keio University School of Medicine Ethics Committee approval #20080016).
Results: ALS patient-derived iPSC-MNs had significantly shorter maximum neurite length after normalization compared to healthy controls (p=4.8E-04, two-tailed Student's *t*-test). *In vitro* ROPI exposure significantly improved post-normalized maximum neurite length (p=9.4E-03, two-tailed paired Mann-Whitney *U*-test). Furthermore, time series RNA-seq analysis revealed that ROPI treatment extensively suppressed gene expression of a group of enzymes involved in the cholesterol synthetic pathway. Transcription factor analysis identified SREBF2 as the largest contributor among the transcription factors common to the ROPI-affected gene cluster. Inhibition of SREBP2 activity with fatostatin and HMG-CoA reductase with atorvastatin and pitavastatin significantly improved post-normalized maximal neurite outgrowth in familial ALS patients with iPSC-MNs (all $p<0.05$, MANOVA test).
Conclusion: Suppression of the cholesterol synthetic pathway in MNs is one of the possible mechanisms by which ROPI attenuates ALS pathogenesis.

Disclosures: **S. Takahashi:** 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.; Japan Society for the Promotion of Science (JSPS) (KAKENHI No. JP22K07500). **S. Morimoto:** 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.; Japan Society for the Promotion of Science (JSPS) (KAKENHI No. JP22K15736), Japan Society for the Promotion of Science (JSPS) (KAKENHI No. JP21H05273). **C. Kato:** None. **S. Nakamura:** None. **F. Ozawa:** None. **D. Ito:** None. **Y. Daté:** None. **K. Okada:** None. **J. Nakahara:** None. **H. Okano:** 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.; JSPS (KAKENHI No. JP20H00485), JSPS (KAKENHI No. JP21H05273), AMED (No. 22bm0804003), AMED (No. 20ek0109395), AMED (No. 20ek0109329), AMED (No. 21ek0109492), AMED (No. 21wm0425009). F. Consulting Fees (e.g., advisory boards); K Pharma Inc., SanBio Co. Ltd..

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.18/S10

Topic: C.06. Neuromuscular Diseases

Title: Phenotypic drug screen in human-derived induced pluripotent stem cell (hiPSC)-derived neurons for modifiers of dipeptide repeat (DPR)-induced toxicity in C9orf72 ALS/FTD

Authors: *M. A. M. YATES[1], C. MARQUES[1], A. H. HELD[1], C. SONG[1], J. SUNG[1], B. J. WAINGER[2];
[1]Neurol., [2]Neurology, Anesthesia, Critical Care & Pain Med., Massachusetts Gen. Hosp., Cambridge, MA

Abstract: ALS is a rapidly progressing neurodegenerative disease characterized by loss of motor neurons, causing muscle weakness and atrophy, and eventually death, usually within three years of onset. Ninety percent of cases are sporadic, and of the ten percent of cases that are familial, a (G_4C_2)-hexanucleotide repeat expansion in the C9orf72 gene is the most common cause. In unaffected individuals, the repeats can number up to thirty, but in ALS patients, repeats in the thousands have been observed. These $(G_4C_2)_n$ repeats can cause neurodegeneration through various mechanisms: aggregation of RNA foci, dipeptide repeat proteins produced via non-canonical repeat-associated non-AUG (RAN) translation, and the loss of function of the proteins produced from the C9orf72 gene. Antisense oligonucleotides (ASOs) targeting the repeat expansion have shown promise in reducing RNA foci and DPR expression but have yet to reduce disease progression in various clinical trials. While genetic modifiers of DPR-induced toxicity have been evaluated in several studies, less attention has been directed to small molecule screens aimed at repurposing known bioactive compounds to treat C9orf72 ALS/FTD. In our study we develop and execute a phenotypic screen for modifiers of DPR-induced toxicity in human-derived induced pluripotent stem cell-derived cortical neurons (hiPSC-CNs).
To model DPR toxicity in neurons, we cultured control hiPSC-CNs expressing both a nuclear blue fluorescent protein (BFP) marker for to allow for longitudinal cell counting and membrane-bound TdTomato to allow for neurite outgrowth measurements. The cells were then treated with increasing concentrations of synthesized hemagglutinin (HA)-tagged poly-GR (GR_{20}) for 48 hours. Unbiased quantification of BFP+ nuclei and TdTomato+ neurite using custom Matlab scripts revealed a dose- and time-dependent decrease in cell survival and neurite outgrowth following GR_{20} treatment, consistent with studies demonstrating toxicity of arginine-rich DPRs (Z' 0.12/-0.31, 0.50/0.32, and 0.58/0.13 cell survival/neurite outgrowth for 1.25uM, 2.5uM, and 5uM GR_{20} as a negative control and 0.1% DMSO as a positive control, 48 wells per condition, 1

biological replicate). We conduct the small molecule drug screen in 384-well plate format using a Molecular Devices ImageXPress Micro Confocal platform and Integra Viaflo robotic liquid handler. Secondary analyses include immunostaining for GR_{20} localization and TDP43 localization. Our experiment helps identify candidates and relevant mechanisms through which poly-GR-induced toxicity in hiPSC-CNs may be mitigated.

Disclosures: **M.A.M. Yates:** None. **C. Marques:** None. **A.H. Held:** None. **C. Song:** None. **J. Sung:** None. **B.J. Wainger:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The Wainger Lab has received research funding from Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity in Quralis. F. Consulting Fees (e.g., advisory boards); Scientific advisory board for Quralis.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.19/T1

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Tdp-43 dysfunction and stmn-2 mis-splicing upon proteasomal inhibition in iPSc-derived neuronal ALS model

Authors: *M. IOVINO[1], L. RITSMA[3], S. COMPTE SANCERNI[3], E. DE KRAA[3], D. MAGNANI[1], L. BUTI[3], A. TURNER[4], T. OOSTERVEEN[4], D. F. FISCHER[2], O. FEDORENKO[1], C. MANSAT[1], M. VLAMING[3];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects both cortical and spinal motor neurons. Most ALS cases are characterized by TDP-43 pathology, being accumulation, nuclear-to-cytosolic mislocalization and phosphorylation of the transactivation response DNA binding protein (TDP)-43. In our work we assessed homozygous or heterozygous CRISPR-edited TDP-43 M337V mutations in induced pluripotent stem cell (iPSC)-derived glutamatergic neurons (a cortical neuron type), generated by bit.bio. Addition of MG-132 proteasomal inhibitor, induced TDP-43 phosphorlyation and mislocalisation, as detected by high content imaging. Moreover, mis-splicing of the neuronal growth associated factor, stathmin 2 (STMN-2) was as also detectable at day 21 *in vitro* by ddPCR. Furthermore, activity of neurons containing homozygous M337V mutation co-cultured with wild type human astrocytes was reduced compared to WT neurons when examined using multi-electrode array (MEA) offering a functional read out for neuronal activity. These data indicate the utility of bit.bio TDP-43 cells for the multiple readouts as an ALS-relevant disease model.

Disclosures: **M. Iovino:** None. **L. Ritsma:** None. **S. Compte Sancerni:** None. **E. de Kraa:** None. **D. Magnani:** None. **L. Buti:** None. **A. Turner:** None. **T. Oosterveen:** None. **D.F. Fischer:** None. **O. Fedorenko:** None. **C. Mansat:** None. **M. Vlaming:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.20/Web Only

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: National Science and Technology Council, Taiwan:MOST 111-2628-B-039-006-MY3

Title: Sigma-1 receptor activation by fluvoxamine restores TFEB-mediated autophagy via nucleoporin POM121 in NSC34 C9ALS/FTD model.

Authors: ***H.-E. WU**[1], S.-M. WANG[2], C.-Y. LIN[2], T.-P. SU[3];
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Abstract: Dysregulated autophagy relates to neurodegenerative diseases, in particular the *C9orf72*-ALS. In *C9orf*72-ALS, (G_4C_2)-hexanucleotide repeats expansion (HRE) does so by disrupting the nucleocytoplasmic transport of TFEB required for autophagy. Recent studies have shown that the Sigma-1 receptor (Sig-1R), a multiple-functional chaperone protein, can provide neuroprotection, particularly in ALS and Alzheimer's disease. However, the exact mechanism underlying Sig-1R on $(G_4C_2)_n$-RNA-induced cell death remains unclear. Here we found that fluvoxamine, a Sig-1R agonist which causes the dissociation of Sig-1R from BiP and inhibits the aggregation of citrate synthase, increased chaperoning nucleoporin POM121 in $(G_4C_2)_{31}$-RNA-expression NSC34 cells, thereby stabilizing POM121 protein expression. Interestingly, fluvoxamine administration increased POM121 expression without affecting transcription. In *C9orf*72-ALS, the TFEB autophagy factor translocation decreases due to nucleocytoplasmic transport defects. HRE causes the dissociation of Sig-1R from POM121, resulting in a deficiency of TFEB and an autophagy marker LC3-II. Our results showed that pretreatment with fluvoxamine promoted TFEB translocation into the nucleus and increased LC3-II expression compared to the overexpression of $(G_4C_2)_{31}$-RNA alone in NSC34 cells. Together, fluvoxamine could be a promising repurposed drug for treating *C9orf*72-ALS, as it stabilizes the nucleoporin POM121 and foster the translocation of TFEB in $(G_4C_2)_{31}$-RNA-expressing NSC34 cells. (Supported by the IRP, NIDA, NIH, and National Science and Technology Council, Taiwan).

Disclosures: **H. Wu:** None. **S. Wang:** None. **C. Lin:** None. **T. Su:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.21/T2

Topic: C.06. Neuromuscular Diseases

Support: NSERC CGS-M
CIHR Project Grant PGT-180294

Title: An in vivo model system to study ALS using human iPSC derived motor neurons

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized, in part, by neuromuscular junction (NMJ) dysfunction and motor neuron death. Multiple model systems have been used to study ALS, including transgenic rodents. A disadvantage of these model systems is that they do not allow for the study of functional human motor neurons under experimental conditions. Human motor neurons, derived from induced pluripotent stem cells (iPSCs), can be studied *in vitro*. However, these cells (here termed iPSCMNs) possess immature transcriptomes and are only viable in culture for a few weeks. To address these limitations, this study investigates human iPSCMNs derived from healthy individuals, and from those with ALS (familial or sporadic), after they are transplanted into transected peripheral nerves of mice. Using this experimental paradigm, the anatomy and electrophysiological properties of the transplanted iPSCMNs, as well as the electrophysiological properties of their NMJs, can be studies for several months *in vivo*. Furthermore, the pathophysiology arising from the various genetic mutations known to cause ALS can be systematically investigated and compared. In this study, we generated iPSCMNs from healthy individuals and from ALS patients with mutations in SOD1, C9orf72 and TDP-43 using a 14-day *in vitro* differentiation protocol. Using this protocol about 30% of the cells in the culture become motor neurons. These cells are then harvested so that approximately 10,000 iPSCMNs can be transplanted into the distal end of transected tibial nerves in SCID mice just proximal to the branch where it innervates the medial gastrocnemius muscle (MG). The proximal end of the tibial nerve is then ligated to prevent endogenous regeneration. To date, we have found that the grafted iPSCMNs from healthy subjects survive in the distal nerve stump for at least 4 months after transplantation. Importantly, transplanted human iPSCMNs were found to functionally innervate denervated muscle fibers. Using an *ex vivo* nerve-muscle preparation we found that the transplanted iPSCMNs can generate approximately 20% of the tetanic force relative to unoperated controls. Future experiments will examine how forces produced by transplanted iPSCMNs from ALS patients compare to iPSCMNs from healthy controls. Additionally, electrophysiological analysis on nerve-muscle preparations will be done to study differences in NMJ transmission and immunohistochemistry will be used to study innervation of transplanted cells. These results suggest that transplanted iPSCMNs may be used as a model to study ALS, as well as the pathophysiology of different ALS variants.

Disclosures: J. Guthrie: None. **V.F. Rafuse:** None.

Poster

PSTR533. ALS: Patient Research and Human-derived Cellular Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR533.22/T3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Unravelling the correlation between stressors and amyotrophic lateral sclerosis onset in an in vitro experimental model

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Abstract: Our lifestyle can strongly affect our health. Nowadays, we constantly undergo physical, social and environmental stressors: in the CNS, a stress can induce several cellular alterations, whose role in triggering neurological diseases is only partially known. Amyotrophic Lateral Sclerosis (ALS) is a motor neuron (MN) disease, determining the progressive degeneration of both upper and lower motor neurons, characterized by weakness, muscle atrophy and premature death. ALS pathophysiological mechanisms include cytotoxicity, oxidative stress and neuroinflammation, which are cellular processes also activated by stressor exposure. The goal of this work is clarifying how stressors can contribute to the onset and progression of ALS. To this aim, an *in vitro* experimental model of stress has been set-up using naive NSC-34 cells (MN-like cells) and NSC-34 cells expressing hSOD1 gene (WT or G93A) under the control of a doxycycline-inducible promoter. On the grounds of cell viability results, cells have been differentiated in MN-like cells with 20µM of retinoic acid (RA) for 4 days, while the hSOD1 expression has been induced adding doxycycline for 24 hours into culture medium. Then, to mimic a stress condition, cells underwent oxygen and glucose deprivation: $CoCl_2$ was used as hypoxic agent and its toxicity was measured by MTT assay. Different concentrations of $CoCl_2$ were evaluated and LC_{50} was obtained using 100µM $CoCl_2$ for 24 hours. Cell damage was demonstrated evaluating protein levels of different markers (HIF1α, UCP4, caspase3) and studying the mitochondrial activity. We observed that mutated cells (hSOD1 G93A) are less able to counteract stress conditions. Then we studied the expression of some ALS-related genes, by performing real-time PCR: genes involved in cytoskeleton organization, cell adhesion, apoptosis, cell cycle regulation and proliferation seem to be deregulated in stressed mutated cells as compared to stressed WT cells. Additional experiments are needed to confirm and extend these results, however we have set-up the conditions for the following analyses, to evaluate genetic/epigenetic alterations and to clarify *in vivo* the stressor impact on the onset and progression of ALS.

Disclosures: D. Rasà: None. **I. Stoppa:** None. **M. Boido:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.01/T4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH Grant R33NS115161
NIH Grant R61NS115161

Title: Loss of TDP-43 facilitates the pathological conversion of endogenous tau protein to drive neurodegeneration

Authors: *G. BURNS[1], M. S. BAGHEL[2], R. TSAPATSIS[3], T. LI[4], P. C. WONG[5];
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Abstract: Nuclear clearance and cytoplasmic aggregation of TAR DNA/RNA binding protein 43kDa (TDP-43) is a pathological hallmark of several mixed etiology dementias (MEDs), including frontotemporal dementia (FTD), Alzheimer's Disease with TAR DNA/RNA binding protein 43kDa (TDP-43) pathology (AD-TDP) and corticobasal degeneration (CBD). Understanding the role of TDP-43 in disease pathogenesis is critical because AD-TDP patients compared to those without TDP-43 pathology exhibit steeper rates of cognitive decline and exacerbated brain atrophy. Previous work in our lab supports the notion that loss of TDP-43 function, particularly its role in splicing repression of cryptic exons, underlies disease pathogenesis in several human diseases, including AD-TDP, but the molecular basis of this connection is unknown. Moreover, recent biochemical evidence shows that TDP-43 interacts with neurofibrillary tangles (NFTs) in the hippocampi of human AD cases, but what (if any) role TDP-43 pathology may play in the progression of tauopathy remains unclear. We previously showed that amyloid-ß (Aß) plaque is necessary but not sufficient for pathological conversion of tau and another risk factor, such as TDP-43 LOF, is likely required. While we showed that young adult mice lacking TDP-43 in forebrain neurons exhibit exacerbated neurodegeneration, there is a need for aged models exhibiting multiple pathologies to faithfully recapitulate the human pathological context of MEDs. Here, we developed an AAV-mediated transgenic model that exhibits TDP-43 and tau pathologies. Mice lacking TDP-43 in forebrain neurons were injected with adeno-associated virus encoding a human four-repeat tau fragment (Tau4R) or Tau4R containing an aggregation-prone deltaK280 mutation (Tau4RdK) in the right hippocampus. We found that loss of TDP-43 function appears to increase mouse phosphorylated tau burden and spreading. These findings can guide pioneering therapeutic approaches and biomarker development for various MEDs with compromised TDP-43 splicing repression.

Disclosures: G. Burns: None. **M.S. Baghel:** None. **R. Tsapatsis:** None. **T. Li:** None. **P.C. Wong:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.02/T5

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: JSPS KAKENHI Grant Number JP22K07370

Title: Reciprocal F1 hybrids of rTg4510 mice show a difference in the pathological tau accumulation in the brain

Authors: *D. YANAGISAWA, S. ISHIGAKI, I. TOOYAMA;
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Abstract: The rTg4510 mouse model is a well-characterized bitransgenic F1 hybrid mouse model of tauopathy. In the present study, we investigated whether there was a difference in pathological tau accumulation between reciprocal F1 hybrids of rTg4510 mice. The rTg4510 mice were obtained by crossing Camk2α-tTA mouse line on a C57BL/6J background with a tetO-MAPT*P301L mouse line on a FVB/NJ background, and therefore, two genetic backgrounds exist in rTg4510 mice, i.e., rTg4510 on the (C57BL/6J × FVB/NJ)F1 background (rTg4510_CxF) and on the (FVB/NJ × C57BL/6J)F1 background (rTg4510_FxC). In histochemical analyses, rTg4510_FxC mice showed significantly high accumulation of AT8-immunoreactivity and Gallyas-positive structure in the cerebral cortex and the hippocampus at 6 months of age, compared with rTg4510_CxF mice. Biochemical analyses also showed that abnormal tau accumulation was accelerated in the brain of rTg4510_FxC mice, compared with rTg4510_CxF mice. Two-way ANOVA revealed that there were strong effects of the genetic background on the differential tau accumulation between reciprocal F1 hybrids of rTg4510 mice, while sex had no apparent effect. Interestingly, *midline-1* was identified as a candidate gene associated with the differential tau accumulation between reciprocal F1 hybrids of rTg4510 mice. Elucidation of the mechanism underlying the differential tau accumulation between reciprocal F1 hybrids of rTg4510 mice would be helpful to understand the pathological progression of tauopathies. Furthermore, it would be informative to develop therapeutic interventions for tauopathies.

Disclosures: D. Yanagisawa: None. **S. Ishigaki:** None. **I. Tooyama:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.03/T6

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH Grant NS117628

Title: Assessment of pathological tau species and structural changes in a rat model of PSP

Authors: K. KALIYAPPAN[1], M. P. LEIGH[3], S. SURESH[4], M. KRZYZANSKA[3], *S. CLARK[2];
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Abstract: Progressive supranuclear palsy (PSP) is a rare neurodegenerative disorder that is clinically characterised by problems with movements, balance, vision, emotional and cognitive functions. It is considered atypical parkinsonism due to symptomatic and pathological overlap with Parkinson's disease. PSP is characterized by the abnormal phosphorylation of tau protein, leading to aggregation of tau into neurofibrillary tangles (NFTs) in neurons and glia throughout various brain regions. The tau accumulation is greatest in the projection sites of pedunculopontine tegmental nucleus (PPTg) cholinergic neurons, which include pons, globus pallidus, caudate, subthalamic nucleus, and substantia nigra (SN). Postmortem assessments have revealed extensive degeneration of PPTg cholinergic, and SN dopaminergic neurons with abnormal levels of insoluble tau aggregates. Neuroimaging assessments of PSP patients have also demonstrated midbrain and cortical atrophy, enlarged ventricles, and changes in white matter and subcortical structures. Currently, treatment options are limited to mainly palliative care. However, the development of an accurate and highly reproducible animal model would significantly accelerate drug discovery efforts aimed at mitigating PSP pathology.
Earlier studies from our lab have demonstrated that selective overexpression of wild-type human tau (1N4R) in the PPTg, using Cre-dependent AAV vectors and Chat-CRE rats, can produce PSP-like behavioral deficits and pathology. However, it remains unclear whether the abnormally phosphorylated tau in the PPTg ChAT neurons can propagate to associated brain regions in a prion-like manner. The proposed study aimed to validate the hypothesis that the abnormal accumulation of tau in cholinergic PPT neurons may represent an early event in the etiology of PSP, which ultimately leads to the development of PSP symptoms and pathology. In this study, the levels of abnormal pathological tau species (AT8 & PHF1) in the form of sarkosyl soluble and insoluble tau aggregates are being assessed in various brain regions through western blotting analysis, while the level of pathological conformational changes (MC1) will be assessed through immunohistochemical analysis at 5 months post-Tau induction. Additionally, we will analyze structural changes in the corpus callosum, cerebral aqueduct and inferior colliculus using MRI imaging. The findings from this study may provide insights into the pathophysiology and mechanism of disease progression, facilitating the identification of potential biomarkers and the development of therapeutic strategies specific to PSP.

Disclosures: K. Kaliyappan: None. **M.P. Leigh:** None. **S. Suresh:** None. **M. Krzyzanska:** None. **S. Clark:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.04/T7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: DoD Defense Health Agency 311661-2.00-66323

Title: Behavioral outcomes after repeated mild CHIMERA traumatic brain injury in tau transgenic rats

Authors: *C. KOSTELNIK[1,3], A. GRILLAKIS[1,3], A. FAN[1,3], J. LIU[1,3], L. B. TUCKER[2,3], A. FU[2,3], G. A. CARLSON[4,5], J. AYERS[4,5], S. B. PRUSINER[4,5,6], J. MCCABE[1];
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Abstract: Progressive tauopathy has been linked to repeated mild traumatic brain injury (rmTBI) cases in the military, with signs of elevated plasma tau levels and associated cognitive, mood, and motor symptoms. Since tauopathies can currently only be diagnosed post-mortem, the mechanistic link between the initial injury and the ongoing degenerative process is still not well understood. The goal of this study is to create a valid preclinical model of a rmTBI-related progressive tauopathy in male and female rats. To accomplish this, we utilized both wild-type (WT) rats and transgenic rats that are either heterozygous (HE) or homozygous (HO) for the abnormal, mutated human tau (htau) P301S gene, which has an increased propensity to develop tau neuropathology. Seventy-five male and female rats were exposed to five rmTBIs using the CHIMERA (Closed-Head Impact Rotational Acceleration) model or sham procedures at four months of age and righting reflex (RR) was recorded immediately after each injury. Behavioral measures, including the Open Field Test (OFT), Novel Object Recognition Test (NOR), and Y-Maze spontaneous alternation task were administered four months after the final injury. We hypothesized that htau rats exposed to rmTBI would present with greater behavioral abnormalities compared to injured WT and sham controls. We found a main effect of genotype on RR, where HO rats had a longer RR compared to HE and WT rats. In addition, we found a main effect of sex, where males had a longer RR compared to females. There was no effect of injury on RR, suggesting the changes seen are related to sex and genotype differences in response to isoflurane exposure. On the OFT, we found a significant interaction between genotype and sex, where HO females displayed hyperactivity, while HO males displayed a reduction in activity levels and spent less time in the center of the arena, suggesting anxiety-like behavior. No differences were seen on the NOR test. On the Y maze, we found a deficit in spatial working memory in HO animals. As we did not see any injury effects on these behavioral tasks, our data suggest behavioral changes in tau rats are related to genotype and sex but do not appear to be affected by exposure to CHIMERA rmTBI early in life.

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Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.05/T8

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: VIEP-BUAP grant to CA en Neuroendocrinología BUAP-CA-288
CONACYT-PRONACES 194171
Fellowship from CONACYT No. 799022

Title: Pregnancy increases spike-wave discharges in the TUBB4A mutant rat

Authors: *E. HERNANDEZ ALVARADO[1], J. R. EGUIBAR, Sr.[2], C. CORTES[3];
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Abstract: The *taiep* rat is a spontaneous mutation from the Sprague-Dawley strain. The rat had a point mutation in the tubulin β 4a gene, being the only available animal model of the human tubulinopathy called hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC). We have shown in long-term EEG recording that they have spike-wave discharges (SWDs) with a sexually dimorphic pattern, being the males more affected than females. Sex hormones such as progesterone, estradiol or prolactin could be responsible for this effect. The gestation period had natural maternal variations in the concentrations of these hormones. The aim of the study was to analyze the SWDs during pregnancy in the *taiep* rat. We used 5-month-old female *taiep* rats implanted with stainless steel screw electrodes in the cerebral cortex. A 24-h EEG was performed before pregnancy. For copulation, they were put in acrylic cage with a sexual expert male and mating was verified by the presence of the spermatic plug. Along pregnancy, a 24-h EEG recording did every week. The frequency and duration of the SWDs were quantified and compared with the control recordings. Our results showed that the number of SWDs increased significantly during the second week of pregnancy, mainly during the light phase (RM-ANOVA, $P< 0.001$). The total duration of SWDs increased in the third week of gestation, relative to non-pregnant conditions (from 3.4 ± 0.1 s to 3.8 ± 0.1 s; RM-ANOVA, $P< 0.05$). In conclusion, natural variations in sex hormones during pregnancy increased the frequency and duration of SWDs in different periods of gestation. In subsequent studies, we will determine the specific role of each hormone in the SWDs. These results clearly have clinical implications in female H-ABC patients.

Disclosures: E. Hernandez Alvarado: None. **J.R. Eguibar:** None. **C. Cortes:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.06/T9

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: ELA International 2019-01212
U54NS115052

Title: Scd1 deficiency results in early-onset severe alopecia and reverses weight increase in an adrenoleukodystrophy mouse model

Authors: *A. QIAN[1], Y. GONG[1], Y. JASPERS[2], S. KEMP[2], F. EICHLER[1];
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Abstract: X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disease caused by mutations in *ABCD1*. Mutations in the *ABCD1* gene result in loss of function of ALDP, a peroxisomal half transporter that facilitates the movement of very long chain fatty acyl-CoA esters from the cytosol into the peroxisome for peroxisome specific beta-oxidation. The biochemical hallmark of X-ALD is the accumulation of saturated very long-chain fatty acids (VLCFAs). To investigate the effect of fatty acid (FA) saturation status in ALD, we characterized a mouse model that is a double knockout for *ABCD1* and stearoyl-CoA desaturase 1 (*SCD1*), a lipogenic enzyme catalyzing the synthesis of monounsaturated fatty acids oleate (C18:1) and palmitoleate (C16:1). Oleate (C18:1) and palmitoleate (C16:1) are important components of membrane phospholipids, triglycerides, wax esters, and cholesterol esters. *ABCD1* knock-out mice develop early obesity and a sensory motor phenotype beginning at 8-10 months of age. Surprisingly, *SCD1ABCD1* double knock-out (dKO) mice exhibit a striking alopecic phenotype at a young age. While *SCD1* knock-out mice typically present with severe alopecia, excessive dandruff, and progressive skin lesions around two months of age, this occurs in the *SCD1ABCD1*dKO as early as 3-4 weeks of age. It is unclear whether small fiber neuropathy is contributing to hair loss in the *SCD1ABCD1*dKO. Furthermore, the long term effects upon the sensory and motor phenotype still remain to be explored. Interestingly, *SCD1* deficiency reduces the high body weight that *ABCD1* knock-out mice exhibit.
In conclusion, the interaction between *SCD1* and *ABCD1* may not only impact lipid metabolism, but also have repercussions for hair loss and neurodegeneration over time. Importantly, the hair loss phenotype of the double-knockout mice mirrors the scanty hair and pre-mature balding of patients with the adrenomyeloneuropathy (AMN) subtype of ALD, and is not seen in *ABCD1* knock-out mice. This suggests that aberrant lipid saturation is involved in the AMN hair loss phenotype. The hypermetabolic state associated with weight loss in *SCD1* deficient mice may also counteract the obese phenotype of the *ABCD1* knock-out mouse. Lipidomics analysis of *SCD1ABCD1*dKO mice are currently under way and will shed further light on this.

Disclosures: A. Qian: None. **Y. Gong:** None. **Y. Jaspers:** None. **S. Kemp:** None. **F. Eichler:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.07/T10

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Applying machine learning on behavioral sequelae after Omicron infection in mice

Authors: *K. K. LY[1], S. R. MILLER[1], T. MA[2], R. SURYAWANSHI[2], R. THOMAS[2], N. ELPHICK[3], K. YIN[2], N. KALISS[1], I. CHEN[2], M. MONTANO[2], B. SREEKUMAR[2], L. STANDKER[5], F. H. DAMRON[6], J. MUNCH[5], M. OTT[4], N. ROAN[2], J. J. PALOP[1]; [1]Neurolog. Dis., [3]Bioinformatics Core, [4]Virology, [2]Gladstone Inst., San Francisco, CA; [5]Inst. of Mol. Virology, Ulm Univ., Ulm, Germany; [6]West Virginia Univ., Morgantown, WV

Abstract: At least 10% of individuals who have successfully recuperated from SARS-CoV-2 infection are estimated to experience Long COVID (LC), which may include cognitive dysfunction. Using standard behavioral approaches (Novel Object Recognition and Morris Water Maze), prior work has shown that intracerebroventricular infusion of SARS-CoV-2 spike protein in mice results in memory and cognitive dysfunction. Our recent work has shown that machine learning can detect subtle behavioral changes associated with Alzheimer's disease in mice that standard behavioral tests (Active Place Avoidance and Morris Water Maze) failed to identify. Here, we sought to determine the post-acute effects of bona fide Omicron infection (104 PFU) in a human ACE2-overexpressing mice model (hACE2 K18 mice). For this, we designed a novel apparatus for Open Field analysis in an ABSL3 setting and applied a newly-developed machine learning behavioral phenotyping method (ethoML) in the COVID-19 convalescent mice. To assess for any post-acute behavioral changes, we evaluated mouse behavior 21 days following intranasal Omicron (BA.1) inoculation, using DeepLabCut (DLC) and Variational Animal Motion Embeddings (VAME) per the ethoML method. Subtle behavioral changes were detected in BA.1 convalescent mice relative to sham-infected mice, including increased time in behavioral motifs associated with locomotion, and impaired habituation to the open arena. We present ethoML as a viable approach for phenotyping behavioral sequelae in rodent models of infectious disease.

Disclosures: K.K. Ly: A. Employment/Salary (full or part-time):; Gladstone Institute. **S.R. Miller:** A. Employment/Salary (full or part-time):; Gladstone Institute, UCSF. **T. Ma:** A. Employment/Salary (full or part-time):; Gladstone Institute. **R. Suryawanshi:** A. Employment/Salary (full or part-time):; Gladstone Institute. **R. Thomas:** A. Employment/Salary (full or part-time):; Gladstone Institute. **N. Elphick:** A. Employment/Salary (full or part-time):; Gladstone Institute. **K. Yin:** A. Employment/Salary (full or part-time):; Gladstone Institute. **N. Kaliss:** A. Employment/Salary (full or part-time):; Gladstone Institute. **I. Chen:** A. Employment/Salary (full or part-time):; Gladstone Institute. **M. Montano:** A. Employment/Salary (full or part-time):; Gladstone Institute. **B. Sreekumar:** A. Employment/Salary (full or part-time):; Gladstone Institute. **L. Standker:** None. **F.H. Damron:** None. **J. Munch:** None. **M. Ott:** A. Employment/Salary (full or part-time):; Gladstone Institute.

N. Roan: A. Employment/Salary (full or part-time):; Gladstone Institute. **J.J. Palop:** A. Employment/Salary (full or part-time):; Gladstone Institute.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.08/U1

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH R01DC017489
NIH R61NS125280
Leon Levy Foundation
Rainwater Charitable Foundation
US Department of Veterans' Affairs BX003168
NIH NS123211
NIH NS125280

Title: Linking molecular abnormalities to behavioral deficits using a zebrafish model for tauopathies

Authors: *Y. ZHU[1], H. GELNAW[1], P. LEARY[1], Q. BAI[2], E. A. BURTON[2,3], D. SCHOPPIK[1]; [1]Neurosci. Inst., NYU Grossman Sch. of Med., New York, NY; [2]Dept. of Neurol., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [3]Geriatric Res. Educ. and Clin. Ctr., Pittsburgh VA Healthcare Syst., Pittsburgh, PA

Abstract: Tauopathies are neurodegenerative diseases characterized pathologically by accumulation of abnormal Tau in the brain. However, it remains unclear how these molecular and cellular dysfunctions lead to behavioral deficits, especially during the early stages of pathogenesis. To dissect disease mechanisms across multiple biological scales, we generated a zebrafish model of progressive supranuclear palsy (PSP), a primary tauopathy causing unexpected falls in patients early in disease progression, by expressing human 0N/4R-Tau in the evolutionarily conserved vestibulospinal (VS) nucleus. Human Tau-expressing zebrafish exhibit impaired balance control during free-swimming while maintaining normal locomotor ability compared to their siblings. Functional imaging of the VS nucleus shows decreased calcium signals in Tau-expressing neurons in response to tilt stimulus. This altered neuronal activity correlates with Tau phosphorylation in VS neurons. Interestingly, we also observed ectopic accumulation of acidic organelles in the cell bodies of Tau-positive neurons, suggesting abnormal lysosomal function. Taken together, our zebrafish PSP model allows us to understand molecular and cellular mechanisms of balance deficits in tauopathies and can be a powerful system for preclinical drug screening and evaluation of potential therapeutic targets.

Disclosures: **Y. Zhu:** None. **H. Gelnaw:** None. **P. Leary:** None. **Q. Bai:** None. **E.A. Burton:** A. Employment/Salary (full or part-time):; UPMC/UPP Physicians, US Department of Veterans Affairs. **D. Schoppik:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.09/U2

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: T32 GM132061
T32 GM008545

Title: Tau-tubulin isotype interaction early in the formation of tau aggregation in C. elegans

Authors: *W. C. AQUINO NUNEZ, B. D. ACKLEY;
Mol. Biosci., The Univ. of Kansas, Lawrence, KS

Abstract: In tauopathies, tau becomes aggregated in areas of the brain that exhibit high levels of synaptic and neuronal degeneration. The primary physiological role of tau is to stabilize microtubules. Loss of tau affects microtubules maintenance and impairs microtubule functions, *e.g.,* cargo transport, *etc*. 80% of tauopathies are sporadic, and aging is a leading risk factor for these disorders. During aging, cellular processes that maintain protein homeostasis of cytoskeletal proteins become less effective, leading to age-dependent decrease of microtubules. This can be exacerbated by tau aggregation and, may contribute to the synaptic loss and neuronal death observed in disease. There are several tubulin isotypes and isotypes can compensate for one another *in vivo, e.g.,* when knocked out, *etc.* Thus, questions remain about how the interaction of tau with microtubules, specifically different isotypes, might cause or exacerbate neurodegeneration. We have created *C. elegans* models to study aging effects on wild-type and disease-associated tau variants. We generated *C. elegans* lines expressing either wild-type tau, rapidly polymerizing tau, or a disease-associated tau variant, each tagged with GFP. We also obtained CRISPR-generated nematode lines tagging the endogenous locus of two α-tubulins, *tba-1* or *tba-2,* and a β-tubulin, *tbb-1,* with RFP. We monitored tau-GFP aggregation and tubulin-RFP stability during aging within neurons using confocal microscopy and quantified the consequence of tau-GFP accumulation on lifespan. We find that distinct tau-GFP variants accumulate in different neuronal compartments, and that aggregation-prone tau leads to a significant reduction in lifespan. Tubulin isotypes also accumulate in different cellular compartments. Specifically, TBA-1 localizes to cell bodies and axons, while TBA-2 and TBB-1 are predominantly enriched in cell bodies. In addition, preliminary data suggest that, TBA-1 levels remain stable during the early adulthood, but, somewhat counterintuitively, when co-expressed with tau-GFP, we observed a significant, age-dependent decrease in TBA-1. Experiments with TBA-2 and TBB-1 are ongoing. We will also examine whether any tau-tubulin-isotype-dependent interactions might play a role in tau aggregation *in vivo.* Overall, our

model allows us to examine the spatial and temporal dynamics of tau aggregation and microtubule stability in an organism that enables genetic, environmental, and pharmacological analyses. Our data can inform us about the kinds of interactions that may lead to neuronal instability in tauopathies.

Disclosures: W.C. Aquino Nunez: None. **B.D. Ackley:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.10/U3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Augusta University Start-up Fund

Title: The synergistic role of tau and α-synuclein in mitochondrial dysfunction and cognitive decline.

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[1]Neurosci. and Regenerative Med., Augusta Univ., Martinez, GA; [2]Neurosci. and Regenerative Med., Augusta Univ., Augusta, GA

Abstract: Alzheimer's disease (AD), followed by Parkinson's disease (PD), are the most common age associated neurodegenerative disorders, both with no cure or disease-modifying treatments. AD is primarily defined by pathologies of amyloid-β and tau proteins, while PD is defined by α-synuclein (α-syn) protein deposits. However, these pathologies often coexist in AD and PD and may potentially act synergistically to promote neuron degeneration. Cholinergic neurons in the basal forebrain degenerate in both AD and PD, and the loss of these cells is thought to contribute to cognitive decline. Yet, it remains unknown what causes protein aggregation and how this leads to neuronal death and cognitive dysfunction. Mitochondrial dysfunction is likely a key player in AD and PD pathogenesis via intracellular transport defects specifically linked to α-syn and tau accumulations. Here, we tested the hypothesis that tau and α-syn synergistically cause severe mitochondrial dysfunction leading to neurodegeneration and cognitive decline in both AD and PD contexts using the rapidly aging and highly manipulable C. elegans model system. Transgenic strains expressing pan-neuronal human tau, α-syn, or a tau;α-syn double transgenic have been tested in learning and memory assays. Compared to non-transgenic controls, α-syn alone showed no learning and memory deficits. Similarly, tau alone had no effect on learning and memory. However, when combined, the presence of tau and α-syn eliminated short term memory, consistent with synergistic toxicity leading to cognitive deficits. To determine the mechanisms by which α-syn and tau induce cognitive decline, single cell RNAseq was performed on a strain expressing α-syn and exhibited a decrease in mitochondrial gene expression when compared to the control. Oxygen consumption rates of the tau expressing and α-syn expressing strains was significantly different when compared to the healthy control,

further confirming mitochondrial disruption. To study cholinergic neurons specifically, a paralysis assay has been performed to assess neurotransmission. It was found that the tau;α-syn double transgenic strain and α-syn expressing strain exhibited cholinergic abnormalities, but the tau expressing strain remained normal when compared to the control. The goal of this work is to shed light on the mechanisms of cholinergic neurodegeneration in AD and PD and identify potential therapeutic targets to treat cognitive decline.

Disclosures: J.E. Vincent: None. **D.E. Mor:** None.

Poster

PSTR534. Animal Models of Proteinopathies and Other Neurodegenerative Disorders

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR534.11/U4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Department of Veterans Affairs Career Development Award Level-2 #BX004341
Department of Veterans Affairs Merit Review Grant #I01BX005742
National Institutes of Health Grant R01NS064131

Title: Sut-6/nipp1 suppresses tau and to a lesser extent tdp-43 in transgenic caenorhabditis elegans models of proteinopathy.

Authors: B. P. HENDERSON[1], A. D. BEALE[1], A. D. SAXTON[1], A. H. BLACK[1], A. L. SCIOCCHETTI[2], B. C. KRAEMER[3,1,4,5], ***R. L. KOW**[1,3];
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Abstract: The microtubule-associated protein tau and the DNA/RNA binding protein TDP-43 accumulate pathologically in multiple neurodegenerative diseases including Alzheimer's disease and frontotemporal dementias. Previously we performed forward mutagenesis screening in a transgenic *Caenorhabditis elegans* model of tau pathology, in which human tau protein is overexpressed in all *C. elegans* neurons. We identified a mutation in the gene *B0511.7 (*which will subsequently be referred to as *sut-6)* that suppressed tau-induced locomotor deficits. *sut-6* is the *C. elegans* homolog of *NIPP1 (Nuclear Inhibitor of Protein Phosphatase 1)*. NIPP1 inhibits Protein Phosphatase 1, binds to RNA, and localizes to nuclear speckles. The identified W292X mutation truncated the last 11 amino acids of the SUT-6 protein, which is predicted to delete part of the RNA binding domain and abrogate RNA binding activity. We characterized the effect of this mutation as well as complete deletion of *sut-6* on tauopathy phenotypes in tau transgenic *C. elegans*. We found that deletion of *sut-6* or *sut-6(W292X)* ameliorated tau-induced locomotor behavior deficits, reduced accumulation of tau protein, reduced neuron loss, but did not rescue

lifespan shortening. Interestingly, *sut-6(W292X)* had a much stronger effect on tau-induced behavioral deficits compared to *sut-6(null)*, but a similar effect on tau protein levels and neuron loss. Neuronal overexpression of SUT-6 W292X protein suppressed tau-induced toxicity while wild type protein had no effect. Similar studies with *sut-6* mutations in transgenic *C. elegans* models of TDP-43 proteinopathy showed that loss of *sut-6* or *sut-6(W292X)* also ameliorated TDP-43 induced locomotor deficits and reduced TDP-43 protein accumulation. SUT-6 overexpression exacerbated (if wild-type) or ameliorated (if SUT-6 W292X) TDP-43 related phenotypes. The suppression of TDP-43 proteinopathy phenotypes by *sut-6* mutations was generally milder than for tau ones. Together these results indicate *sut-6/NIPP1* modulates tau and to a lesser extent TDP-43 proteinopathy suggesting overlapping mechanisms by which *sut-6/NIPP1* loss of function rescues tau and TDP-43 toxicity.

Disclosures: B.P. Henderson: None. **A.D. Beale:** None. **A.D. Saxton:** None. **A.H. Black:** None. **A.L. Sciocchetti:** None. **B.C. Kraemer:** None. **R.L. Kow:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.01/U5

Topic: C.06. Neuromuscular Diseases

Support: JSPS KAKENHI JP20K16580
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The Serika Fund
The SENSHIN Medical Research Foundation
NOVARTIS Foundation for the Promotion of Science.

Title: CGG repeat expansion in LRP12 in amyotrophic lateral sclerosis

Authors: *K. KUME[1], T. KURASHIGE[2], K. MUGURUMA[3], H. KAWAKAMI[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the degeneration of motor neurons. Although repeat expansion in *C9orf72* is its most common cause, the pathogenesis of ALS isn't fully clear. In this study, we show that repeat expansion in

LRP12, a causative variant of oculopharyngodistal myopathy type 1 (OPDM1), is a cause of ALS. We identify CGG repeat expansion in *LRP12* in five families and two simplex individuals. These ALS individuals (*LRP12*-ALS) have 61-100 repeats, which contrasts with most OPDM individuals with repeat expansion in *LRP12* (*LRP12*-OPDM), who have 100-200 repeats. Phosphorylated TDP-43 is present in the cytoplasm of iPS cell-derived motor neurons (iPSMNs) in *LRP12*-ALS, a finding reproduces the pathological hallmark of ALS. RNA foci are more prominent in muscle and iPSMNs in *LRP12*-ALS than in *LRP12*-OPDM. Muscleblind-like 1 aggregates are observed only in OPDM muscle. In conclusion, CGG repeat expansions in *LRP12* cause ALS and OPDM, depending on the length of the repeat. Our findings provide insight into the repeat length-dependent switching of phenotypes.

Disclosures: K. Kume: None. **T. Kurashige:** None. **K. Muguruma:** None. **H. Kawakami:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.02/U6

Topic: C.06. Neuromuscular Diseases

Support: NIH (K22NS09131401)
Brooklyn College
CUNY

Title: Histone ptm crosstalk in a yeast als/ftd model

Authors: *C. P. REYNOSO FERNANDEZ, M. TORRENTE, S. N. COBOS, R. FREDERIC; City Univ. of New York Brooklyn Col., Brooklyn, NY

Abstract: Amyotrophic lateral sclerosis (ALS) and Frontotemporal Dementia (FTD) form a fatal, incurable neurodegenerative disease continuum involving the death of neurons. Previous work in our lab has discovered that epigenetic mechanisms -namely histone post-translational modifications (PTMs)- are connected to ALS/FTD. In particular, we have discovered that the levels of phosphorylation on Histone H3 on Serine 10 (H3S10ph) are increased in yeast models of the disease. The goal of this project is to examine histone PTM levels when Ipl1 (the kinase responsible for installing H3S10ph) is knocked down in yeast. We hypothesize that removing Ipl1 might affect the levels of H3S10ph and as well as other PTMs via crosstalk. Crosstalk between *histone* modifications occurs when a *histone* PTM modulates the status of another modification on the same or a different histone. H3S10ph is known to be involved in a few histone crosstalk examples, specifically with H3K9ac, H3K14ac, and H4K16ac. We hypothesize that we should also detect the levels of these PTMs decrease when Ipl1 is knocked down. We will test this hypothesis by way of immunoblotting. We hope that this research will expand our

knowledge of epigenetic mechanisms in ALS/FTD and open new avenues for new treatments for this disease.

Disclosures: C.P. Reynoso Fernandez: 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.; Mariana P. Torrente. Other; Brooklyn College, CUNY, NIH (K22NS09131401). **M. Torrente:** 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.; Mariana P. Torrente. Other; Brooklyn College, CUNY, NIH (K22NS09131401). **S.N. Cobos:** Other; Brooklyn College, CUNY. **R. Frederic:** Other; Brooklyn College, CUNY.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.03/U7

Topic: C.06. Neuromuscular Diseases

Title: Investigating Nucleoporin coding variation and nucleocytoplasmic defects in ALS

Authors: *C. M. FARE[1], L. JIN[1], O. ROMAN[2], A. N. COYNE[1], L. DUPUIS[2], J. D. ROTHSTEIN[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Univ. de Strasbourg, Strasbourg, France

Abstract: Amyotrophic lateral sclerosis (ALS) is an incurable, fatal neurodegenerative disease that is characterized at the cellular level by severe disruptions to nucleocytoplasmic transport and nuclear pore complex (NPC) injury. The overwhelming majority of ALS cases are sporadic, and the number of identified disease-causing genetic mutations only accounts for a small portion of all cases. Using sequencing data available from more than 1,000 patients included in the Answer ALS (AALS) repository, we have identified a large number of coding variants in the proteins that comprise the nuclear pore complex which occur exclusively in cases of sporadic ALS (sALS). Specifically, we have identified protein coding variants in nucleoporins (Nups) within each domain of the NPC, including the central ring, transmembrane ring, and nuclear basket. Importantly, protein coding variants in nuclear basket Nup, Nup50, were recently reported by Megat and colleagues (*Nat. Comm. 2023*) in a study analyzing whole genome sequencing data from patients with sALS. In addition to the variants described by Megat et al., the AALS dataset revealed additional Nup50 coding variants associated with disease. We plan to use these authentic patient lines to study the true pathology of Nup50 coding variants, their role in NCT dysfunction, and downstream consequences, such as TDP-43 loss-of-function. We hope to share some of these early data to describe a molecular mechanism for some cases of sALS.

Disclosures: **C.M. Fare:** None. **L. Jin:** None. **O. Roman:** None. **A.N. Coyne:** None. **L. Dupuis:** 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.; sponsored research agreements with Inflectis Bioscience and Cytokinetics. **J.D. Rothstein:** 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 from Expansion Therapeutics, Calico, Fralin Biomedical Research Institute, Answer ALS, and TEDCO (Maryland Technology Development Corporation). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CHMP7 ASO patent pending. F. Consulting Fees (e.g., advisory boards); Expansion Therapeutics and Team Gleason.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.04/U8

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS116143

Title: Nuclear envelope homeostasis is altered in ALS cellular models

Authors: R. SIRTORI, M. GREGOIRE, L. DONATELLI, B. CHATRAGADDA, R. CULLEN, *C. FALLINI;
Univ. of Rhode Island, Kingston, RI

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that primarily affects motor neurons, leading to progressive muscle weakness and loss of voluntary muscle control. While the exact cause of ALS is not fully understood, emerging research has identified nucleocytoplasmic transport impairment, accumulation of mislocalized proteins, nuclear morphology abnormalities, and activation of cellular stress and DNA damage response as key drivers of neuronal death. Together, these observations point to the dysfunction of the nuclear envelope (NE) as a unifying element, suggesting it may play an important but not well-understood role in disease progression. Mechanical stresses generated by cytoskeletal forces on the nucleus can cause transient NE ruptures which disrupts essential NE structures such as nuclear pore complex (NPC). Under normal conditions, NE ruptures are remodeled and repaired by the endosomal sorting complex required for transport (ESCRT) machinery with the aid of BROX. BROX (BRO1 domain and CAAX motif containing protein) binds Nesprin-2G, a component of the linker of nucleoskeleton and cytoskeleton complex (LINC) and facilitates its ubiquitination, thereby decreasing the mechanical stress imposed by the actin-LINC complex at the rupture site. Thus, by rebalancing excessive cytoskeletal forces in cells experiencing NE instability, BROX can promote effective repair and cell survival. In the present work we confirm

that alteration of NPC distribution are early and key phenotypes in different sporadic and familial ALS cellular models. We also show that alterations to the NE tensional homeostasis leads to NPC injury and alters nuclear morphology. Importantly, we show that BROX can modulate these phenotypes in ALS models. Together, our data suggests that modulation of nuclear envelope homeostasis and repair may represent a novel and promising therapeutic target for ALS.

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Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.05/U9

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant U01NS114156
NIH Grant ZIAHG200409
NIH Grant P30 DK020579

Title: Application of a pentasaccharide biomarker to access treatment efficacy of gene therapy for GM1 gangliosidosis

Authors: P. KELL[1], S. MISHRA[1], N. ELENA-RALUCA[2], P. D'SOUZA[2], C. TIFFT[2], ***X. JIANG**[1,2];
[1]Washington Univ. Sch. of Med., Saint Louis, MO; [2]NIH, Bethesda, MD

Abstract: GM1 gangliosidosis is a rare and lethal neurodegenerative disorder caused by mutations in the GLB1 gene. These mutations lead to a deficiency in β-galactosidase enzyme activity, resulting in the accumulation of glycoconjugates with a terminal β-galactose. Promising advancements have been made in the treatment of this condition using adeno-associated viral (AAV) gene therapy. In a cat model of GM1 gangliosidosis, this therapy demonstrated the ability to delay the onset of symptoms, reduce storage in the brain and peripheral tissues, and increase lifespan. These favorable outcomes have laid the foundation for early-stage AAV gene therapy trials. Given the slow progression of the disorder, particularly in late-infantile and juvenile forms, the availability of validated biomarkers would significantly enhance the assessment of therapeutic efficacy in AAV gene therapy. Recently, we have successfully identified and synthesized a pentasaccharide biomarker called H3N2b, which exhibited an elevation of over 18-fold in patient plasma, cerebrospinal fluid (CSF), and urine. Importantly, H3N2b is a natural substrate of β-galactosidase. To ensure accurate quantification of this biomarker in biospecimens, we have developed fully validated assays. In a Phase 1/2 intravenous AAV9 gene therapy trial for GM1 gangliosidosis, we observed a significant reduction of H3N2b levels in patient urine, plasma, and CSF samples. This reduction correlated with an increase in β-galactosidase activity. These findings strongly indicate that the pentasaccharide H3N2b can serve

as a valuable pharmacodynamic biomarker for monitoring therapeutic response. Moreover, its implementation may have far-reaching implications for expediting drug approval processes in this rare genetic disease.

Disclosures: **P. Kell:** None. **S. Mishra:** None. **N. Elena-Raluca:** None. **P. D'Souza:** None. **C. Tifft:** None. **X. Jiang:** Other; I am an inventor on a patent application pertaining to use of pentasaccharide biomarkers in GM1 gangliosidosis.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.06/U10

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS116143
URI Undergraduate Research Grant

Title: Cytoskeletal dysfunction in ALS motor vs. cortical iPSC-derived neurons

Authors: *L. DONATELLI[1], M. GREGOIRE[2], A. BURNS[2], C. FALLINI[2];
[1]Interdisciplinary Neurosci. Program, Univ. of Rhode Island, Kingston, RI; [2]Univ. of Rhode Island, KIngston, RI

Abstract: Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disorder affecting motor neurons in the brain and spinal cord and is commonly comorbid with frontotemporal dementia (ALS/FTD). ALS/FTD leads to progressive cell weakness and death of cortical and motor neurons. The C9ORF72 mutation accounts for ~33.7% of familial ALS; up to 40% of FTD patients carry this mutation. Previous research has uncovered functional and morphological alterations linked to ALS/FTD, including changes in the nuclear envelope and lamina, alterations in neuronal excitability, and disruptions in the nuclear pore. However, whether motor or cortical neurons are more or uniquely vulnerable to these defects is not known. To address this question, we compared cortical and motor neurons differentiated from the same set of isogenic iPSC lines carrying the C9ORF72 hexanucleotide repeat expansion using the I3 method, that relies on the overexpression of a set of transcription factors to induce cortical (i.e. NGN2) or motor neuron (i.e. NGN2-ISL1-LHX3) fate. Using live and fixed cell imaging, we have characterized the timing of appearance and frequency of changes to the nuclear envelope and pore in both neuronal types, and investigated changes in neuronal excitability using a genetically encoded calcium indicator. Interestingly, most of the assessed phenotypes show little difference between the two neuronal populations, however we have determined that cellular age plays a major role in the development of these changes. Overall, our study will shed more light on the cellular mechanisms driving disease pathologies in motor and cortical neurons, contributing to a better understanding of vulnerability to disease of different neuronal populations in ALS patients.

Disclosures: **L. Donatelli:** None. **M. Gregoire:** None. **A. Burns:** None. **C. Fallini:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

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Program #/Poster #: PSTR535.07/V1

Topic: C.06. Neuromuscular Diseases

Support: NSF Grant DGE2139757

Title: Identifying Mechanisms That Maintain TDP-43 Splicing Fidelity

Authors: ***I. R. SINHA**, K. D. BOWDEN, K. E. IRWIN, P. C. WONG, J. P. LING;
Johns Hopkins Univ., Baltimore, MD

Abstract: Splicing fidelity of RNA is essential for cellular processes across all eukaryotes. Splicing repressors, such as transactive response DNA binding protein 43 kDa (TDP-43), play an important role in ensuring the inclusion of "correct" canonical exons and the thousands of "incorrect" cryptic exons found within introns. The nuclear clearance and cytoplasmic aggregation of TDP-43 is a pathological hallmark of the amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) disease spectrum. Although the disruption of TDP-43 function leads to the inclusion of hundreds of cryptic exons detrimental to protein expression, there is currently little understanding of the mechanisms regulating TDP-43-associated splicing fidelity. Elucidating these regulatory mechanisms is crucial in understanding the pathological progression of ALS-FTD and the maintenance of protein isoform expression and specificity within cells. Here, we mined public RNA sequencing (RNA-seq) databases for TDP-43-associated alternative splicing. We found that immune cells are susceptible to splicing errors when treated with environmental and compound stressors and display aberrant splicing phenotypes similar to those identified when TDP-43 is dysregulated. Furthermore, we found that compound treatment led to depletion of TDP-43 protein within the treated cells. Our results demonstrate a mechanism of TDP-43 dysregulation that can be compared with neurodegenerative models to elucidate shared characteristics and pathways. These experiments are a significant start to understanding the regulators of TDP-43 in normal and disease contexts and could lead to potential therapeutic strategies involving identified upstream targets.

Disclosures: **I.R. Sinha:** None. **K.D. Bowden:** None. **K.E. Irwin:** None. **P.C. Wong:** None. **J.P. Ling:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.08/V2

Topic: C.06. Neuromuscular Diseases

Support: FAPESP Grant 2021/05194-4
FAPESP Grant 2013/08028-1
CNPq

Title: Molecular characterization of VRK1 R321C mutation in autosomal recessive Amyotrophic Lateral Sclerosis using iPSC derived motor neurons

Authors: *D. OLIVEIRA[1], L. M. ALVES[2], B. GHIROTTO NUNES[3], A. ASSONI[2], A. TELES E SILVA[4], A. SERTIÉ[4], A. SAKUGAWA[2], M. R. FERRARI[2], M. ZATZ[2];
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Abstract: Amyotrophic Lateral Sclerosis is a severe motor neuron disease, which so far has no treatment. In spite of having already been associated with hundreds of pathogenic mutations, located at more than 30 genes, its genetic etiology is far from being fully understood. Vaccinia-related kinase 1 (*VRK1*) is a gene located at 14q32.2 which has been implicated in the pathological process of a broad range of neurodevelopmental disorders as well as neuropathies, including microcephaly, spinal muscular atrophy with cerebellar hypoplasia (SMA-PCH) and Amyotrophic Lateral Sclerosis (ALS). Here we report an endogamic family presenting ALS in an autosomal recessive mode of inheritance, segregating with a homozygous missense mutation located at *VRK1* gene (p.R321C; Arg321Cys). The proband's age of onset was at 38 years, after referring weakness in legs, which progressed to the upper limbs. After two years, the patient was wheelchair confined. Patellar, radial and tricipital brisk reflexes were described, suggesting upper motor neuron impairment. Similar findings were also observed in the other two affected ALS siblings. After informed consent, cultured fibroblasts were obtained from skin biopsies and used for cellular reprogramming. iPSCs were shown to express cellular markers of pluripotency (*OCT4*, *SSEA4*, *SOX2*, *NANOG*) and possess a normal chromosomal complement through MLPA assays. iPSC derived -motor neurons from two patients and two unrelated controls were then differentiated, and were also observed to express cellular specific markers (*ISL1*, *MAP2*, *ISL1* and *MNX1*). A label-free proteomics assay was performed using the motor neurons, and we were able to identify major pathways enriched in both affected cell lineages. The main molecular pathways associated to *VRK1* R321C pathological process were MTORC1 signalling, MYC targets v1, E2F pathway, Glycolysis and UV response pathway. Further analyses are underway in order to characterize de effects of this pathogenic mutation, in the context of ALS physiopathology. To our knowledge, it is the first Brazilian family reported with Amyotrophic Lateral Sclerosis caused by a mutation in *VRK*1 gene.

Disclosures: D. Oliveira: None. **L.M. Alves:** None. **B. Ghirotto Nunes:** None. **A. Assoni:** None. **A. Teles e Silva:** None. **A. Sertié:** None. **A. Sakugawa:** None. **M.R. Ferrari:** None. **M. Zatz:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.09/V3

Topic: C.06. Neuromuscular Diseases

Support: RF1NS127407
NIH DP2-NS106664

Title: Neuronal stimulator of interferon genes (STING) pathway activation in C9orf72 and sporadic amyotrophic lateral sclerosis

Authors: *S. POWLEY[1], C. MARQUES[1], K. DORFMAN[1], J. SUNG[1], C. SONG[1], A. H. HELD[1], M. ADLER[1], M. YATES[1], A. KAVUTURU[1], C. I. AGUILAR[1], I. ROBEY[3], D. OAKLEY[2], L. PETRUCELLI[4], B. T. HYMAN[1], C. LAGIER-TOURENNE[1], B. WAINGER[1]; [1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Pathology, Massachusetts Gen. Hosp., Boston, MA; [3]Southern Arizona VA Healthcare Syst., VA Biorepository Brain Bank, Tuscon, AZ; [4]Neurosci., Mayo Clin., Jacksonville, FL

Abstract: The role of the stimulator of interferon genes (STING) in initiating the type-I interferon (IFN) pathway has been well-established as an innate immune response to pathogen infection, and more recently implicated in neurodegenerative diseases, including the rapidly progressive and fatal motor nervous system disorder amyotrophic lateral sclerosis (ALS). Previous studies have focused on immune cells themselves, while the effect of this STING-dependent cell-autonomous activation in neurons has not been studied. We hypothesize that dysregulation of STING signaling within neurons contributes to their degeneration in both familial ALS, which represents around 10% of all cases and is most often caused by chromosome 9 open reading frame 72 (C9orf72) repeat intron expansion, and sporadic ALS representing the other 90% of cases. Immunohistochemistry analyses in human postmortem samples showed an increase in STING protein in vulnerable layer 5 cortical pyramidal neurons and ventral horn spinal motor neurons of both familial and sporadic ALS patients relative to Alzheimer's and non-neurological controls. Culture of primary mouse cortical neurons and human induced pluripotent stem cell (hiPSC)-derived neurons with STING agonists and antagonists lead to an increase and decrease, respectively, of type-I IFN response gene expression, confirming functionality of the neuronal STING pathway by qRT-PCR. Using hiPSC-derived spinal motor and cortical neurons from a range of familial ALS patients and isogenic gene mutation-edited controls derived from these lines, we demonstrated an increase of STING activation in ALS neurons compared to controls. These findings were validated *in vivo* as adeno-associated virus (AAV) C9orf72 model mice had increased STING levels in layer 5 corticospinal motor neurons compared to controls. These studies have shown STING pathway activation in familial ALS in human postmortem brains, hiPSC-derived cortical and motor neurons, and mouse models, and suggest this increased neuronal STING may contribute to degeneration in vulnerable neurons. Interestingly, preliminary analysis of hiPSC-derived spinal motor neurons from sporadic ALS patients now suggests a similar increase in STING pathway activation compared to controls. As we further investigate other components of the canonical and non-canonical STING pathways in neurons and expand our studies to sporadic ALS models, the

function of neuronal STING in the selective degeneration of vulnerable neurons in ALS will be better understood.

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Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.10/V4

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant T32 NS077888

Title: Als vapb p56s variant impairs recovery and adaptability to external stressors in human ipsc-derived motor neurons

Authors: *C. A. LANDRY[1], J. COSTANZO[1], M. HATZOGLOU[1], A. R. MUOTRI[2], H. MIRANDA[1];
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[2]Pediatrics/Cellular Mol. Med., UCSD, La Jolla, CA

Abstract: Vesicle-associated membrane protein-associated protein-B (VAPB) is an ER membrane bound protein. Under non-pathological conditions VAPB is known by its tethering properties anchoring organelles to the ER but is most often studied in association to amyotrophic lateral sclerosis Type VIII (ALS8). Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is the most common adult–onset motor neuron (MN) disease. There is currently no cure for this devastating disease which is characterized by rapidly progressing motor deficiency with muscle atrophy, accompanied by muscular weakness, cramps, and fasciculation. An autosomal dominant c.166C>T mutation in the *VAPB* gene leads to a change of a proline to a serine at amino acid position 56 (P56S) and is known to be the causative mutation of ALS8. However, the mechanism through which this mutation causes disease remains unknown. We hypothesized that the VAPB P56S mutation reduces the ability to bind to the mitochondria, this in turn reduces the cell's ability to respond to stress in human MNs derived from patient iPSC. We have performed a surface sensing of translation (SUnSET) and observed that the VAPB P56S mutation leads to decreased mRNA translation throughout the lifespan of the iPSC-derived

MNs. We also identified iPSC-derived MN age dependent decreased mitochondrial membrane potential, through a JC-1 dye with flow cytometry as well as hypoexcitability, measured using a multielectrode array (MEA) system . Both the translation phenotype and mitochondrial dysfunction are reversible using an inhibitor of the Integrated Stress Response (ISRIB), implying activation of the ISR as a causative factor for MN pathology. IPSC-derived MN carrying VAPB P56S display increased sensitivity to tunicamycin exposure and slowed recovery from this challenge compared to controls. In addition, as the VAPB P56S MNs age, their response to the tunicamycin exposure worsens, indicating the initial age-dependent phenotypes could arise from impaired stress responses. Taken together, these data suggest that VAPB P56S mutation impairs MN's ability to adapt and recover from environmental stressors encountered throughout life, which could lead to MN degeneration in ALS patients.

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Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.11/V5

Topic: C.06. Neuromuscular Diseases

Support: NINDS Research Education Grant (R25) NS065743

Title: Genome editing for CSF1R-related disorder: the case of Adult Onset Leukodystrophy with Neuroaxonal Spheroids and Pigmented Glia

Authors: *J. METOVIC[1], Y. LI[1], Y. GONG[1], A. QIAN[1], C. R. R. ALVES[3,1], L. HA[3,2], B. P. KLEINSTIVER[3,2], F. EICHLER[1];
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Abstract: Adult onset leukodystrophy with axonal spheroids and pigmented glia (ALSP) is a rare neurodegenerative disease caused by autosomal dominant mutations in the colony-stimulating factor 1 receptor (*CSF1R*) gene located on chromosome 5q32. CSF1R is a tyrosine kinase receptor, essential for regulating the proliferation and activation of microglial cells. This disorder is characterized by white matter destruction and brain atrophy that result from severe myelin loss, axonal swelling with abundant neuroaxonal spheroids, pigmented macrophages, and mild gliosis. Clinically, these patients experience a rapid and relentless progression of neuropsychiatric behavioral changes, memory loss, and extrapyramidal/pyramidal motor dysfunction, all of which inevitably culminate in premature death. There is currently no cure, and at best, available treatments can only slow disease progression. Precision genome editing is a newly developed tool with the potential to correct pathogenic point mutations and precisely edit DNA sequences by avoiding double stranded DNA breaks. We explored several genome editing

methods targeting the most common *CSF1R* mutation (p.Ile794Thr; c.2381 T>C). Different cytosine base editor strategies with the potential to restore normal CSF1R activity were employed on a patient-derived fibroblast cell line with minimal off-target editing effects. Currently, we are determining the most efficient *CSF1R* gene editing strategy. We anticipate that genome editing holds great potential for precise gene correction in ALSP patients and will pave the way for personalized treatments of this currently incurable and fatal disease.

Disclosures: **J. Metovic:** None. **Y. Li:** None. **Y. Gong:** None. **A. Qian:** None. **C.R.R. Alves:** None. **L. Ha:** None. **B.P. Kleinstiver:** None. **F. Eichler:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.12/V6

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

Support: NIH NS114221
VA RX003865

Title: Delayed and brain region specific manipulation of the NMDA receptor subunit GluN3A causes AD-like functional and behavioral changes in mice

Authors: *M. Q. JIANG[1,2,4], T. ESTABA[2], J. PATEL[2], T. S. LIN[2], K. BERGLUND[3], X. GU[2,4], L. WEI[2], S. YU[2,4];
[2]Anesthesiol., [3]Neurosurg., [1]Emory Univ., Atlanta, GA; [4]Ctr. for Visual & Neurocognitive Rehabil., VA Med. Ctr., Atlanta, GA

Abstract: Alzheimer’s disease (AD) and related dementias (ADRD) severely affect aging populations. The Ca^{2+} hypothesis for AD proposes that Ca^{2+} dysregulation in the brain is a chronic pathophysiology in AD. Hyperactivity of N-methyl-D-aspartate (NMDA) receptors (NMDARs) in excitatory neurons has been implicated as both a potential cause and outcome of AD/ADRD but instigating factors for the receptor dysregulation remain undefined. NMDAR overactivation is uniquely restrained by the GluN3 subunits (GluN3A and GluN3B, or NR3A and NR3B). Our previous studies revealed significant GluN3A expression in the adult mouse brain, showing imperative roles in Ca^{2+} homeostasis, Ca^{2+}-dependent signaling pathways, and normal brain functions. In the GluN3A knockout (KO) mouse where the subunit is globally absent throughout the lifespan, we have recently disclosed age-dependent chronic degenerative excitotoxicity, progressive cognitive decline, and endogenous Aβ/tau pathology during the aging process. In an effort to delineate the critical time window and brain regional specificity of GluN3A deficiency-induced sporadic AD/ADRD development, we now examine delayed GluN3A deficiency achieved by CRISPR knockdown in specific brain regions at young adult ages. Using an AAV9 packaged CRISPR targeting GluN3A, young adult wild-type mice (3-month-old) were subjected to GluN3A selective knockout (sKO) in the hippocampus and cortex

via stereotaxic injection. Reduced GluN3A levels in the cortex and hippocampus were verified using Western blotting. Similar to global KO mice, 3 to 6 months after sKO, these animals spontaneously developed dysfunction in the olfactory discrimination test, followed by the progression of psychological/cognitive declines such as anxiety-like behavior in the open field test and sociability abnormalities in the social novelty test. Learning and memory deficits were later identified in sKO mice in the Y-maze test, fear conditioning test, and Morris water maze test. In a gain-of-function study, GluN3A expression was restored in the cortex and hippocampus of global GluN3A KO mice via AAV9 CRISPR GluN3A knock-in (KI) injections. The restoration of GluN3A at 3 months of age prevented or attenuated age-dependent psychological and cognitive declines in the open field test, the Morris water maze test, and the fear conditioning memory test. Thus, our previous and current investigations provide consistent evidence supporting a causal mechanism of sporadic AD/ADRD mediated by GluN3A deficiency in age- and brain region-specific manners and implicating potential disease-modifying therapies at an early stage of the disease development.

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Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.13/V7

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

Support: NIH/NIA Grant U01AG073323
NIH/NIA Grant R01AG066707
NIH/NIA Grant R01AG076448
NIH/NIA Grant R56AG074001

Title: Combining genetics and real-world patient data fuel ancestry-specific target and drug discovery in Alzheimer’s disease

Authors: *F. CHENG[1], Y. HOU[2], P. ZHANG[3];
[2]Genomic Med. Inst., [1]Cleveland Clin., Cleveland, OH; [3]Indiana Univ., Indianapolis, IN

Abstract: Background: Although high-throughput DNA/RNA sequencing technologies have generated massive genetic and genomic data in Alzheimer’s disease (AD), translation of these findings into new patient treatment has not materialized. **Methods:** To address this problem, we have used Mendelian randomization (MR) and large patient’s genetic and functional genomic data to evaluate druggable targets using AD as a prototypical example. We utilized the genetic instruments from 9 expression quantitative trait loci (eQTL) and 3 protein quantitative trait loci (pQTL) datasets across five human brain regions from three human brain biobanks and tested the outcome of MR independently across 7 genome-wide association studies (GWAS) datasets of

European-American (EA) and African-American (AA) ancestries, with 275,540 AD cases and 1.55 million controls. **Results:** We identified 25 drug targets in EAs and 6 new drug targets in AAs. We pinpointed that the inflammatory target of epoxide hydrolase 2 (EPHX2) emerged as a potent AD target in EAs, and treatment of AD transgenic rats with an EPHX2 inhibitor was therapeutic. We also identified 23 candidate drugs associated with reduced risk of AD in mild cognitive impairment (MCI) patients after analysis of ~80 million electronic health records. Using a propensity score-matched design, we identified that usage of either apixaban (hazard ratio [HR] = 0.74, 95% confidence interval [CI] 0.69 - 0.80) and amlodipine (HR = 0.91, 95% CI 0.88 - 0.94) were both significantly associated with reduced progression to AD in people with MCI. **Conclusion:** In summary, combining genetics and real-world patient data identified ancestry-specific therapeutic targets and medicines for AD and other neurodegenerative diseases if broadly applied.

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Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.14/V8

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

Support: NIH Grant 1F31AG074673-01A1

Title: The opposing effects of microRNA-33 deletion on amyloid and Tau pathology

Authors: *M. TATE, B. KIM, S. WIJERATNE, Y. YOU, A. D. SHARIFY, J. KIM; Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of beta-amyloid plaques and neurofibrillary tangles consisting of hyper-phosphorylated tau. Recently, ATP Binding Cassette Subfamily A Member 1 (*ABCA1*) has been identified as a novel risk gene for developing AD. ABCA1 transfers lipids onto lipid-poor APOE, the major cholesterol transport protein in the brain. The APOEε4 allele is the strongest genetic risk factor for developing AD, directly implicating the ABCA1-APOE pathway in AD onset and/or progression. microRNAs (miRs) are small, inhibitory molecules that regulate the expression of their target genes. They have emerged as promising therapeutic targets given their important role in gene regulation. miR-33 negatively regulates ABCA1 expression, and loss of miR-33 increases ABCA1 protein levels in the brain. Given that there is no current mouse model that faithfully recapitulates both the amyloid and tau pathology observed in humans, we aimed to utilize two separate mouse models to determine if miR-33 deletion ameliorates amyloid and tau pathology, in part, by increasing ABCA1 levels. We generated miR-33 knockout (KO) and miR-33 wildtype (WT) mice and either crossed them with an Aβ-amyloidosis mouse model (APP/PS1) or injected them with an adeno-associated virus expressing *MAPT* with the P301L

mutation (AAV-Tau). In both models, we confirmed that the deletion of miR-33 increases ABCA1 protein levels similarly. The loss of miR-33 ameliorates the amyloid pathology observed in the APP/PS1 mouse model. miR-33KO;APP/PS1 mice have decreased Aβ40 and Aβ42 levels as well as decreased Aβ-positive and X-34-positive plaque deposition. Interestingly, we found that the loss of miR-33 exacerbates the tau pathology observed in the AAV-Tau mouse model. miR-33KO;AAV-Tau mice have increased phosphorylation of Tau compared to miR-33WT;AAV-Tau mice. Additionally, the deletion of miR-33 increases the levels of insoluble Tau and increases the seeding capacity of Tau. These results highlight the continued need to study the mechanisms that regulate Alzheimer's disease pathogenesis. Identifying pathways that have similar or opposing effects on amyloid and tau pathology can help our understanding of the relationships between these two pathologies and aid in the more informed development of therapeutic strategies.

Disclosures: M. Tate: None. **B. Kim:** None. **S. Wijeratne:** None. **Y. You:** None. **A.D. Sharify:** None. **J. Kim:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.15

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

Support: NIH Grant R01NS101745

Title: Lipophorin Receptors Genetically Modulate Neurodegeneration Caused by Psn Knockdown in the Aging Drosophila Brain

Authors: *J. KANG[1], C. ZHANG[1], Y. WANG[2], J. FENG[3], B. BERGER[2], N. PERRIMON[4], J. SHEN[1];
[1]Harvard Med. School/BWH, Boston, MA; [2]MIT, Cambridge, MA; [3]Univ. of Illinois at Urbana-Champaign, Champaign, IL; [4]Dept. of Genet., Harvard Med. School/HHMI, Boston, MA

Abstract: Mutations in the *Presenilin* (*PSEN*) genes are the most common cause of early-onset familial Alzheimer's disease (FAD). Studies in cell-free biochemical systems, cell culture, and knockin mice showed that *PSEN* mutations are loss-of-function mutations, impairing γ-secretase activity. Mouse genetic analysis highlighted the importance of Presenilin (PS) in learning and memory, synaptic plasticity and neurotransmitter release, and neuronal survival, and *Drosophila* studies further demonstrated an evolutionarily conserved role of PS in neuronal survival during aging. However, the molecular mechanisms by which PS protects neurons during aging remain unclear. To identify genetic modifiers that modulate PS-dependent neuronal survival, we developed a new *Drosophila Psn* model that exhibits age-dependent neurodegeneration and increases of apoptosis. Following a bioinformatic analysis, we tested the top ranked 25 candidate genes by selective knockdown (KD) of each gene expression in adult neurons using two

independent RNAi lines. Interestingly, among the 9 genes that enhanced neurodegeneration caused by *Psn* KD, 4 of them, *lpr2*, *lpr1*, *arr*, and *mgl*, encode proteins that belong to the low-density lipoprotein receptor family, which is involved in lipid transport and metabolism. Specifically, neuron-specific KD of lipophorin receptors (LpR1 or LpR2) results in neurodegeneration and worsens *Psn* KD phenotypes. Furthermore, heterozygotic deletions of *lpr1* and *lpr2* or homozygotic deletions of *lpr1* or *lpr2* also lead to age-dependent neurodegeneration and further exacerbate neurodegeneration in *Psn* KD flies. These findings show that proteins involved in lipid transport and metabolism, such as LpRs, modulate *Psn*-dependent neuronal survival and are critically important for neuronal integrity in the aging brain.

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Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.16/V9

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

Support: R01MH106575
R01MH116281
R01AG063175

Title: Alzheimer’S disease risk allele at clu locus affects chromatin accessibility and mediates the interplay of clu and apoe to promote excitability and neuropathy in human neurons

Authors: *X. ZHAO[1,2], H. ZHANG[1], S. ZHANG[1,2], A. SUDWARTS[4,5], A. KOZLOVA[1,2], X. SUN[3], W. WOOD[1], Z. P. PANG[6], X. HE[3], A. R. SANDERS[1,2], G. THINAKARAN[4,5], J. DUAN[1,2];
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Abstract: Genome-wide association studies (GWAS) of Alzheimer’s disease (AD) have identified 75 risk loci; however, how variants in these loci confer risk remains largely elusive. We have recently developed an approach to identify functional GWAS risk variants that show differential allelic chromatin accessibility, i.e., allele-specific open chromatin (ASoC) in human iPSC-derived neurons. Here, with a relatively large sample size and in more iPSC-derived cell types including neurons, microglia and astrocytes, we first analyzed ASoC to evaluate for AD GWAS risk enrichment. We found that the noncoding AD risk SNP rs1532278 at the Clusterin (CLU) locus showed strong ASoC in hiPSC-derived excitatory neurons (iEx), but not in

microglia and astrocytes. To test whether this SNP was functional and to identify its cis-target gene(s), we employed CRISPR/Cas9 editing to engineer two heterozygous (C/T) hiPSC lines into isogenic homozygous lines (C/C and T/T), and differentiated them into iEx. We found the AD risk allele T of rs1532278 was associated with increased CLU mRNA levels. Interestingly, we found that editing rs1532278 affected the expression of the well-known AD risk gene APOE on a different chromosome, and this "trans-effect" was more pronounced in neuron-glia co-cultures. To further corroborate the SNP editing effects, we also CRISPR-engineered a 200-bp deletion of the open chromatin region (OCR) flanking rs1532278. We found that OCR deletion reduced CLU expression by ~50% in iEx, suggesting an enhancer activity of the rs1532278-flanking OCR. Like the rs1532278 editing, the OCR deletion reduced APOE mRNA expression as expected. To examine whether the "trans-effect" of the rs1532278-flanking OCR on APOE expression was mediated by CLU expression, we overexpressed CLU in the OCR deletion lines and found that APOE expression was indeed rescued by CLU, suggesting an interesting interplay of the CLU and APOE through the cis-regulatory effect of rs1532278. We next elucidated whether the functional rs1532278 affects neural phenotypes relevant to AD. We found that iEx with risk allele T exhibited higher phosphorated-Tau levels, an elevated expression of presynaptic marker (synaptophysin), and hyperexcitability as assayed by calcium imaging. Similar cellular phenotypes have also been reported for the strongest AD risk allele APOE4. Overall, our data suggests the AD risk SNP (rs1532278) at the CLU locus may confer AD risk through altering chromatin accessibility and increasing the expression of CLU and APOE in excitatory neurons, providing novel mechanistic insight into how CLU and APOE may interact to promote the risk for developing AD.

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Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.17/V10

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

Support: 5R21AG062378
5U01AG074960

Title: Mapt combined with app and psen1 worsens behavior in mice

Authors: *K. SAMBAMURTI[1], V. PADMARAJU[2], D. CROWDER[3], I. CROMWELL[3], P. PADHI[4], N. H. GREIG[5], D. LAHIRI[6], A. KANTHASAMY[4];
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Develop. Section, LNS, Intramural Res. Program, Natl. Inst. On Aging, NIH, Baltimore, MD; [6]Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Background: Familial AD (FAD) mice expressing mutant forms of the Amyloid β protein (Aβ) precursor (APP) and presenilin-1 (PS1) have been key models for AD research. While other studies combined FAD and the Tau protein, none, except 3xTgAD, have been made freely available or characterized well. APP and Tau were cosegregated in 3xTgAD mice, confounding their independent evaluation. This study used mice expressing human wild-type MAPT after disrupting mouse Mapt (hTau) combined with FAD mutant APP and PS1. **Method:** We generated HTAP by crossing hTau mice in C57BL6/J (JAX #005491) with FAD mutant APP-PS1 (MMRRC #034833-JAX) and HTAP2 with 5xFAD (#032882-JAX). Both FAD models deposit Aβ after 5 mo, and hTau hyperphosphorylates Tau at 3 mo. We compared mouse spatial memory with either radial arm water or Barnes maze. We also compared the levels of norepinephrine in the hippocampus, cortex, and striatum of HTAP2 with the parental C57BL6/J mice by HPLC analysis **Result:** HTAP and HTAP2 mice deposit plaques similar to parental FAD mice and hTau hyperphosphorylated Tau. The combined HTAP mice showed hyperphosphorylated Tau, but the structures appeared distorted. We noted that after 7 months of age, HTAP mice performed poorly in the radial arm water maze compared to wild-type, FAD, and hTau mice. We observed a similar deficit in HTAP2 in the Barnes Maze. HTAP2 mice showed a significant drop in norepinephrine in all evaluated brain regions, reminiscent of early AD-related changes. **Conclusion:** HTAP/2 mice have exacerbated behavioral and neurochemical deficits, suggesting that it is a more robust model for studying AD-related dysfunction. However, these mice still perform well in activities of daily living, such as grooming and feeding. Such novel tau mice, which contain the human MAPT gene, would accelerate our ongoing translational and mechanistic studies including novel bioengineered live-biotherapeutic as well as human microRNAs that regulate human 3'UTR *MAPT* mRNA without interference from the host *Mapt* gene. We propose that this system better recapitulates the behavioral deficits in AD.

Disclosures: K. Sambamurti: None. **V. Padmaraju:** None. **D. Crowder:** None. **I. Cromwell:** None. **P. Padhi:** None. **N.H. Greig:** None. **D. Lahiri:** None. **A. Kanthasamy:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.18/V12

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

Support: NIA U54 AG054345

Title: Combining genetic and environmental risk to create preclinical animal models of late-onset Alzheimer’s disease

Authors: *M. SASNER[1], A. OBLAK[2], K. P. KOTREDES[1], R. PANDEY[1], A. REAGAN[1], C. RANGEL-BARAJAS[2], G. W. CARTER[1], G. HOWELL[1];
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Abstract: The IU/JAX/PITT MODEL-AD Center initially focused on phenotyping mouse models expressing strong genetic risk factors including the E4 allele of apolipoprotein E (*APOE4*) and the R47H allele of triggering receptor expressed on myeloid cells (*Trem2*R47H*). To do this, we created the LOAD1 strain that was double homozygous for *APOE4* and *Trem2*R47H* on C57BL6J (B6) and used a human-relevant phenotyping pipeline (including *in vivo* imaging, multi-omics, and immunofluorescence) to assess LOAD1 and control mice between four and 24 months. We also used LOAD1 mice as a sensitizer strain to determine the effect of additional genetic and environmental risk factors for LOAD. This included determining the effect of humanizing the Aβ sequence in the mouse *App* gene – we call this strain LOAD2 (*APOE4*.*Trem2*R47H*.hAβ). We also used gene editing of LOAD1 to assess putative variants in 12 genes associated with LOAD, work that identified three variants – *Plcg2*M28L*, *Abca7*A1527G*, and *Mthfr*677C>T* – as mediating transcriptional changes like those observed in human LOAD (manuscript in preparation).
Finally, we determined that chronic consumption of a high fat/high sugar diet (HFD) increased alignment of mouse models to human LOAD, but in an Aβ genotype-specific manner. Collectively, these data have led us to create and phenotype a series of mouse models of LOAD centered around the LOAD2 model.
Models being tested are LOAD2, LOAD2 fed a high fat diet (HFD), LOAD2.*Plcg2*M28L* fed a HFD, LOAD2.*Abca7*A1527G*, and LOAD2.*Mthfr*677C>T*. For each model, males and females are being assessed from four to 24 months of age using a combined longitudinal and cross-sectional phenotyping pipeline. *In vivo* assays include frailty, cognition and behavior, MRI and PET/CT, and fluid biomarkers. Postmortem assays include multi-omics (transcriptomics, proteomics, and metabolomics), biochemistry, and immunofluorescence to evaluate neuronal cell loss, glial activation and cerebrovascular changes.
Results to date show that, compared to LOAD2 mice fed a regular diet, LOAD2 mice fed a HFD show higher levels of insoluble Aβ, cortical neuronal cell loss, elevated Nfl in the plasma and brain, and proteomic changes like those observed in human LOAD. Interestingly, PET/CT using ^{64}Cu-PTSM and ^{18}F-FDG revealed a greater degree of dyshomeostasis of blood flow and glucose uptake in LOAD2.*Abca7*A1527G* than LOAD2 mice.
All MODEL-AD data will be made available through the AD knowledge portal and MODEL-AD Explorer, and all mouse models are available (with no restrictions on for-profit use) from the Jackson Lab.

Disclosures: M. Sasner: None. **A. Oblak:** None. **K.P. Kotredes:** None. **R. Pandey:** None. **A. Reagan:** None. **C. Rangel-Barajas:** None. **G.W. Carter:** None. **G. Howell:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.19/V13

Topic: C.06. Neuromuscular Diseases

Support: SPF, GR018686
CreATe Scholar U54

Title: Dna damage as a disease mechanism in primary lateral sclerosis (pls)

Authors: *J. LAVERDE PAZ[1,2], K. MAKI[3], N. RICCIARDI[3,2], C.-H. VOLMAR[3,2,4], M. BENATAR[5], J. WUU[5], H. A. ALI[4,6,7,8], P. KAPRANOV[9], C. WAHLESTEDT[3,2,4], Z. ZEIER[3,2,4]; [1]Psychiatry, Univ. of Miami Neurosci. Grad. Program, Miami, FL; [2]Ctr. for Therapeut. Innovation, [3]Psychiatry, [4]Sylvester Comprehensive Cancer Ctr., [5]Neurol., [6]Katz Family Div. of Nephrology and Hypertension, [7]Peggy and Harold Katz Family Drug Discovery Ctr., Univ. of Miami Miller Sch. of Med., Miami, FL; [8]Neurolog. Surgery, The Miami Project to Cure Paralysis, Miami, FL; [9]Inst. of Genomics, Sch. of Biomed. Sciences, Huaqiao Univ., Xiamen, China

Abstract: Primary Lateral Sclerosis (PLS) is a rare motor neuron disease (MND) characterized by the degeneration of upper motor neurons (UMN). Due to a required 4-year period of exclusive UMN syndrome, and clinical similarities with the more prevalent amyotrophic lateral sclerosis (ALS) in the early stages, a conclusive diagnosis may require up to 5 years. The causes and mechanisms of PLS have been understudied, thus constituting a challenge for developing effective therapies. DNA damage has been identified as an important trigger to neurodegenerative diseases and evidence from our lab showed impairment of critical DNA double-strand break repair pathways in C9ORF72 ALS-induced pluripotent stem cells (iPSCs) motor neurons (Andrade, 2020). Cellular reprogramming methods were used to generate iPSCs from three PLS patients. After establishment and characterization, the iPSCs were differentiated into cortical neurons along with three C9ORF72-ALS patient-derived lines, and their respective isogenic control lines (corrected repeat expansion). To confirm neuronal identity, cells were immunostained for the neuronal markers MAP2, NEUN, and TUJ. Next, we assessed DNA strand break frequency using a general marker of DNA breakage (γH2AX), activation of repair pathways (pP53), and markers of specific double-strand break repair (e.g. RAD52 and Ku70), and single-strand break repair (e.g. APE1, MSH2, and DDB1). The immunolabeled cells were visualized on a Confocal Microscope and the Signal intensity values were measured for each cell within the imaged fields, with a minimum of 5 fields per biological replicate, and analyzed using the open-source Fiji software. We found that levels of DNA strand break were similar when comparing iPSCs-derived cortical neurons from PLS patients versus C9ORF7-ALS, but higher when compared to isogenic iPSCs-derived cortical neurons. Indicating activation of single and double-strand break repair pathways levels of pP53 were higher in disease-derived iPSCs cortical neurons when compared to isogenic controls. Furthermore, markers of single-strand break repair pathways showed elevated expression in both PLS and C9ORF7-ALS iPSCs-derived cortical neurons, when compared to the isogenic controls, without major differences between diseases. This is the first DNA damage assessment in iPSCs-derived cortical neurons, revealing a common feature of increased DNA breakages in both ALS and PLS. Our ongoing research aims to elucidate the underlying mechanisms responsible for the elevated DNA damage observed in PLS, providing further insights that could potentially contribute to establishing a therapeutic strategy.

Disclosures: **J. Laverde Paz:** None. **K. Maki:** None. **N. Ricciardi:** None. **C. Volmar:** None. **M. Benatar:** None. **J. Wuu:** None. **H.A. Ali:** None. **P. Kapranov:** None. **C. Wahlestedt:** None. **Z. Zeier:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.20/V14

Topic: C.06. Neuromuscular Diseases

Support: Active Against ALS

Title: Characterizing the functional consequences of YAF2-RYBP fusion in amyotrophic lateral sclerosis.

Authors: *G. SADRI-VAKILI, T. PETROZZIELLO, S. HUNTRESS, A. CASTILLO-TORRES, D. GAO, M. CUDKOWICZ, J. BERRY, R. MOURO PINTO, M. TALKOWSKI; Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Characterizing the functional consequences of YAF2-RYBP fusion in amyotrophic lateral sclerosis.
Ghazaleh Sadri-Vakili, Tiziana Petrozziello, Sommer S. Huntress, Ayleen L. Castillo-Torres, Dadi Gao, Merit E. Cudkowicz, James D. Berry, Ricardo Mouro Pinto, Micheal Talkowski
Although over 30 genes have been linked to amyotrophic lateral sclerosis (ALS), the underlying genetic cause of more than 80% of the disease remains unknow. One potential genetic cause may be structural variants, including gene fusion events. Recently we demonstrated an enrichment of gene fusion events in ALS post-mortem brain and spinal cord samples using STAR-Fusion to assess publicly available RNA-Seq datasets. Specifically, we have identified 90 rare gene fusion events enriched in ALS that were absent in known cancer databases as well as in control samples. We have now begun to characterize the functional consequences of these events in cellular models to determine whether fusion genes contribute to pathogenesis in ALS. One of the most recurrent gene fusions identified in ALS was the inter-chromosomal fusion between YY1 Associated Factor 2 (YAF2) and RING1 and YY1 Binding Protein (RYBP), genes involved in chromatin remodeling and transcriptional regulation. Specifically, YAF2 and RYBP exert their transcriptional activity through ubiquitination of histone H2 (H2AK119ub1) and interaction with the polycomb (PcG) proteins. Human neuroblastoma SH-SY5Y cells were transfected with YAF2, RYBP, or YAF2-RYBP fusion plasmids and binding to the PcG proteins, Ezh2, Ring1A and $H3K27me_3$ were measured, demonstrating an interaction between the fusion gene and the PcG complex. Furthermore, YAF2-RYBP fusion significantly decreased H2AK119ub1 levels, suggesting that YAF2-RYBP may alter the chromatin landscape. Importantly, SH-SY5Y cells transfected with the fusion gene demonstrated a significant decrease in cell viability, suggesting a toxic role of the fusion gene. Ongoing RNA-Seq and transposase-accessible chromatin with

sequencing (ATAC-Seq) will determine whether YAF2-RYBP fusion alters chromatin accessibility and thereby gene expression in ALS contributing to motor neuron loss.

Disclosures: G. Sadri-Vakili: None. **T. Petrozziello:** None. **S. Huntress:** None. **A. Castillo-Torres:** None. **D. Gao:** None. **M. Cudkowicz:** None. **J. Berry:** None. **R. Mouro Pinto:** None. **M. Talkowski:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.21/V15

Topic: C.06. Neuromuscular Diseases

Title: C9orf72 poly(PR) mediated neurodegeneration is associated with nucleolar stress

Authors: *M. CICARDI[1], D. TROTTI[2];
[1]Thomas Jefferson university, Philadelphia, PA; [2]Thomas Jefferson Univ., Philadelphia, PA

Abstract: The ALS/FTD-linked intronic hexanucleotide repeat expansion in the *C9orf72* gene is aberrantly translated in the sense and antisense directions into dipeptide repeat proteins among which polyPR displays the most aggressive neurotoxicity *in-vitro* and *in-vivo*. PR partitions to the nucleus when heterologously expressed in neurons and other cell types. We show that by lessening the nuclear accumulation of PR, we can drastically reduce its neurotoxicity. PR has a strong tendency to accumulate in the nucleolus which has a critical role in regulating the cell stress response. We determined that, in neurons, PR caused nucleolar stress and increased levels of the transcription factor p53. Downregulating p53 levels also prevented PR-mediated neurotoxicity both in *in-vitro* and *in-vivo* models. We then investigated whether PR could cause the senescence phenotype in neurons but observed no sign of it. Instead, we found evidence for the induction of programmed cell death *via* caspase-3 activation.

Disclosures: M. Cicardi: None. **D. Trotti:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.22/V16

Topic: C.06. Neuromuscular Diseases

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NIH MH109978
NIH MH119509
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Title: Elucidating mechanisms of cell type-specific vulnerability in ALS and FTLD through single-cell profiling of the human cortex

Authors: *S. PINEDA[1,4], H. LEE[2], B. FITZWALTER[3], R. LINVILLE[3], E. COOK[5], D. W. DICKSON[5], V. BELZIL[5], M. KELLIS[1,4], M. HEIMAN[3];
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Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are devastating and fatal neurodegenerative diseases that share many clinical, pathological, and genetic signatures. However, the mechanistic basis of their shared and distinct circuitry remains unknown at the molecular level. To uncover cell type-specific transcriptional changes, underlying biological pathways, and putative upstream regulators, we conducted high-resolution single-cell profiling of transcriptional alterations in the primary motor and dorsolateral prefrontal cortices of 75 sporadic and *C9orf72+* familial ALS and FTLD donor individuals and unaffected controls, providing the most comprehensive-to-date characterization of Brodmann areas 4 and 9, and yielding insights of unprecedented resolution into both ALS and FTLD. Our analysis revealed enhanced cross-region, and cross-phenotypic vulnerability of an extratelencephalic layer V population that includes the ALS and FTLD-implicated upper motor and Von Economo neurons and uncovered a potentially novel L3/5 excitatory subtype that is similarly dysregulated. We identified novel and highly-specific marker genes for these previously ill-defined populations, found that most of these are preserved across brain regions, and identified a molecular fingerprint of the selectively depleted cell populations. We found that several genetically-linked ALS and FTLD associated genes are enriched in motor and spindle neurons and related populations in the human cortex, and uncovered a dramatic loss in expression of numerous structural and signaling components of the primary cilia in these cell types. Several casually linked genes, including *C9orf72*, were dysregulated across phenotypes and some with regional and cell type specificity. Across vulnerable populations, we noted a dysregulation of innate and adaptive immune response genes, and in endothelial cells, we observed a reduction to and mislocalization of tight junction proteins that we propose contributes to vascular dysfunction. Finally, we identify genes whose baseline expression in neurons serves as a

predictor of gene expression dysregulation in ALS/FTLD, and which thus we predict likely confer intrinsic disease vulnerability to ALS and FTLD in these cells. Overall, our study represents the largest and most accurate molecular atlas of these two human brain regions to date, and the first cell type-specific molecular characterization of ALS and FTLD in either.

Disclosures: **S. Pineda:** None. **H. Lee:** None. **B. Fitzwalter:** None. **R. Linville:** None. **E. Cook:** None. **D.W. Dickson:** None. **V. Belzil:** None. **M. Kellis:** None. **M. Heiman:** None.

Poster

PSTR535. ALS and Motor Neuron Disease: Human Genetics and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR535.23/V17

Topic: C.06. Neuromuscular Diseases

Support: NIH R35 NS122140

Title: Impaired nucleolar liquidity and transcriptional dysregulation cause neurotoxicity in amyotrophic lateral sclerosis type 4

Authors: *C. M. STOCKFORD[1], F. J. ARNOLD[1], A. R. LA SPADA[2];
[1]Pathology and Lab. Med., UC Irvine Sch. of Med., Irvine, CA; [2]Pathology & Lab. Med. and Neurol., Univ. of California Irvine, Irvine, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder with a prognosis of 3-5 years. Rare, dominantly inherited mutations in the DNA/RNA helicase senataxin (SETX) cause a juvenile-onset, slow-progressing form of ALS called ALS4. Because RNA binding proteins (RBPs) are among the most frequently mutated proteins in familial ALS, and nearly 97% of all ALS cases converge on mislocalization of the RBP TDP-43, it is believed that neuronal loss in ALS is partly caused by dysfunctional RNA metabolism and RBP dynamics. To explore the mechanism by which mutations in SETX cause ALS4, we differentiated an isogenic set of human SETX induced pluripotent stem cell (iPSC) lines - SETX wild-type (WT), SETX knockout (KO), and SETX L389S - into motor neurons (MNs). We performed RNA-sequencing to identify differentially expressed genes (DEGs) and altered cellular pathways associated with ALS4 (n = 3). Altogether, we found 759 DEGs in SETX L389S MNs versus WT MNs and 101 DEGs between SETX KO and WT neurons. This experiment revealed significant dysregulation of genes that function in cell adhesion and the cytoskeleton. We next performed cross-linking followed by immunoprecipitation (CLIP)-sequencing of WT and mutant SETX to define the RNA clients directly bound to SETX in human MNs (n = 2). This experiment exposed that wild-type, but not mutant SETX, strongly recognizes the TDP-43 RNA binding motif (UGUGUG). Furthermore, we discovered that SETX directly binds to tRNAs, and this association is reduced for mutant SETX. This is a new aspect of SETX biology with potential relevance to ALS4, as abnormal tRNA processing can lead to neurodegeneration. In parallel, we found that SETX can modify disease phenotypes in the most

common form of familial ALS caused by an expansion of a noncoding GGGGCC (G_4C_2) repeat in the first intron of the chromosome 9 open reading frame 72 (*C9orf72*) gene. The G_4C_2 repeat RNA often escapes degradation after transcription and is aberrantly translated into dipeptide repeat proteins (DPRs) that form pathological aggregates. Arginine-rich DPRs can enter the nucleolus and disrupt its dynamic, liquid-like properties. To investigate nucleolar stress when SETX is mutated, we transduced mature MNs with an arginine-rich DPR and a nucleolar marker to assess the fluidity of the nucleolus through fluorescence recovery after photobleaching analysis (n = 38). Both KO and mutant SETX MNs exhibited impaired nucleolar dynamics compared to WT SETX MNs. All of these findings suggest SETX may regulate nucleolar liquidity, tRNA processing, and transcriptional homeostasis in ALS4 and must be explored further to ascertain therapies to cease degeneration.

Disclosures: C.M. Stockford: None. **F.J. Arnold:** None. **A.R. La Spada:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.01/V18

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS115748
NIH Grant R01NS124813

Title: C698r mutation in lrsam1 gene impairs peripheral nerve regeneration in a cmt2p mouse model

Authors: *B. HU[1], D. MOISEEV[2], J. LI[1];
[1]Neurol., Houston Methodist Res. Inst., Houston, TX; [2]Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: Introduction: Missense mutation C694R in the RING domain of the *leucine rich repeat and sterile alpha motif 1* (*LRSAM1*) gene results in a dominantly inherited peripheral polyneuropathy, Charcot-Marie-Tooth disease type 2P (CMT2P). The C694R mutation altered the RNA-binding protein nuclear translocation likely by disrupting the protein-protein interaction between LRSAM1 and the RNA-binding proteins, a potential mechanism in CMT2P pathogenesis. To further explore this mechanism *in vivo*, we have generated a $Lrsam1^{C698R}$ knock-in mouse model, the amino acid substitution equivalent to the human C69**4**R mutation. **Methods:** A $Lrsam1^{C698R}$ knock-in mouse model was produced through CRISPR/Cas9 technology. The C698R *Lrsam1* knock-in mice were clinically evaluated using Rotarod and hindlimb clasping tests, physiologically assessed by nerve conduction studies, and morphologically examined on nerve sections. **Results:** Both heterozygous ($Lrsam1^{+/C698R}$) and homozygous ($Lrsam1^{C698/C698R}$) knock-in mice exhibited normal motor functions on behavioral tests as well as normal on nerve conduction studies. Axonal density and myelin thickness were

not significantly different between mutants and wild-type mice by sciatic nerve morphometric analysis up to 17 months of age. In line with these normal findings, protein-protein interactions between mutant LRSAM1 and RNA-binding proteins (such as FUS and G3BP1) were still present in mouse cells, which differs from the disrupted interactions between these proteins in human CMT2P cells. However, after crush sciatic nerve injury, *Lrsam1*$^{+/C698R}$ mice had a mild, but statistically significant, reduced compound nerve action potential and conduction velocity during recovery. **Conclusions:** C698R mutation results in a mild impaired nerve regeneration in mice. While the phenotype is not robust, mild abnormality in nerve repair provides a helpful clue toward the slowly progressing polyneuropathy in CMT2P.

Disclosures: B. Hu: None. **D. Moiseev:** None. **J. Li:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.02/V19

Topic: C.06. Neuromuscular Diseases

Title: Systemic AAV9-mediated Gene Addition for Canavan Disease (CANaspire Trial): Lowering N-acetylaspartate Levels, Impacting Disease Severity, and Managing Immune Responses

Authors: *F. EICHLER[1], A. NAGY[1], G. A. LAFORET[2], E. J. MALLACK[3], A. FAY[4], P. R. HARMATZ[4], Z. ERGONUL[3], C. BURTON[2], K. KIRBY[2], T. B. KINANE[1], E. L. TOWNSEND[1], M. KIEFER[1], B. LEIRO[2], R. WILLIAMS[2], J. BALSER[2], L. KRATZ[5], A. SHAYWITZ[2], A. BLEY[6];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Aspa Therapeut., Palo Alto, CA; [3]Weill-Cornell Med. Col., New York, NY; [4]Univ. of California San Francisco, San Francisco, CA; [5]Kennedy Krieger Inst., Baltimore, MD; [6]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Canavan disease (CD) is caused by mutations in the *ASPA* gene, leading to accumulation of N-acetylaspartate (NAA), a profound early-onset spongiform leukodystrophy, and impaired psychomotor development. We report a first-in-human open-label study evaluating the safety, pharmacodynamic (PD) properties, and clinical activity of BBP-812, a systemically administered recombinant AAV9 h*ASPA* vector for the treatment of CD (CAN*aspire*, NCT04998396). Data from the CAN*inform* CD natural history study (NCT04126005) are used as a comparator. As a PD marker of ASPA activity, NAA levels are quantified in urine and cerebrospinal fluid (CSF) by gas chromatography-mass spectrometry and in brain by magnetic resonance spectroscopy. Clinical outcome measures include the disease-specific CD Rating Score that ranks the severity of 11 characteristic features of CD, achievement of developmental milestones , and performance-based and parent-reported motor and developmental scales. Effects on white matter and brain volumes are quantified by magnetic resonance imaging (MRI). All 6 dosed participants (median age at dosing 18.8 months, range 9.6 - 29.2 months) exhibited robust,

persistent decreases in urine, CSF and brain NAA when compared to baseline. Early MRI findings reveal resolution of white matter swelling and evidence of new myelination in the brainstem and cerebellar peduncles. In several participants, this has corresponded with acquisition of clinical milestones. All participants experienced elevated liver enzymes presumably related to an acquired anti-BBP-812 immune response, and a transient early thrombocytopenia presumably linked to an innate immune response. These responses have been managed with high-dose steroids in all 6 patients as well as complement inhibition (eculizumab) in the last 2 participants dosed. In conclusion, our preliminary data demonstrate that BBP-812 achieves robust and durable NAA reductions in urine, CSF and brain along with an early suggestion of clinical stabilization. Most adverse events (AEs) have been well-managed with steroids and complement inhibition, with no treatment-related serious AEs to date. While the literature and the ongoing natural history study suggest an association between NAA levels and phenotype, longer follow-up is needed to assess whether the intervention translates to clinical efficacy.

Disclosures: **F. Eichler:** None. **A. Nagy:** None. **G.A. Laforet:** A. Employment/Salary (full or part-time):; Aspa Therapeutics. **E.J. Mallack:** None. **A. Fay:** None. **P.R. Harmatz:** None. **Z. Ergonul:** None. **C. Burton:** A. Employment/Salary (full or part-time):; Aspa Therapeutics. **K. Kirby:** A. Employment/Salary (full or part-time):; Aspa Therapeutics. **T.B. Kinane:** None. **E.L. Townsend:** None. **M. Kiefer:** None. **B. Leiro:** A. Employment/Salary (full or part-time):; Aspa Therapeutics. **R. Williams:** A. Employment/Salary (full or part-time):; Aspa Therapeutics. **J. Balser:** A. Employment/Salary (full or part-time):; Aspa Therapeutics. **L. Kratz:** None. **A. Shaywitz:** A. Employment/Salary (full or part-time):; Aspa Therapeutics. **A. Bley:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

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Program #/Poster #: PSTR536.03/V20

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant NINDS K99126576
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Title: Central degeneration of monosynaptic connectivity between muscle afferents and motoneurons in Charcot-Marie-Tooth Disease Type 2D

Authors: *T. ROTTERMAN[1], R. SIERRA[1], A. L. TADENEV[2], C. E. FIX[1], R. W. BURGESS[2], T. COPE[1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]The Jackson Lab., Bar Harbor, ME

Abstract: Charcot-Marie-Tooth Disease Type 2D is an inherited peripheral neuropathy resulting from missense and small in-frame indel mutations in *GARS*, encoding glycyl-tRNA synthetase. Mutations in the *GARS* gene can result in a variety of motor abnormalities including loss of the

stretch reflex, which is normally mediated by activation of stretch-sensitive muscle Ia afferents that project from the muscle directly to motoneurons located in the ventral horn of the spinal cord. Furthermore, mice carrying a patient-associated mutation (*GARS*^{ETAQ}) show partial denervation of the neuromuscular junction, reduced nerve conduction velocities, and a decrease in sensory axon diameter. However, these partial peripheral deficits alone cannot explain the complete absence of stretch-evoked reflexes we observe in mice with this mutation. Here, we hypothesized that central disconnection between Ia afferents and motoneurons contributes to the absence of the stretch reflex in *GARS*^{ETAQ}. We first generated a transgenic mouse line that expressed a fluorescent tdTomato protein in muscle afferents, including Ia afferents, and injected a retrograde tracer directly into the sciatic nerve of both control and *GARS*^{ETAQ} mice to retrogradely label motoneurons. Spinal cords were then collected, motoneurons were imaged, then reconstructed in 3D, and tdTomato+ synapses were quantified on both the soma and proximal dendrites to compare densities across the two groups. We found a significant depletion of tdTomato+ synapses from Ia muscle afferents on both the soma and dendrites in *GARS*^{ETAQ} mice, while no differences in synaptic inputs from spinal interneurons was detected. We concluded, from these data, that the ETAQ mutation not only impacts peripheral axon function but results in the central degeneration of Ia afferents that monosynaptically project to motoneurons and encode proprioceptive information regarding the body's position in space which will absolutely result in motor dysfunction.

Disclosures: T. Rotterman: None. **R. Sierra:** None. **A.L. Tadenev:** None. **C.E. Fix:** None. **R.W. Burgess:** None. **T. Cope:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.04/V21

Topic: C.06. Neuromuscular Diseases

Title: Muscle Activity Patterns in Patients with Diabetic Peripheral Neuropathy during Sit-to-Stand

Authors: *M. ALIGHANBARI[1], L. GRIFFIN[2], A. JAHDI[2], I. VIRANI[2];
[1]Univ. of Texas, Austin, Austin, TX; [2]Kinesiol & Hlth. Edu, Univ. Texas Austin, Austin, TX

Abstract: Diabetic peripheral neuropathy (DPN) affects large, myelinated nerve fibers in the distal extremities, causing a decrease in conduction velocity and an increase in the risk of falling by up to 64%. DPN affects each leg differently and may affect neuromuscular control during transitional tasks. We compared electromyographic (EMG) activity between older adults with and without DPN during a sit-to-stand task. Six healthy older adults (70 ± 10 yrs) and 3 older adults with DPN (72 ± 10 yrs) participated. EMG of the tibialis anterior (TA), soleus (SOL), peroneus longus (PRL), rectus femoris (RF), biceps femoris (BF), gluteus maximus (Gmax), adductor longus (ADL), and gluteus medius (Gmed) was measured. Participants performed 3 sit-

to-stand trials at a self-selected speed. Then, 3 maximal voluntary isometric contractions were obtained for each muscle for EMG normalization. Movement velocity and kinematics were recorded with a Vicon system. There was no significant difference in sit-to-stand velocity between the groups. In the DPN group, the RF peak amplitude (R_{RMS}: 1.75 ± 0.02, L_{RMS}: 0.92 ± 0.2) and normalized EMG integral were significantly higher for the left leg than in the right leg (R_{INT}: 1451.2 ± 199.4, L_{INT}: 647.8 ± 163.9). RF-Gmax co-activation was also significantly higher for the left than right leg in the DPN group (R: 39.5 ± 1.5, L: 56.3 ± 2.7). However, the healthy group had significantly higher RF-BF co-activation for right than for the left leg (R: 58.1 ± 5.9, L: 47.9 ± 4.8) with no significant difference in peak amplitude and EMG integral. There was greater normalized EMG in the left TA (DPN: 1.36 ± 0.06, H: 0.99 ± 0.1), right PRL (DPN: 0.8 ± 0.2, H: 0.4 ± 0.8), right RF (DPN: 1.7 ± 0.05, H: 0.7 ± 0.35), and right ADL (0.94 ± 0.1, H: 0.38± 0.1) for both groups. However, the DPN group had lower peak EMG amplitude for the BF (DPN:2.896 ± 1.2, H: 0.38 ± 0.2) than the healthy group. The DPN group had a lower EMG integral in BF (DPN: 3457.3 ± 665.3, H: 410.29 ± 284.5), PRL (DPN: 76.6.6 ± 12.2, H: 445.8 ± 153.5), and RF (DPN: 1702.9 ± 102.7, H: 644.4 ± 337.9) in the right leg than the healthy group. The DPN group had lower co-activation in left leg TA-SOL muscle pairs than the healthy group (DPN: 18.8 ± 1.8, H: 49.85 ± 6.8). However, there was no significant difference in co-activation of the muscles of the right leg between groups. We found that DPN affects each limb differently. Higher muscle activity in the right limb and lower co-activation in the left limb indicate higher reliance on the right limb for controlling balance during sit-to-stand.

Disclosures: M. Alighanbari: None. **L. Griffin:** None. **A. Jahdi:** None. **I. Virani:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.05/V22

Topic: C.06. Neuromuscular Diseases

Support: Charcot Marie Tooth Research Foundation
Department of Veteran Affairs (RX002305)

Title: Targeted Drug Delivery of Leukomimetic Nanoparticles in Charcot-Marie-Tooth 1x

Authors: *H. KONDETI;
Loyola Univ. Chicago, Chicago, IL

Abstract: TARGETED DRUG DELIVERY OF LEUKOMIMETIC NANOPARTICLES IN CHARCOT-MARIE-TOOTH 1X

Authors*H. Kondeti[1,2], M.H. Cabe[1,2], L. Soehlke[1,2], K.A. Langert[1,2]
DisclosuresH. Kondeti: None. M.H. Cabe: None. L. Soehlke: None. K.A. Langert: None.
[1]Research Service, Edward Hines, Jr., VA Hospital, Chicago IL; [2]Pharmacology Graduate Program, Department of Molecular Pharmacology and Neuroscience, Loyola University

Chicago, Stritch School of Medicine
AbstractCharcot Marie Tooth disease is a disabling, progressive, inherited peripheral neuropathy with no effective cure. Potential therapies are often limited due to systemic toxicity or a lack of efficacy at the site of interest, due to the blood nerve barrier (BNB). To address this, current work in the Langert Laboratory is focused on targeted delivery systems that use endogenous mechanisms of transendothelial migration to circumvent the BNB. It is established that circulating immune cells accumulate in affected nerves in the CMT1X subtype, and that they are recruited across the BNB by the chemokine CCL2. We hypothesize that polymeric nanoparticles (NPs) coated with monocyte plasma membranes enriched with CCR2 (termed *leukomimetic NPs*) will similarly accumulate in affected nerves in a mouse model of CMT1X (Connexin32 knockout (Cx32KO) mice). To test this hypothesis, we first used high throughput flow cytometry and multispectral labeling to quantify the magnitude and kinetics of immune cell infiltration and its association with BNB activation across the lifespan of Cx32KO mice compared to age matched WT controls. We demonstrate that these techniques allow for assessment of 10^6 cells per nerve, as opposed to 10^1 cells per nerve section by immunohistochemistry previously. We then obtained plasma membrane vesicles from WEHI mouse monocytes using dounce homogenization and subcellular fractionation. Plasma membrane vesicles retain CCR2 after sonication to 191 ± 4 nm. With an *in vitro* transwell migration assay, we demonstrate that CCR2-expressing monocyte plasma membrane vesicles migrate towards a gradient of CCL2. These studies will inform the timing of administration of leukomimetic NPs *in vivo* to Cx32KO mice. This study is funded by research awards from the Charcot Marie Tooth Research Foundation and the Department of Veterans Affairs (RX002305).

Disclosures: H. Kondeti: None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.06/V23

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

Support: NIH grant 5R01NS094402

Title: Dlk jnk signaling is required for cytoskeletal integrity in drg neurons

Authors: *B. ROTANZ[1], G. THOMAS[2];
[1]Temple Univ., Philadelphia, PA; [2]Shriners Pediatric Res. Ctr., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Dual leucine-zipper kinase (DLK) signals via the downstream MAP kinase c-Jun N-terminal kinase (JNK) to drive diverse forms of neurodegeneration. DLK action was thought to be largely restricted to these pathological contexts, making DLK an attractive therapeutic target. However, recent studies suggest that DLK is also required for intrinsic integrity of the neuronal

cytoskeleton. The reason for this requirement was previously unclear, but we found that acute inhibition of DLK or JNK rapidly distends axons of dorsal root ganglion neurons. The axonal distension correlates with dysregulation of axonal tubulin and, to a lesser extent, neurofilaments, and several vesicle cargoes accumulate at these regions of cytoskeletal disruption. The effect of DLK inhibition on axonal distension was reversible, as normal morphology was restored by DLK inhibitor washout. At the molecular level, we provide evidence that the effect of DLK inhibition requires the scaffold protein JNK-interacting protein-1 (JIP1) but not JIP3. Together, these findings suggest caution with regard to acute inhibition of DLK's kinase activity as a therapeutic strategy. However, more subtle interventions to target specific pools of DLK could still be therapeutically effective.

Disclosures: B. Rotanz: None. **G. Thomas:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.07/V24

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

Title: HD-MEA-based CIPN model for investigating cisplatin-induced axon degeneration in human iPSC-derived motor neurons and the neuroprotective effect of HDAC6 inhibitor ACY-1215

Authors: *C. INGENSIEP[1], S. ZACH[2], P. NICKLIN[3], J. YE[4], B. HENGERER[2];
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is one of the most common side effects experienced by cancer patients undergoing chemotherapeutic treatment. The symptoms include numbness and pain in the extremities starting in fingers and toes and progressively spreading to arms and legs. Patients may also experience oversensitivity and impairment of movements. Chemotherapeutic agents like cisplatin trigger a "dying-back" axon degeneration in neurons of the peripheral nervous system (PNS). This study focuses on examining the impact of cisplatin on motor neurons derived from human induced pluripotent stem cells (iPSCs). The functionality of the cells is investigated by high-density multielectrode array (HD-MEA) recordings. An axon tracking assay is used to identify axons of single neurons and analyze their length and conduction velocity. Cisplatin (10 - 50 µM) is applied for 2 - 8 h and the recovery is monitored for 48 h. In addition, the study investigates axon degeneration after cisplatin administration (10 - 100 µM, 24 - 48 h) by performing β-III-tubulin staining. An immunoassay is utilized to detect neurofilament light chain (NF-L) as a biomarker for axon degeneration in the supernatant. To protect the axons and maintain their functionality, the effect of histone deacetylase 6 (HDAC6) inhibitor ACY-1215 (ricolinostat) is tested. Immunostaining of the

motor neurons revealed slight axon damage after 24 h at high cisplatin concentrations. After 48 h, severe damage was observed across all tested concentrations. First MEA assay results showed a decrease in axon length by 42% following short-term cisplatin exposure, whereas the spike amplitude (30.66 ± 1.66 µV vs. 30.85 ± 2.33 µV) remained stable. Firing frequency, spike amplitude at initiation site, and conduction velocity of action potentials did not significantly change. Since a loss in function precedes the degeneration process, monitoring the functionality of motor neurons following a chemotherapy-induced insult allows for a more sensitive and quicker approach to detecting axon degeneration *in vitro*. The axon tracking readout is a powerful tool for investigating the mechanisms of axon degeneration on a functional level at an early stage. Moreover, the cultivation of iPSC-derived neurons on MEA chips for up to 12 weeks enables (i) the functional characterization of the cells over time and (ii) testing of potential therapeutics besides ACY-1215 in a chronic approach to address CIPN in the future.

Disclosures: C. Ingensiep: A. Employment/Salary (full or part-time):; BI Pharma GmbH & Co. KG. **S. Zach:** A. Employment/Salary (full or part-time):; BI Pharma GmbH & Co. KG. **P. Nicklin:** A. Employment/Salary (full or part-time):; BI Pharma GmbH & Co. KG. **J. Ye:** A. Employment/Salary (full or part-time):; BI Pharma GmbH & Co. KG. **B. Hengerer:** A. Employment/Salary (full or part-time):; BI Pharma GmbH & Co. KG.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.08/V25

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

Support: BBSRC Industrial CASE CSV PhD Studentship BB/V509322/1

Title: Characterising SARM1 Activation and Pathogenic Mutation in Axon Loss: Insights from Structural Investigation

Authors: *E. L. HOPKINS[1], J. GILLEY[1], F. RAMOND[2], M. P. COLEMAN[1];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Service de Genetique Clinique et Biologique, CHU de Saint-Etienne, Saint-Etienne, France

Abstract: SARM1 is a pro-degenerative NADase that is a key executor of the programmed axon degeneration pathway, after nerve injury and in diseases including polyneuropathies. The ARM domain of SARM1 regulates its NADase activity; attenuating this activity delays axon degeneration, so SARM1 has become an important drug target. This project aims to characterise the ARM domain allosteric site, where NMN and NAD bind in competition, respectively activating SARM1 and blocking its activation. In addition, this project is investigating whether a rare, natural ARM domain mutant ($SARM1^{W253C}$) in a patient with a complex disorder with motor and retinal symptoms, confers a gain-of-function consistent it having a causative role. Site-directed mutagenesis was used to modify the ARM domain allosteric site with artificial

variants, or to introduce the SARM1^{W253C} natural mutation. Variants were expressed in HEK cells to determine how they affect intracellular NAD levels and mutant proteins isolated using immunoprecipitation for NADase assays of basal and NMN-induced activity.
Several artificial mutants in the SARM1 ARM domain influence NAD levels in transfected HEK cells and alter basal and/or induced SARM1 NADase activity. Interesting patterns are emerging that will help understand how SARM1 becomes activated and potentially how to block activation therapeutically. Further characterisation of these residues is ongoing to understand more fully how they influence activation, including the making of CryoEM SARM1 mutant structures. The SARM1^{W253C} natural variant was shown to decrease NAD levels in HEK cells to similarly low levels as known gain-of-function variants. Purified SARM1^{W253C} NADase assays have also shown it to be constitutively active mutant, with comparable activity compared to those reported previously in ALS (Gilley *et al*., 2021).
The SARM1 ARM domain allosteric site residues are vital in regulating NADase activity, helping to understand how this site could be targeted to block activation. Additionally, data are consistent with SARM1^{W253C} conferring gain-of-function, but further parallel clinical work is needed to confirm whether this is contributing to patient symptoms.

Disclosures: **E.L. Hopkins:** None. **J. Gilley:** None. **F. Ramond:** None. **M.P. Coleman:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); SARM1 antisense oligonucleotides from Ionis Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); Consulting for Nura Bio.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.09/W1

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

Support: NIH IRP ZIA-HD008966

Title: Dual leucine zipper kinase regulates onset and maintenance of nerve injury signaling, neuroinflammation and mechanical allodynia in a neuropathic pain model

Authors: *S. HAREENDRAN[1], J. WLASCHIN[2], C. E. LE PICHON[3];
[1]Natl. Inst. of Hlth. (NIH), Bethesda, MD; [2]NIH, Bethesda, MD; [3]NICHD, NIH, NICHD, Bethesda, MD

Abstract: Peripheral nerve lesions can cause neuropathic pain; however, the key molecule(s) that coordinate the process from initiation to perpetuation of pain transmission are still poorly understood. We have shown that dual leucine zipper kinase (DLK) is a critical upstream regulator of transcriptional changes in injured sensory neurons and of a microglial response, leading to pain sensitization. Inhibition of DLK by GNE-3511 effectively prevents pain signaling and the development of mechanical allodynia (heightened sensitivity to innocuous stimuli) after

sciatic nerve damage. Here, we sought to address two questions with clinical relevance: (1) is initial suppression of DLK signaling sufficient to block pain response? (2) is delayed inhibitor treatment helpful in controlling neuropathic pain? We used a spared nerve injury (SNI) model of neuropathic pain and assessed the induction of injury and microglial activation markers either in the dorsal root ganglia (DRG), where the cell bodies of the sensory neurons reside or the spinal cord, the site of projection of these neurons. The hypersensitive pain behavior was evaluated using the von Frey assay, which measures the paw withdrawal thresholds to filaments of varying forces. To determine if a defined period of GNE-3511 treatment is sufficient to attenuate the injury cascade, we utilized an 'on/off' approach, where we administered DLK inhibitor twice daily for five days post SNI, and then discontinued the drug for the next five days. A reverse 'off/ on' scheme was adopted to determine the effect of delayed GNE-3511 treatment on pain suppression. Activation of the DLK pathway was assessed by quantitating the nuclear expression of pc-Jun in the DRG and ATF3 levels in the motor neurons. Both pc-Jun and ATF3 are transcription factors involved in the DLK-dependent neuronal stress response. We analyzed the spinal microglial response to injury by determining the expression of neuronal CSF1 and microglial IBA1 markers. Our data revealed that the DLK pathway can be activated to initiate pain signaling days after the actual neuronal insult, and that continuous DLK inhibition is required to prevent chronic pain. Further we found that DLK inhibition is nevertheless beneficial in treating neuropathic pain days after the injury occurs. In conclusion, we show that DLK is essential not only for the onset of neuropathic pain signaling but also for the disease maintenance. Insights gained from this study are clinically relevant in the development of DLK inhibitors for pain management.

Disclosures: S. Hareendran: None. **J. Wlaschin:** None. **C.E. Le Pichon:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.10/W2

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

Support: NIH GRANT 13161291
The Wagner Fellowship Fund
The Raven Fellowship
The University of Virginia Program in Fundamental Neuroscience

Title: Spheroids on degenerating neurites are eliminated via engulfment, inhibiting degeneration

Authors: *S. HUNTER-CHANG[1], T. VEGIRAJU[2], Y. YONG[3], S. C. KUCENAS[3], C. DEPPMANN[4];
[1]Univ. of Virginia Neurosci. Program, Charlottesville, VA; [3]Univ. of Virginia, [4]Biol., [2]Univ. of Virginia, Charlottesville, VA

Abstract: Up to 40% of our neurites die as we age, eliminating compensatory circuitry and making us susceptible to neurodegenerative diseases. Furthermore, axonal dysfunction and death precede neuronal death on an order of years in many chronic degeneration contexts. However, there are currently no therapies targeting neurite loss. Axonal spheroids are bubble-like structures that form along all degenerating axons universally and along dendrites in many disease contexts including hypoxia, excitotoxicity, and tauopathies. Functional implications of spheroids are just beginning to be understood, with recent work finding that spheroids disrupt axon conductance early in Alzheimer's Disease progression. Additionally, previous work from our lab showed that spheroids rupture and release an as-yet unknown prodegenerative factor in vitro. A physiological mechanism for spheroid elimination would therefore confer adaptive protection of the nervous system. We hypothesize that spheroids are eliminated by engulfment, which slows axon degeneration by preventing prodegenerative factor release. To test this hypothesis, we established acute injury models in vivo in zebrafish and in vitro with mouse cell cultures in microfluidic devices. Using time-lapse, confocal microscopy in these models, we are identifying the phagocytes that engulf spheroids and finding that engulfment indeed slows axon degeneration. Ongoing work seeks to determine whether spheroid engulfment protects axons by preventing spheroid rupture and pro-degenerative factor release. These data identify a new process by which spheroids are eliminated and axon degeneration is regulated. As such, future work will address topics such as whether disruptions in spheroid engulfment contribute to disease pathogenesis, and whether promoting spheroid clearance can rescue disease progression and circuit function.

Disclosures: S. Hunter-Chang: None. **T. Vegiraju:** None. **Y. Yong:** None. **S.C. Kucenas:** None. **C. Deppmann:** None.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.11/Web Only

Topic: B.10. Demyelinating Disorders

Support: JSPS 21H02821
JSPS 19J40281
Intramural Research Grants (3-5, 3-9) for Neurological and Psychiatric Disorders of NCNP

Title: Identification of chemical compounds for promotion of peripheral nerve myelination by screening of a compound library

Authors: Y. KOBAYASHI-UJIIE, S. WAKATSUKI, ***T. ARAKI**;
Peripheral Nervous Syst. Res., Natl. Inst. Neurosci, NCNP, Tokyo, Japan

Abstract: Charcot-Marie Tooth disease type 1 (CMT1) is a hereditary demyelinating neuropathy. Although it is one of the most common inherited neurological disorders, there is no effective treatment developed for the disease. In this work, we screened an annotated chemical compound library to identify compounds that promote myelination in CMT1. For this purpose, we utilized our in vitro myelination system using dorsal root ganglia explants (IVMDE) obtained from Trembler-NCNP mice (1). By using this system, we found that Trembler-NCNP mice-derived explants give shorter myelination segments with lower frequency and weaker mylelin basic protein (MBP) immunoreactivity compared with those in wild type control. Among the screened compounds, we found that serotonin 1B/1D receptor agonists increased size and frequency of myelination segments in Trembler-NCNP-derived IVMDE culture. We also found that serotonin 1B/1D receptor agonists increased expression of p75 and Oct6 in cultured Schwann cells. These data suggest that serotonin 1B/1D receptor agonists may increase myelination by promoting number of promyelinating Schwann cells along axons.
Ref.1: Numata-Uematsu Y et al. PLOS One 10.1371/journal.pone.0285897

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

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.12/W3

Topic: C.06. Neuromuscular Diseases

Support: Medical Research Council award MR/S006990/1
Wellcome Trust Sir Henry Wellcome Postdoctoral Fellowship 103191/A/13/Z
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NIH R35 award GM139627
Wellcome Trust award 107116/Z/15/Z
Wellcome Trust award 223022/Z/21/Z
UK Dementia Research Institute Foundation award UKDRI-1005

Title: Boosting peripheral BDNF rescues impaired in vivo axonal transport in aminoacyl-tRNA synthetase-related neuropathies

Authors: *J. N. SLEIGH[1], D. VILLARROEL-CAMPOS[1], S. SURANA[1], T. WICKENDEN[1], Y. TONG[2], R. L. SIMKIN[1], J. N. S. VARGAS[1], E. R. RHYMES[1], A. P. TOSOLINI[1], S. J.

WEST[1], Q. ZHANG[2], X.-L. YANG[2], G. SCHIAVO[1];
[1]Univ. Col. London, London, United Kingdom; [2]Scripps Res., La Jolla, CA

Abstract: Gain-of-function mutations in the housekeeping genes *GARS1* and *YARS1*, which lead to the expression of toxic versions of glycyl-tRNA (GlyRS) and tyrosyl-tRNA (TyrRS) synthetases, respectively, cause the selective motor and sensory nerve pathology characteristic of Charcot-Marie-Tooth disease (CMT). Aberrant interactions between GlyRS and TyrRS mutants and different proteins, including neurotrophin receptor TrkB, underlie CMT type 2D (CMT2D) and dominant intermediate CMT type C (DI-CMTC); however, our pathomechanistic understanding of these untreatable peripheral neuropathies remains incomplete. Through intravital imaging of the sciatic nerve, we show that CMT2D and DI-CMTC mice display disturbances in axonal transport of neurotrophin-containing signalling endosomes *in vivo*. We discovered that BDNF-TrkB impairments correlate with transport disruption and overall CMT2D neuropathology, and that inhibition of this pathway at the nerve-muscle interface perturbs endosome transport in wild-type axons. Accordingly, supplementation of muscles with BDNF, but not other neurotrophins, completely restores physiological axonal transport in neuropathic mice. Together, these findings suggest that selectively targeting muscles with BDNF-boosting therapies could represent a viable therapeutic strategy for aminoacyl-tRNA synthetase-related neuropathies.

Disclosures: **J.N. Sleigh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The technology described in this work has been protected in the patent GB2303495.2 (patent applicant, UCL Business Ltd., status pending). **D. Villarroel-Campos:** None. **S. Surana:** None. **T. Wickenden:** None. **Y. Tong:** None. **R.L. Simkin:** None. **J.N.S. Vargas:** None. **E.R. Rhymes:** None. **A.P. Tosolini:** None. **S.J. West:** None. **Q. Zhang:** None. **X. Yang:** None. **G. Schiavo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The technology described in this work has been protected in the patent GB2303495.2 (patent applicant, UCL Business Ltd., status pending).

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.13/W4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Characterization of two rodent models of CMT1A to aid the preclinical evaluation of potential new Charcot-Marie Tooth therapies

Authors: H. FERNANDES[1], B. HANER[2], E. K. BENSON[3], G. J. KIDD[3], M. SCHEIDELER[4], *T. HANANIA[2];
[1]Behavioral Pharmacol., Psychogenics Inc, Paramus, NJ; [2]Behavioral Pharmacol., PsychoGenics,

Inc., Paramus, NJ; [3]3DEM Ultrastructural Imaging and Computation Core, Lerner Inst., Cleveland, OH; [4]HumanFirst Therapeut. LLC/CMTA, Silver Spring, MD

Abstract: Charcot-Marie-Tooth (CMT) disorders are a family of related peripheral neurodegenerative diseases that produce progressive distal neuropathy. While CMT Type 1 patients generally live a full lifespan these disorders produce significant morbidity throughout life, representing significant health challenges for patients, their families and society. CMT1A is the most common form of CMT caused by duplication of the peripheral myelin protein 22 (PMP22) gene, causing demyelination of peripheral nerves. Mouse and rat models of CMT1A that overexpress PMP22 are critical tools that support preclinical therapeutic development. Psychogenics, in partnership with the Charcot-Marie-Tooth Association (CMTA) and the Cleveland Clinic has performed extensive characterization of these models. Male CMT1A HET and littermate WT mice were tested at 5, 12-13 and 20-21 weeks of age. Throughout this age range CMT1A mice did not differ in body weight compared to WT, and generally presented as healthy. Grip strength (both fore- and hindlimb) was significantly decreased in CMT1A HET mice as early as 5 weeks of age and these differences persisted throughout the study, albeit with the differences growing smaller in forelimb grip strength and larger in hind limb grip strength with increased age. By 12 weeks of age CMT1A HET mice showed deficits on the tapered beam compared to WT mice, taking significantly longer to traverse the full beam length with a significantly higher number of foot slips. These deficits were still present at 20 weeks of age. Comparison of compound action potentials (CAP) in the predominantly sensory nerves of the tail revealed a severe reduction in amplitude of nerve responses in CMT1A HET mice by 12 weeks of age which persisted through 20 weeks of age. The onset latency of responses was significantly longer in CMT1A HET mice, and nerve conduction velocity was likewise impaired by 12 weeks and this impairment remained through 20 weeks. CMT1A rats showed similar deficits in grip strength, tapered beam performance, and nerve conduction impairments that once established did not improve with age. Electron microscopic analysis of femoral nerve motor branch indicated that large diameter (>3.5µm) axons in CMT1A HET mice were hypomyelinated, with few or no myelin wraps. In WT nerve, in contrast, no large axons were hypomyelinated. Similar results were observed in the 1A rat. These clear phenotypic differences in longitudinal profiling indicate the suitability of these animal models for testing therapies targeting CMT1A, where the ability to affect progression of the disease, or repair existing damage, can be measured

Disclosures: H. Fernandes: A. Employment/Salary (full or part-time):; PsychoGenics 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.; PsychoGenics Inc. **B. Haner:** A. Employment/Salary (full or part-time):; PsychoGenics 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.; PsychoGenics Inc. **E.K. Benson:** None. **G.J. Kidd:** None. **M. Scheideler:** None. **T. Hanania:** A. Employment/Salary (full or part-time):; PsychoGenics 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.; PsychoGenics Inc.

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.14/W5

Topic: C.04. Movement Disorders other than Parkinson"s Disease

Title: Characterization of a rat model of CMT2A to aid the preclinical evaluation of potential new Charcot-Marie-Tooth therapies

Authors: *H. B. FERNANDES[1], B. HANER[1], E. K. BENSON[2], G. J. KIDD[2], M. SCHEIDELER[3], T. HANANIA[1];
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Abstract: Charcot-Marie-Tooth (CMT) disorders are a family of related peripheral neurodegenerative diseases that produce progressive distal neuropathy. CMT Type 2 patients typically experience significant morbidity early in life, representing significant health challenges for patients, their families and society. CMT2A is a form of CMT Type 2 caused by dominant mutations in the mitofusin 2 (MFN2) gene, resulting in progressive axonopathy. The creation of two rat models of CMT2A expressing knock-ins of authentic human mutations has provided critical tools to support the preclinical evaluation of potential new therapies. Psychogenics, in partnership with the Charcot-Marie-Tooth Association (CMTA) and Cleveland Clinic have performed extensive characterization of both rat models to this end. Male CMT2A and littermate WT rats were bred and raised at Psychogenics, dosed with a vehicle starting at 16 weeks of age and tested in various assays at 22-23 and 30-31 weeks of age. Throughout this age range CMT2A rats did not differ in body weight compared to WT littermates, and generally presented as healthy without obvious health concerns in the home cage. Grip strength (both fore- and hindlimb) was significantly decreased in CMT2A HET animals at 16 weeks of age and this difference persisted throughout the tested age range, albeit somewhat diminished in forelimbs by 22 weeks of age. Gait analysis using Psychogenics proprietary NeuroCube gait analysis system found significant differences between WT and CMT2A HET animals by 22 weeks of age when considering all features of gait, and these differences were more pronounced by 30 weeks of age. At this latter age notable deficits in CMT2A HET animals were observed in gait features, average gait speed, and gait rhythmicity. Comparison of compound action potentials (CAP) in the predominantly sensory nerves of the rat tail revealed a significant reduction in response amplitude of nerve responses in CMT2A HET animals by 22 weeks of age which persisted through 30 weeks of age. While the onset latency of responses was not different between WT and CMT2A HET animals at 22 weeks of age, by 30 weeks of age the latency was significantly longer in HET animals. A similar pattern was observed for nerve conduction velocity, in that there were no differences between genotypes at 22 weeks of age but a significant reduction was observed in CMT2A HET rats by 30 weeks of age. Electron microscopic analysis of distal tibial nerves from CMT2A HET animals indicated many shrunken axons, notably among large diameter myelinated fibers. The longitudinal phenotypic differences observed point to the suitability of these models for use in the evaluation of therapies targeting CMT2A.

Disclosures: **H.B. Fernandes:** A. Employment/Salary (full or part-time):; Psychogenics, 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.; Psychogenics, Inc. **B. Haner:** A. Employment/Salary (full or part-time):; Psychogenics, Inc.. **E.K. Benson:** None. **G.J. Kidd:** None. **M. Scheideler:** None. **T. Hanania:** A. Employment/Salary (full or part-time):; Psychogenics, 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.; Psychogenics, Inc..

Poster

PSTR536. Peripheral Nerves: Normal Biology, CMT, and Other Pathologic Conditions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR536.15/W6

Topic: C.04. Movement Disorders other than Parkinson"s Disease

Title: Characterization of a mouse model of CMT2E to aid the preclinical evaluation of potential new Charcot-Marie-Tooth therapies

Authors: H. B. FERNANDES[1], A. KOSHY CHERIAN[2], E. K. BENSON[3], G. J. KIDD[3], *M. SCHEIDELER[4], T. HANANIA[1];
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Abstract: Charcot-Marie-Tooth (CMT) disorders are a family of related peripheral neurodegenerative diseases that produce progressive distal neuropathy. CMT Type 2 patients typically experience significant morbidity early in life, representing significant health challenges for patients, their families and society. CMT2E is a form of CMT caused by dominant mutations in the NEFL gene that encodes the neurofilament light protein. The creation of a mouse model of CMT2E (Lancaster et al., 2018) presented a tool to support the preclinical evaluation of potential new therapies. Psychogenics, in partnership with the Charcot-Marie-Tooth Association (CMTA) and the Cleveland Clinic has performed longitudinal characterization of this mouse model of CMT2E. Male CMT2E and littermate WT mice were dosed with vehicle starting at 8 weeks of age and tested in various assays at 14, 20 and 26 weeks of age. Throughout the study CMT2E mice did not differ significantly in body weight compared to WT littermates, generally presenting as healthy. By 14 weeks of age CMT2E HET mice showed deficits in the tapered beam compared to WT littermates, taking significantly longer to traverse the full beam length with a higher number of foot slips while doing so. These differences were more pronounced at 20 weeks of age. Gait analysis using Psychogenics proprietary NeuroCube gait analysis system found significant differences between WT and CMT2E HET animals by 20 weeks of age, which were more pronounced at 26 weeks of age; notable deficits were observed in gait features, body motion, gait rhythmicity and paw positioning. Compound muscle action potentials (CMAP) elicited by sciatic motor nerve stimulation and measured in the gastrocnemius muscle were

analyzed to assess the extent of axonal degeneration and demyelination in motor nerves. While the amplitude of the muscle responses were not different between genotypes at 20 or 26 weeks of age, the onset latency of CMT2E HET animals was significantly longer at these time points and motor nerve conduction velocity was significantly slower. Examination of compound action potentials (CAP) in the predominantly sensory nerves of the mouse tail revealed severe deficits in response latency, amplitude, and nerve conduction velocity in CMT2E HET mice. Electron microscopic analysis of distal tibial nerves from CMT2E HET mice indicated that large axons were prominently affected. In addition, axons exhibited quantifiable evidence of dysfunction and degeneration, such as mitochondrial accumulation and increased axoplasmic staining. These substantial phenotypic differences demonstrate the suitability of this animal model for use in the evaluation of therapies.

Disclosures: **H.B. Fernandes:** A. Employment/Salary (full or part-time):; Psychogenics, 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.; Psychogenics, Inc.. **A. Koshy Cherian:** None. **E.K. Benson:** None. **G.J. Kidd:** None. **M. Scheideler:** None. **T. Hanania:** A. Employment/Salary (full or part-time):; Psychogenics, 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.; Psychogenics, Inc..

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.01/W7

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

Support: NRF 2022R1C1C2006049
KHIDI HI19C1347
MSIT 2020R1C1C1010505

Title: Focused ultrasound as a novel non-invasive method for the delivery of gold nanoparticles to retinal ganglion cells

Authors: *J. SHIN[1,2], Y. PARK[4], J. PARK[5], I. AUBERT[3], K. EOM[4], W. CHANG[5];
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Abstract: Delivery of gold nanoparticles (AuNPs) to retinal ganglion cells is gaining attention as a therapeutic and diagnostic approach for retinal diseases. However, intravitreal injection of

AuNPs is invasive and thus is not optimal. Focused ultrasound with microbubbles (FUS) is a non-invasive method for systemic delivery of viral vectors to retinal Müller glia; however, whether metallic nanoparticles of various sizes and shapes can be delivered via FUS remains unknown. Here, we report FUS-assisted delivery of AuNPs of varying shapes and sizes to retinal ganglion cells. Magnetic resonance imaging and histological analyses show that 0.3 MPa is the optimal sonication parameter for safe blood-retinal barrier modulation. FUS can also deliver dextran (70 kDa) to the retinal layer, especially the retinal ganglion cell layer and inner nuclear layer cells. Two-photon microscopic imaging of AuNPs injected into the retinal ganglion cell layer confirms that spherical- and rod-shaped AuNPs with maximum dimensions <80 nm are effectively delivered without damage. The amount of detected AuNPs varies with size. Spherical nanoparticles of small diameter (10 nm) are ~20-fold more abundant than larger nanoparticles (55 nm). Our findings provide a novel approach for delivering nanometer-sized metallic and organic nanomaterials without damaging retinal tissue.

Disclosures: J. Shin: None. **Y. Park:** None. **J. Park:** None. **I. Aubert:** None. **K. Eom:** None. **W. Chang:** None.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.02/W8

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

Support: RS-2020-KD000103

Title: Role of P2X7 receptor in focused ultrasound and microbubble mediated blood-brain barrier opening

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Abstract: Role of P_2X_7 receptor in focused ultrasound and microbubble mediated blood-brain barrier opening Junwon Park[1†], Byungwook Song[3†], Younghee Seo[2], Jin Woo Chang[1,2], Youngcheol Na[3*] and Won Seok Chang[1*]
Purpose: Cavitation of microbubbles affected by ultrasound energy allows the non-invasive, transient, and localized disruption of the blood-brain barrier (BBB) which is used in diverse studies. Especially temporal modulation of BBB is being studied for efficient drug delivery in various diseases. Under this circumstances, this study observe a series of signal transductions altered by FUS in the perspective of P_2X_7 receptor and provides important insights of BBB modulation mechanism induced by FUS.
Materials & Methods: Sprague-Dawley rats (270 ± 20g) were fixed to the stereotactic frame. Right hippocampus was sonicated using a single-element transducer (frequency 0.5 MHz) with

microbubble (Definity, 20ul/kg), and the group was divided with time after sonication; Ctrl,1,4,24h. The P_2X_7 receptor and its corresponding proteins including NLRP3, IL-1β and NF-kB were quantified through Western blotting. In addition, quantification of proteins related to the BBB including Zonula occludens-1 (ZO-1), Occuldin, and metalloproteinase9 (MMP9) were also carried out. Disruption of BBB was measured through Evans blue quantification using spectrophotometry.
Results: Comparing the sonicated region of each group, significant protein expression level increase 1 hour after FUS was confirmed related to the P_2X_7 receptor ($p<0.05$). A pattern of normalization over time could be observed. On the contrary, proteins related to tight junction protein were significantly reduced at 1 hour and then normalized ($p<0.005$). In the case of Evans Blue quantification, correspondingly, the most leakage was found in an hour ($p<0.05$), and after 24 hours, it was confirmed that it returned to its normal state. **Conclusion:** Although FUS is frequently used clinically, the mechanism related to BBB modulation has not been accurately identified. To use this technique with a better understanding, we focused on the P_2X_7 receptor and its cascade after induction of FUS. As a result, activation of P_2X_7 and related factors showed its peak intensity at 1 hour after application of FUS, and tight junction proteins were decreased. In addition, compared to the group treated with antagonist, the degree of expression increased slightly. These results indicate that the P_2X_7 receptor plays an important role in BBB modulation.

Disclosures: J. Park: None. **B. Song:** None. **Y. Seo:** None. **J. Lee:** None. **J. Chang:** None. **Y. Na:** None. **W. Chang:** None.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.03/W9

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

Support: RS-2020-KD000103

Title: Modulation of the septo-hippocampal circuit for enhancement of cognitive function by focused ultrasound

Authors: *Y. SEO[1,2], S. HAN[1], J. BAEK[1], Y. NA[3], J. CHANG[1,2], W. CHANG[1];
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Abstract: Introduction Focused ultrasound (FUS) is a treatment that can promote functional recovery with non-invasive stimulation of the neuron in the deep-brain area. In particular, there are various studies confirming an increase in brain-derived neurotrophic factor (BDNF) when stimulated by FUS. It is known that the cholinergic projection of Medial septum (MS) is mainly directed to hippocampal formation, and the decrease in cholinergic neurons of MS is related to

hippocampal dependent learning and memory. The input from MS to the hippocampus consists of at least three routes, namely, cholinergic, GABAergic, and glutamatergic fibers, and it is known that this projection affects the activity of the hippocampus. Therefore, in this study, it was compared whether there was an improvement in cognitive function when stimulating the MS and Hippocampus (septo-hippocampal pathway) with FUS.
Materials and methods In this study, male C57BL/6 mice were used. After fixing the mouse to the stereotaxic frame, the scalp was incised, and Medial septum (MS; AP + 0.6, ML 0), Hippocampus (HP; AP -2, ML ± 1.3), and MS+HP were targeted based on Bregma. Low-intensity focused ultrasound was applied to parameters of 515 kHz FF, 50% DC, 1 kHz PRF, 0.5 ms TBD, 300 ms SD, 2 s ISI. They were sacrificed 1 hour, 1 day, 2 days, and 3 days after sonication, respectively.
Results To determine brain modulation depending on the changes in pulse repetition frequency (PRF) in a constant duty cycle of 50%, the expression of c-fos, a neuronal activity marker, was confirmed in response to 30, 300, 1000, 2500, and 4000 Hz. As a result, when the PRF is 1000 Hz, the c-fos and BDNF increase the most. After FUS stimulation, TrkB was activated, and the elevation of BDNF protein levels in astrocytes was confirmed. As a result, it was confirmed that the function of the synaptic NMDA receptors was also improved. In addition, it was confirmed that cell death was reduced through immunohistochemistry of cholinacetyltransferase (ChAT). To determine whether there is a difference in the degree of AChE, an Ellman assay was performed, and the level was increased. The Y-maze was performed to compare the effects for each sonicated target site. As a result of comparing spontaneous alternation in the Y-maze, it increased after FUS in all groups, and a significant increase was confirmed, especially on the 2nd day of the MS+HP group.
Conclusion Focused ultrasound is a non-invasive and safe way to stimulate specific brain areas. The results of this study showed that the modulation of the septo-hippocampal pathway by FUS can improve spatial learning and memory by protecting cholinergic neurons.

Disclosures: **Y. Seo:** None. **S. Han:** None. **J. Baek:** None. **Y. Na:** None. **J. Chang:** None. **W. Chang:** None.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.04/W10

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

Support: RS-2020-KD000103

Title: Enhancing Photodynamic Therapy for Brain tumor: Amplifying Anti-tumor effects through Inhibition of Autophagy mechanisms

Authors: *S. HAN, J. PARK, C. KONG, W. CHANG;
Dept. of Neurosurg., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Introduction Glioblastoma (GBM) is the most common primary malignant glioma in adults and is characterized by high mortality, frequent recurrence, and aggressive invasion. Photodynamic therapy (PDT) has emerged offering advantages over traditional approaches by minimizing damage to normal tissues. The therapeutic effects of PDT primarily stem from the direct effects of singlet oxygen and reactive oxygen species (ROS). ROS can induce oxidative damage to subcellular organelles and disrupt microvasculature, leading to cell death. Notably, PDT-induced tumor eradication involves various cell death mechanisms, including necrosis, apoptosis, ferroptosis, and autophagy. Autophagy, unlike other processes, exhibits a dual role in tumor suppression and promotion and contributes to cancer cell development and proliferation. Consequently, the anti-tumor effects utilizing each aspect of autophagy have been extensively investigated over an extended period. In this study, the anti-tumor effect of PDT was compared with the combination therapy using hydroxychloroquine (HCQ), an autophagy inhibitor.
Materials and methods Male Sprague-Dawley rats weighing 230-250g were used in this study. C6 glioma cells were injected into the rats' cortical region. The study included three groups: a non-treatment, PDT, PDT with HCQ injected groups, respectively. The photosensitizer (PS) used of PDT was Chlorin e6 (Ce6) and a laser at a wavelength of 660 nm was irradiated by 100 J/cm^2. Tumor size was monitored using Magnetic resonance imaging (MRI). The activity of Autophagy was compared using LC3B and P62/SQSTM1, and cell damages including apoptosis is confirmed with Caspase-3.
ResultsBased on the MRI, PDT and HCQ-PDT groups demonstrated a significant decrease in tumor size. LC3B, which is involved in autophagosome formation, showed a decreasing trend in the HCQ group and an increasing in the PDT group. P62/SQSTM1 exhibited an increase in the HCQ-PDT group and a decrease in the PDT group. Caspase-3 showed a higher increase in the HCQ-PDT group compared to the PDT group.
ConclusionThis research demonstrates that combination therapy of HCQ and PDT can enhance the efficacy of brain tumor treatment. Several studies have suggested that the activation of autophagy is implicated in tumor recurrence. Therefore, future investigations should focus on the effect of HCQ on residual tumors to assess its impact on mechanisms of tumor recurrence suppression. Additional research is warranted to further elucidate the potential influence of this combination therapy on tumor recurrence inhibition.

Disclosures: **S. Han:** None. **J. Park:** None. **C. Kong:** None. **W. Chang:** None.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.05/W11

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

Support: KDDF Grant, Project number:1711195913, KDDF2118217617-22
NRF Korea Grant 2020R1F1A107410413
KMDDF Grant, RS-2022-00141392

Title: Extremely low frequency stimulation of ventrolateral periaqueductal gray induces activation of descending serotonergic system in the peripheral neuropathic pain

Authors: *M. PARK[1,2], C. KOH[1], H. CHANG[1], W. MUN[1], T. KIM[1], J. CHANG[1,2], H. JUNG[1]; [1]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Dept. of Neurosurgery, Grad. Sch. of Med. Science, Brain Korea 21 Project, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Introduction: Neuropathic pain is a type of chronic pain, which involves severe prolonged sensory dysfunctions caused by a lesion of the somatosensory system. Compelling evidence have suggested that pain is associated with malfunction of descending inhibitory pathway, so the imbalance of descending system may critically exacerbate the chronic pain condition. The role of serotonin in the descending inhibition has been controversial because it varies depending on behavioral conditions and types of pain. In this study, in order to understand more details on descending serotonergic modulation, we mainly focused on descending pain modulation, especially mediated by PAG and nucleus raphe magnus (RMg). **Methods**: Adult male Sprague-Dawley rats (200g-220g) were used. Rats were grouped into three groups: Stim (n=16), SNI (n=8), Control (n=4). On post-operative 16th day (POD), two stimulation electrodes for bipolar stimulation were implanted in the ventrolateral periaqueductal gray. The stimulation system was turned on at 09:00 and turned off after 9 hours of stimulation. The level of antinociception was measured by the von Frey test. For analysis, RMg and spinal dorsal horn were harvested for western blot and immunohistochemistry. Electrophysiological recording was also conducted immediately after 9-hour stimulation.**Results**: Immediately after 9-hour stimulation, electrophysiological recording showed significant differences in firing rates and after-discharge rates were observed (Stim: post-press 8.070 ± 3.768; SNI: post-press 16.26 ± 7.260) and stimulation effects were most remarkable at 9-hour on the last day of stimulation (D1 vs. D5: 5.581 ± 0.9315 vs. 8.807 ± 0.9784). In the RMg, stimulation restored serotonergic release though it still shows lower level compared to control group (Control: 1.488 ± 0.1653, SNI: 0.6466 ± 0.0801, Stim: 1.023 ± 0.0738) At the spinal level, the expression level of 5-HT1A receptor was remarkably increased in the Stim group compared to nerve-injured group (Control: 1.354 ± 0.0993, SNI: 0.3647 ± 0.1433, Stim: 1.232 ± 0.0511).**Conclusion**: Extremely low frequency DBS of vlPAG is clearly associated with the activation of descending pain modulation in the spared nerve injury. This work would possibly gives us lower chances of side effects from repeated high-frequency stimulation or long-term use of medication.

Disclosures: M. Park: None. **C. Koh:** None. **H. Chang:** None. **W. Mun:** None. **T. Kim:** None. **J. Chang:** None. **H. Jung:** None.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.06/W12

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

Support: HI19C0060
2020R1A2C1011839
2020-KD000103
NRF-2022R1C1C2006049

Title: Enhanced delivery of a low dose of aducanumab via FUS in 5xFAD mice,an AD model

Authors: *C. KONG[1], E.-J. YANG[2], J. SHIN[3], S.-H. KIM[4], J. PARK[1], S.-W. PARK[4], W. CHANG[1], C.-H. LEE[4], H. KIM[4], H.-S. KIM[5], J. CHANG[1];
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Abstract: Aducanumab (Adu), which is a human IgG1 monoclonal antibody that targets oligomer and fibril forms of beta-amyloid (Aβ), has been reported to reduce amyloid pathology and improve impaired cognition after the administration of a high dose (10 mg/kg) of the drug in Alzheimer's disease (AD) clinical trials. The purpose of this study is to investigate the effects of a lower dose of Adu (3mg/kg) with enhanced delivery via focused ultrasound (FUS) in an AD mouse model.The FUS with microbubbles opened the blood-brain barrier (BBB) of the hippocampus for the delivery of Adu. The combined therapy of FUS and Adu was performed three times in total and each treatment was performed biweekly. Y-maze test, Brdu labeling, and immunohistochemical experimental methods were employed in this study. In addition, RNA sequencing and ingenuity pathway analysis were employed to investigate gene expression profiles in the hippocampi of experimental animals.The FUS-mediated BBB opening markedly increased the delivery of Adu into the brain by approximately 8.1 times in the brains. The combined treatment induced significantly less cognitive decline and decreased the level of amyloid plaques in the hippocampi of the 5xFAD mice compared with Adu or FUS alone. Combined treatment with FUS and Adu activated phagocytic microglia and increased the number of astrocytes associated with amyloid plaques in the hippocampi of the 5xFAD mice. Furthermore, RNA sequencing identified 4 enriched canonical pathways such as phagosome formation, neuroinflammation signaling, CREB signaling and reelin signaling was altered in the hippocami of 5xFAD given the combined treatment.In conclusion, the enhanced delivery of a low dose of Adu (3mg/kg) via FUS decreased amyloid deposits and attenuated cognitive function deficits. FUS-mediated BBB opening increases adult hippocampal neurogenesis as well as drug delivery. We present an AD treatment strategy through the synergistic effect of the combined therapy of FUS and Adu.

Disclosures: C. Kong: None. **E. Yang:** None. **J. Shin:** None. **S. Kim:** None. **J. Park:** None. **S. Park:** None. **W. Chang:** None. **C. Lee:** None. **H. Kim:** None. **H. Kim:** None. **J. Chang:** None.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.07/W13

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

Title: Effects of Plasma Fraction therapy on Chemotherapy Induced Cognitive Impairment

Authors: *I. D. GALLAGER, N. SMITH, J. KIM, M. CAMPBELL;
Alkahest Inc., Half Moon Bay, CA

Abstract: Cisplatin is a platinum-based chemotherapeutic used to treat solid-state tumors, however, 35-85% of patients suffer from side effects including long term cognitive dysfunction, impaired memory and attention, and decreased executive function. Cisplatin administration in juvenile rats and adult mice induces blood-brain barrier dysfunction, resulting in cisplatin crossing into the brain parenchyma, inhibition of neuronal stem cell proliferation and decreased neurogenesis, changes in white matter, increased reactive oxygen species (ROS), accelerated biological aging, increased neuroinflammatory state and reduction in cognitive function. Therefore, therapeutics which could address these neurotoxic effects resulting from Cisplatin treatment, dubbed Chemotherapy Induced Cognitive Impairment (CICI), is paramount. Plasma contains many beneficial factors which have been shown in animal models to ameliorate multiple age-related deficits across varied organ systems, including the brain. We focused on age-related hippocampal-dependent cognitive deficits, as the hippocampus is well accessed by the vascular system, especially within the subgranular zone, making it an attractive target for evaluation of plasma-derived factors. In previous studies we have demonstrated the benefits of a fractionated plasma product (PF) to induce reversal of age-related cognitive decline, enhanced hippocampal neurogenesis, reduced neuroinflammation and cell survival in aged immunocompetent mice. In this study, we investigated whether PF can therapeutically reverse the concomitant CNS dysfunctions induced by Cisplatin treatment, many of which parallel impacts occurring in biological aging. These data warrant further investigation into plasma fraction treatment for brain fog or Chemotherapy Induced Cognitive Impairment.

Disclosures: I.D. Gallager: A. Employment/Salary (full or part-time):; Alkahest Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest Inc. **N. Smith:** A. Employment/Salary (full or part-time):; Alkahest Inc. **J. Kim:** A. Employment/Salary (full or part-time):; Alkahest Inc. **M. Campbell:** A. Employment/Salary (full or part-time):; Alkahest Inc..

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.08/W14

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

Title: Machine learning-based method of quantifying blood-brain barrier dysfunction in a mouse model of chemotherapy-induced cognitive impairment.

Authors: *N. SMITH, J. KIM, R. BRITTON, M. KERRISK CAMPBELL, I. GALLAGER; Pre-Clinical, Alkahest, San Carlos, CA

Abstract: Chemotherapy-induced cognitive impairment (CICI) is an often-reported chronic neurological condition amongst those undergoing cancer treatment. Approximately 20-33% of surviving patients describe the loss of cognitive processing speed, memory impairment, and general “brain fog” for years following the completion of their chemo regime. Platinum-containing compounds are common anti-cancer drugs, which are associated with CICI, with one of the most common being Cisplatin. Cisplatin induces dysfunction of the blood-brain barrier (BBB) resulting in apoptosis of dividing neuronal precursor cells. These findings, in association with concurrent reactive oxygen species generation, are common hallmarks of many neurological diseases, including CICI. However, a comprehensive characterization of the severity of BBB dysfunction in CICI is lacking. Common methods like the use of fluorescein-conjugated molecules result in high signal in control animals and require a dedicated fluorophore channel for the purpose of quantification. An alternative is the measurement of serum proteins within the brain parenchyma, however, histological analysis is prone to inter-investigator variability, suffers from low sampling size, and is exceedingly time-consuming. In this study, we employed the use of a machine learning-based segmentation program and quantified mislocalized AQP4 and extravascular IgG. The methodology utilized demonstrates a streamlined and reproducible approach to quantitating BBB dysfunction in a mouse model of Cisplatin/chemotherapy-induced cognitive impairment. Using Zeiss’ Intellesis trainable segmentation tool, we categorized regions of histological images as vascular (Lectin/Laminin-positive) or extravascular (Lectin/Laminin-negative) segments. Within the extravascular segment, we quantified IgG and AQP4 within 5 µm shells up to 30 µm of the nearest vascular segment. In a mouse model of CICI, AQP4 signal intensity was increased within each 5 µm shell up to 25 µm from the vascular region. Our data suggest that the automated segmentation of lectin/laminin to enable the analysis of AQP4 and IgG staining around brain microvasculature is an optimized methodology compared to the standard technique such as line scan, yielding a robust, reproducible and standardized measurement of BBB permeability to enable therapeutic discovery.

Disclosures: **N. Smith:** A. Employment/Salary (full or part-time):; Alkahest, inc. **J. Kim:** A. Employment/Salary (full or part-time):; Alkahest, inc. **R. Britton:** A. Employment/Salary (full or part-time):; Alkahest, inc. **M. Kerrisk Campbell:** A. Employment/Salary (full or part-time):; Alkahest, inc. **I. Gallager:** A. Employment/Salary (full or part-time):; Alkahest, inc.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.09/W15

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

Title: Altered Oligodendrocyte Lineage Contributes to Cisplatin-based Chemotherapy Induced Cognitive Impairment

Authors: *J. KIM, N. SMITH, M. K. CAMPBELL, I. GALLAGER;
Alkahest, San Carlos, CA

Abstract: Altered Oligodendrocyte Lineage Contributes to Cisplatin-based Chemotherapy Induced Cognitive Impairment

Authors: **J. Kim**, N. Smith, M. Kerrisk Campbell, I. Gallager.

Cisplatin-based chemotherapy patients often experience cognitive impairment, also known as "chemo fog." This adverse neurological sequela, defined as Chemotherapy Induced Cognitive Impairment (CICI), can be associated with various physiochemical changes in the brain. One of these changes is found to affect cells of oligodendrocyte lineage that follows the stages of oligodendrocyte progenitor cell then pre- to mature myelinating oligodendrocyte. Previous studies saw high vulnerability of oligodendrocytes to Cisplatin treatment compared to other cell types, such as neurons or astrocytes, and identified significant increase in apoptotic oligodendrocytes upon treatment. With known implications of oligodendrocyte lineage on cognition, it is crucial to map the changes in population, differentiation, and myelination capacity of oligodendrocytes following chemotherapy.

Metformin, a treatment for type II diabetes, has recently received attention for its ability to increase sensitivity to anti-cancer drugs. Co-administration of Metformin with Cisplatin in animal models have shown to prevent Cisplatin-induced cognitive impairments, although its mechanism of action is not yet well-defined. Direct impact of Metformin on oligodendrocytes have been explored in relation to AMPK-pathway while the impacts of *in vivo* prophylactic metformin on oligodendrocyte lineage cells has yet to be elucidated.

Here we characterize an *in vivo* CICI mouse model by evaluating changes in cells of oligodendrocyte lineage upon Cisplatin treatment. We performed time-dependent and dose-dependent analysis to observe change in oligodendrocyte population size and to map out the patterns of differentiation and myelination capacity post-treatment. We further investigated the oligoprotective effects of Metformin against Cisplatin treatment and correlation with reduction of ROS. Depletion and deficits in oligodendrocytes following Cisplatin treatment will be characterized to identify oligodendrocyte lineage as a potential therapeutic target to attenuate CICI.

Disclosures: **J. Kim:** A. Employment/Salary (full or part-time):; Alkahest. **N. Smith:** A. Employment/Salary (full or part-time):; Alkahest. **M.K. Campbell:** A. Employment/Salary (full or part-time):; Alkahest. **I. Gallager:** A. Employment/Salary (full or part-time):; Alkahest.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.10/W16

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

Title: Assessing angiogenin neuroprotection in a chemotherapy-induced cognitive impairment mouse model.

Authors: *A. AL OMRAN[1], J. SIN[1], I. GALLAGER[2], M. KERRISK CAMPBELL[1], C. F. YANG[1];
[1]Alkahest Inc, San Carlos, CA; [2]Alkahest Inc., Half Moon Bay, CA

Abstract: Assessing angiogenin neuroprotection in a chemotherapy-induced cognitive impairment mouse model. Alzahra J. Al Omran, Jung H. Sin, Ian Gallager, Meghan Kerrisk Campbell, Cindy F. Yang
Angiogenin (ANG) is a stress-induced ribonuclease known for its potent angiogenic and cell proliferative activity. ANG loss-of-function genetic mutations are associated with several neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. Under conditions of cellular stress, ANG may serve as a neuroprotective factor, inducing translational arrest and stress granule formation. However, *in vitro* and *in vivo* studies investigating ANG as a neuroprotectant have focused on motoneuron survival, and it is unclear if ANG confers protection across multiple CNS cell types. In this study, we evaluated the effects of ANG delivery in reducing neurotoxicity in the chemotherapy-induced cognitive impairment (CICI) mouse model. In humans, chemotherapy imposes several detrimental effects on the nervous system resulting in substantial memory deficits manifesting as short/long memory impairment and delaying in mental processing. Hence, the animal model of CICI allows a critical evaluation and assessment of the underlying neuronal toxicities associated with cognitive impairment such as neuronal death. Eight-month-old C57BL/6J male mice were dosed with a chemotherapy regimen of cisplatin, a platinum-based chemotherapeutic agent commonly used in the treatment of solid tumors. We evaluated cognitive impairment in a Y-maze assay and anxiety-like behaviors and locomotion in the open field assay. We examined the effects of ANG on neuronal apoptosis, oxidative stress, and neuroinflammation, including astrogliosis and microgliosis. Furthermore, we assessed the effect of ANG in neurogenesis and proliferation using the histological markers doublecortin and Ki67. Collectively, this study supports the mechanistic understanding of the role of ANG in neuroprotection.

Disclosures: **A. Al Omran:** A. Employment/Salary (full or part-time):; Alkahest, Inc. **J. Sin:** A. Employment/Salary (full or part-time):; Alkahest, Inc. **I. Gallager:** A. Employment/Salary (full or part-time):; Alkahest, Inc. **M. Kerrisk Campbell:** A. Employment/Salary (full or part-time):; Alkahest, Inc. **C. F. Yang:** A. Employment/Salary (full or part-time):; Alkahest, Inc.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.11/W17

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

Support: NeuraStasis, Inc.

Title: Transcutaneous stimulation of trigeminal and vagus nerves: Safety, tolerability, and effects on cerebral blood flow in individuals at high risk for stroke

Authors: *N. KIM[1,2], S. WARD[3], C. HOWELL[3], C. NIESER[3], J. UPCHURCH[1], K. GILL[1], A. V. ALEXANDROV[4], P. GUPTA[3];
[1]NeuraStasis, Inc., Houston, TX; [2]Dept. of Psychological Sci., Rice Univ., Houston, TX; [3]Neurol. Consultants of Dallas, Dallas, TX; [4]Dept. of Neurol., Univ. of Arizona Col. of Med., Phoenix, AZ

Abstract: A multi-target therapy is required to protect the entire neurovascular unit during ischemia. Electrical stimulation of the vagus and/or trigeminal nerves in animals has shown to increase cerebral perfusion and vasodilatory peptide release while mitigating neuronal death and cytotoxic depolarization events. As a first step to clinical translation, we aim to assess the safety and tolerability of transcutaneous stimulation of the supraorbital branch of the trigeminal nerve (soTNS) and the auricular branch of the vagus nerve (taVNS) in individuals at high risk for stroke. To inform whether stimulation improves cerebral perfusion, we also explore the effects on cerebral blood flow velocity. **Methods** Our custom device produced non-invasive, current-controlled, pulsed biphasic soTNS of up to 12 mA and taVNS of up to 5 mA on four independent channels. Ten participants (67.1 ± 6.7 years of age, n=5 females, n=2 prior transient or cerebrovascular attack, n=7 hypertension, n=8 hyperlipidemia, n=3 diabetes mellitus) received sub-maximum tolerable stimulation over 45-minutes (50% duty cycle, 3-minute pulse trains) and reported subjective comfort ratings from 1 to 10 (least to most comfort). We used transcranial doppler ultrasonography to record the M1 segment of the middle cerebral artery and compare blood flow metrics before and during stimulation. **Results** No unanticipated adverse events were reported. Participants rated stimulation with a mean comfort score of 8.8 ± 1.3 at intensity levels of 52 ± 18% of device maximum. During tolerability assessment, 10% increase in stimulation intensity predicted a 1.7% increase in mean flow velocity compared to initial baseline, $\beta = .017$, $R^2 = .29$, $F(2,210) = 86.3$, $p < .001$, and a 1.6% increase in end diastolic velocity (EDV) compared to initial baseline, $\beta = .016$, $R^2 = .22$, $F(2,210) = 58.5$, $p < .001$. Changes in min-pulsatility index (PI) were not sustained. However, we observed more immediate effects during each 3-minute pulse train, wherein stimulation was associated with a 17% reduction in min-PI relative to the prior inter-stimulus interval, $\beta = -.173$, 95% CI [-.273, -.073], $p < .001$. **Conclusion** Our findings demonstrate the safety and tolerability of soTNS and taVNS in high-risk stroke individuals. Furthermore, stimulation increases EDV and decreases PI—indicating vasodilation. Since preserved autoregulatory responses can overcome short-term gains, future investigations should explore the cumulative effects of longer stimulation durations on blood flow metrics. These results provide a foundation for the clinical translation of non-invasive neurostimulation as a multi-target therapy for ischemic stroke.

Disclosures: **N. Kim:** A. Employment/Salary (full or part-time):; NeuraStasis, Inc.. **S. Ward:** None. **C. Howell:** None. **C. Nieser:** None. **J. Upchurch:** A. Employment/Salary (full or part-time):; NeuraStasis, Inc. **K. Gill:** A. Employment/Salary (full or part-time):; NeuraStasis, Inc. **A.V. Alexandrov:** F. Consulting Fees (e.g., advisory boards); NeuraStasis, Inc. **P. Gupta:** 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.

Poster

PSTR537. Neuroprotective Mechanisms: Neurostimulation, Fus, and Chemotherapeutics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR537.12/W18

Topic: D.05. Auditory & Vestibular Systems

Support: NRF-2022R1A2C2006061
NRF-2017R1A5A2014768
NRF-2021R1A2C2092038

Title: A novel BCAP31 variant associated with non-syndromic deafness shows mitochondrial dysfunction and high sensitivity to cisplatin

Authors: *Y. KIM;
Paean Biotech., Seoul, Korea, Republic of

Abstract: A novel *BCAP31* variant associated with non-syndromic deafness shows mitochondrial dysfunction and high sensitivity to cisplatin Yujin Kim[1,*], Yehree Kim[2*], Bong Jik Kim[3*], Shin-Hye Yu[1], Jin Hee Han[2], Minyoung Kim[2], Seoeun Lee[1], Young Cheol Kang[1], Chun-Hyung Kim[1,#] and Byung Yoon Choi[2,#1]Paean Biotechnology, Inc. 5 Samil-daero8-gil, Jung-gu, Seoul, Korea [2]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Korea[3]Department of Otolaryngology—head and Neck Surgery, Chungnam National University College of Medicine, Republic of Korea
B-cell receptor-associated protein 31 (BAP31 or BCAP31) is an integral ER membrane protein, which involves the transport and quality control of transmembrane proteins. BAP31 is also important for the cross-talk of apoptotic signals between the ER and mitochondria. Due to its critical role in cellular physiology, the BAP31 dysfunction has been associated with numerous human diseases including deafness, dystonia, and central hypomyelination (DDCH) syndrome, cancer, metabolic syndrome, cystic fibrosis, and neurodegenerative diseases. Recently, we have found a novel in-frame insertion variant in the *BCAP31* gene from a family segregating only non-syndromic hearing loss in an X-linked, recessive fashion. It is not known how this variant contributes to the hearing loss, which is an important issue to understand the molecular pathogenesis. To address it, we compared the mitochondrial function between the patient-derived lymphoblastoid cell lines (LCLs) and normal LCLs. The patient-derived LCLs showed the elevation in ROS, and the decrease in ATP and membrane potential intracellularly compared to normal LCLs. Surprisingly, the administration of mitochondria (PN-101) isolated from umbilical cord mesenchymal stem cells (UC-MSC) was able to rescue the mitochondrial dysfunction in the patient-derived LCLs. Furthermore, patient-derived LCLs demonstrated more pronounced cisplatin-induced cell death than did normal LCLs by confirming the increase in the expression of pro-apoptotic genes. Taken together, the novel *BCAP31* variant may contribute to the pathogenesis of the impaired hearing due to the mitochondrial dysfunction. Key words: BAP31/BCAP31, mutation, hearing loss, mitochondria, apoptosis*Support Contributed By:* NRF-2022R1A2C2006061 to C.H. Kim and NRF-2017R1A5A2014768, 2021R1A2C2092038 to B.Y. Choi

Disclosures: Y. Kim: None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.01/W19

Topic: C.09.Stroke

Support: NIH GM104941

Title: Understanding the role of proprioception in obstacle avoidance reaching behavior in individuals with chronic stroke

Authors: *D. AUSTIN[1], J. A. SEMRAU[2];
[2]Univ. of Delaware, [1]Univ. of Delaware, Newark, DE

Abstract: Every day we make arm movements that allow us to interact with, avoid, and navigate around objects in our environment. This process requires sensory information to create a plan to avoid colliding with an object, and subsequent use of sensory feedback to divert our path when an object unexpectedly appears. We know that sensory function is commonly impacted in individuals with stroke, with over 50% of individuals with stroke having proprioception impairments. While impairments in motor execution are apparent after stroke, sensory contributions to arm dysfunction are unclear. We aimed to use an object avoidance task to understand sensory contributions to motor execution after a stroke. We predicted that individuals with stroke would take longer to initiate corrections, hit obstacles more often and use more distance to clear the obstacle when reaching without vision. We tested 8 controls and 2 individuals with stroke using an obstacle avoidance task. Participants made 20 cm reaching movements to 1 of 3 target locations with 3 different trial types: baseline (60%), static obstacle (20%), and dynamic obstacle (20%) over 225 trials. In obstacle (3 cm rectangle) trials, either a static obstacle appeared at the beginning of the trial 18 cm from a start target; or a dynamic obstacle appeared suddenly once the hand crossed a 10% speed threshold. Participants were instructed to make movements as quickly and accurately as possible without hitting the obstacle. Participants performed the task once with vision of the hand (V) and once without (NV) to examine the contributions of sensory information.Preliminary analyses of the dynamic condition revealed no differences with or without vision in controls for when timing corrections (V: 12.0 ± 6.7% NV: 12.8 ± 9.0% $p = 0.8$) or distance taken to clear the obstacle (V: 3.0 ± 1.1 cm NV: 3.2 ± 0.9 cm $p = 0.4$). As expected, controls hit the obstacle more often in the without vision condition (V: 95.8 ± 4.5% NV: 90.2 ± 6.12% $p < 0.01$). In contrast, individuals with stroke initiated corrections later (V: 4.9 ± 1.3% NV: 2.8 ± 1.9% $p < 0.01$) and used more distance to avoid the obstacle (V: 4.2 ± 1.9 cm NV: 5.57 ± 1.91 cm $p < 0.01$). Unexpectedly, individuals with stroke hit similar amounts of obstacles with and without vision (V: 95.6 ± 4.4% NV: 88.6 ± 11.36% $p = 0.5$). Overall, we observed that individuals with stroke have an impaired ability to initiate early corrective responses and make compensatory corrective responses that are significantly larger when relying predominantly on proprioception in the without vision condition. A better

understanding of the sensory contributions to impairments in motor execution after stroke will better inform rehabilitation processes.

Disclosures: D. Austin: None. **J.A. Semrau:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.02/W20

Topic: C.09.Stroke

Support: NSF Grant 1934650
University of Delaware Research Foundation Seed Grant

Title: Targeted training of proprioception using an integrated robotic-joystick approach improves sensorimotor function in chronic stroke

Authors: *D. TULIMIERI[1], G. KIM[1], F. SERGI[2], J. SEMRAU[1];
[2]Biomed. Engin., [1]Univ. of Delaware, Newark, DE

Abstract: Stroke is known to significantly impact both motor and proprioceptive function of the upper limb. Typical rehabilitation programs generally fail to treat proprioception, despite many individuals with stroke (~50%) having proprioceptive impairments. Targeted rehabilitation of proprioception is difficult, as these impairments are challenging to identify and decouple from existing motor impairments. Recent studies have demonstrated effectiveness of proprioceptive training in controls and some clinical populations, but other approaches in stroke have shown mixed results. Here, we piloted a proprioceptive training paradigm combining passive movement of the affected limb and active guidance via an integrated robot-joystick approach that minimizes existing motor confounds of the more affected limb. We hypothesized that reliance on proprioception in the absence of vision requires participants to repeatedly sample proprioception of their more-affected arm, resulting in a reduction of proprioceptive error. We predicted that individuals with stroke would show improved proprioception following a one-day training protocol.

We pilot tested three individuals with chronic stroke and two controls on a proprioceptive training paradigm using the KINARM Exoskeleton. To assess pre- and post-training proprioception and motor control, we used two previously established robotic tasks (Arm Position Matching (PM) and Visually Guided Reaching (VGR)). Participants performed 108 training trials to 3 goal targets. To quantify the effects of training, we calculated Task Scores representative of overall task performance for PM and VGR. To test our prediction, we compared changes from pre- to post-training in PM and VGR Task Scores (Δ = Post-Task Score - Pre-Task Score) within groups.

Our initial findings show that the training paradigm significantly improved proprioception in individuals with stroke (Δ PM: -0.7 ± 0.1, $p < 0.001$). Additionally, individuals with stroke had

significant improvements in motor control (Δ VGR: -1.4 ± 1.1, $p < 0.001$). These same effects were not observed in the control group (Δ PM: -0.4 ± 0.6, $p = 0.3$; Δ VGR: -0.1 ± 0.5, $p = 0.8$). The positive effects we observe for proprioceptive and motor function demonstrate that this approach is feasible for improving proprioceptive impairments after stroke. Further, this suggests that using repeated, self-guided transformations of proprioceptive information is a potential treatment avenue for rehabilitation programs.

Disclosures: **D. Tulimieri:** None. **G. Kim:** None. **F. Sergi:** None. **J. Semrau:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.03/W21

Topic: C.09.Stroke

Support: NSF 1934650

Title: Proprioceptive signaling from the upper limb influences oculomotor function and may suggest impaired multisensory integration after stroke

Authors: ***A. DECARIE**[1], D. TULIMIERI[1], T. SINGH[3], J. A. SEMRAU[2];
[2]Univ. of Delaware, [1]Univ. of Delaware, Newark, DE; [3]Pennsylvania State Univ., Pennsylvania State Univ., University Park, PA

Abstract: After stroke, many individuals (~50%) experience proprioceptive deficits in the upper limb. Typically, providing visual feedback of the upper limb during proprioceptive tasks improves performance, but it has been shown that it does not for many individuals with stroke. This may suggest problems with multisensory integration, where after stroke, people have difficulty combining visual and proprioceptive signals. Therefore, our goal was to assess multisensory integration after stroke in a proprioceptive arm and eye matching task. We hypothesized that individuals with stroke would perform worse on measures of oculomotor function compared to age-matched controls. Additionally, we hypothesized that results would be similar for oculomotor function post-stroke regardless of which arm is passively moved (i.e., more affected vs. less affected limb).
In Experiment 1, we tested 20 individuals with stroke (S1) and 20 age-matched controls (C) in an upper limb arm and eye mirror-matching task using a KINARM Exoskeleton with eye-tracking. In this task, the more affected limb was passively moved by the robot to one of 5 target locations, and the passively moved limb was randomized for controls. Individuals were then asked to mirror-match the end position of the passively moved limb with the opposite limb, or make an active eye movement (i.e., saccade). In Experiment 2, we retested 12 individuals with stroke (S2) from Experiment 1. Here, the robot moved their less affected limb, and they matched the movement with their more affected limb or their eyes. To quantify oculomotor function, we determined serval measures, including eye end point error (EEPE) and the total number of

saccades (NS) made during matching.
Results from Experiment 1 revealed that individuals with stroke had significantly greater EEPE compared to age-matched controls (S1: 11.0 ± 3.9 cm, C: 7.2 ± 2.8 cm, $p < 0.001$) and they made significantly more NS (S1: 36.3 ± 19.0 cm, C: 20.7 ± 9.7 cm, $p < 0.001$). Results from Experiment 2 showed no significant differences in EEPE when the more affected limb post-stroke was passively moved compared to the less affected limb (S1: 11.4 ± 4.5 cm, S2: 12.4 ± 5.2 cm, $p = 0.437$) and there were no significant differences in the NS (S1: 33.9 ± 14.3 cm, S2: 28.9 ± 12.1 cm, $p = 0.323$). Results from Experiment 1 and 2 suggest that proprioceptive impairments from stroke affect not only proprioceptive signals originating from the stroke affected arm, but also the less affected arm. This suggests that proprioceptive impairments from stroke may be multisensory in nature, and that upper limb proprioceptive impairments observed may be reflective of underling sensorimotor integration deficits.

Disclosures: A. Decarie: None. **D. Tulimieri:** None. **T. Singh:** None. **J.A. Semrau:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.04/W22

Topic: C.09.Stroke

Support: NS100294 to H.W.
NS123195 to J.W.

Title: Repurposing the $K_{Ca}3.1$ inhibitor senicapoc for treatment of acute ischemic stroke

Authors: *J. R. WEINSTEIN[1], R. LEE[2], Y.-J. CHEN[2], L. SINGH[2], M. ADLER-WACHTER[1], B. SCHWEITZER[1], A. MCDONOUGH[1], H. WULFF[2];
[1]Neurology/Neuroscience, Univ. Washington, Seattle, WA; [2]UC Davis, Davis, CA

Abstract: Title: Repurposing the $K_{Ca}3.1$ inhibitor senicapoc for treatment of acute ischemic stroke
Authors: Jonathan R. Weinstein, MD PhD[#]; Ruth D. Lee, PhD[¶]; Yi-Je Chen, MSc, DVM, PhD[¶]; Latika Singh, PhD[¶]; Mitzi Adler-Wachter[#]; Brendan Schweitzer[#]; Ashley McDonough, PhD[#]; Heike Wulff, PhD[¶]
Author affiliations:
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Background: Acute ischemic stroke (AIS) is a leading cause of death and long-term disability. Both microglia (MG) and macrophages (MP) are critical effector cell types in ischemic brain injury. $K_{Ca}3.1$ is a calcium-activated potassium channel that is up-regulated in reactive MG and MP. Studies using genetic deletion or pharmacological inhibition of $K_{Ca}3.1$ demonstrate this channel is critical for pro-inflammatory activation of MG/MP and exacerbation of stroke

pathophysiology. Senicapoc is a $K_{Ca}3.1$-specific inhibitor that has been used in human clinical trials for non-neurological indications and was proven safe. Here we evaluate the potential for repurposing senicapoc for AIS. **Methods:** Young adult male mice underwent 60 min middle cerebral artery occlusion (MCAO)/reperfusion. Senicapoc's pharmacokinetic (PK) profile was determined using HPLC/MS. Drug levels in plasma and brain were quantified at multiple time points. Effects of senicapoc on post-stroke release of cytokines/chemokines was determined by multiplex ELISA. Inflammatory infiltrates were quantified with immunofluorescent microscopy (IFM). Efficacy studies included: (i) infarct volume (MRI T2 and IFM), white matter integrity (MRI DTI) and longitudinal neurobehavioral outcomes (NBO). NBO studies included grid test and alternating T-maze. *In-vitro* chromogenic assay was used to assess senicapoc's effect on proteolytic activity of tissue plasminogen activator (tPA). **Results:** Administration of senicapoc (40 mg/kg, i.p.) twice daily for 7 d starting 12 h after MCAO resulted in ~55% reduction in infarct volume with corresponding improvements in NBO. Free senicapoc levels in brain ranged from 20 - 200 nM in stroked mice at 1, 4 & 12 hours post administration (exceeding senicapoc's IC_{50} (11 nM) for $K_{Ca}3.1$). Senicapoc attenuated stroke-induced: (i) infiltration of MG/MP and T-cells and (ii) up-regulation of IL-1β, TNFα and IFNγ. Senicapoc had no effect on tPA activity. **Conclusions:** We provide proof-of-concept data that senicapoc, administered in an extended temporal window, can reduce infarct volume, improve NBO and attenuate stroke-induced neuroinflammation. Senicapoc has a favorable PK profile, good CNS penetration and no effect on tPA.

Disclosures: **J.R. Weinstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-holder of United States patent No. 11,395,807 [Application #16/481, 779] granted by patent office on July 26, 2022 for use of senicapoc for treatment of stroke.. **R. Lee:** None. **Y. Chen:** None. **L. Singh:** None. **M. Adler-Wachter:** None. **B. Schweitzer:** None. **A. McDonough:** None. **H. Wulff:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.05/W23

Topic: C.09.Stroke

Support: FRANCE BED MEDICAL HOME CARE RESEARCH SUBSIDY
PUBLIC INTEREST INCORPORATED FOUNDATION

Title: Musculo-neurophysiological characteristics of voluntary muscle contraction disorders in the recovery phase from stroke

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Abstract: Muscle contraction disorders are typical sequelae of stroke patients and cause a lack of control of muscle activity on the affected side of the extremities. The efferent pathway from central commands controls muscle contraction and physical movement. However, the efferent pathway includes brain activity, pyramidal tract neurons, neuro-muscular junction, and muscle contraction; all of these components affect the ability of muscle contraction. To facilitate the recovery of this function in rehabilitation for stroke patients, it is essential to reveal the role and change of each component. In this study, we aimed to characterize the chronological recovery change of musculo-neurophiysiological function in stroke patients during the recovery phase. Ten stroke patients (The age of 50-80, 9-20 weeks after onset) participated. We attached wireless surface electromyography (sEMG; 2,222Hz) sensor on their biceps brachii to detect muscle activity. Subjects performed force control tasks after they carried out the maximum voluntary contraction(MVC). Motor unit (MU) activities were estimated using a Neuromap (Delsys Inc.). We also measured MVC Force, root mean square error (RMSE), MU mean firing rate (MFR), and MU recruitment range (Slope). Larger values of Slope indicate an abnormal recruitment style of limiting to smaller-sized MU. SIAS upper extremity scores were used as clinical assessment data. As a result, six subjects could be measured MU activity chronologically through their recovery phase. MVC Force on the affected side was lower than the less affected side at all time points, but the two subjects showed a tendency to improve gradually. The relative change in MFR on the affected side was higher than that on the less affected side at the first half of the time point, but at the later half of the time point, this relationship was reversed dependent on improving MVC Force. In contrast, the relative change in the Slope of all subjects on the affected side was higher than that on the less affected side at all the time points. These results showed that modulation of MU firing rate might be related to the recovery phase, but muscle fiber inactivation due to abnormal recruitment tending to smaller-sized MU may have long-term effects irrespective of the recovery phase. These findings may provide a fundamental contribution to rehabilitation for stroke patients and fill the gap between received motor commands from the brain and generated force. It is important to explore not only the recovery of the central nervous system but also the MU activities in detail and present rehabilitation interventions aimed at preserving or recovering their functions.

Disclosures: **M. Ito:** None. **T. Ito:** None. **T. Kokubun:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.06/W24

Topic: C.09.Stroke

Support: NIH NICHD grant: HD096071

Title: Precise measures of stroke-related impairments and reaching performance using a mechatronic device and protocol designed for use in a clinical environment.

Authors: *R. IBRAHIM[1], J. GYARMATY[2], A. ACOSTA[2], M. D. ELLIS[2];
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Abstract: High-resolution measurements of upper extremity impairment and activity limitation can be safely conducted on a mechatronic system in individuals who have had a stroke. Existing laboratory-based protocols are time-consuming, limiting application in the inpatient rehabilitation setting. The present study evaluated test-retest reliability and responsiveness to change for outcomes administered on a mechatronic device implementing a protocol that was efficiently designed to accommodate the busy inpatient rehabilitation environment. Twenty-five individuals (13 females) aged 56.4 ± 17.8 years with chronic stroke (5.37 ± 7.55 years) took part in the study that was approved by the local ethics board. Strength, shoulder/elbow flexion synergy expression, and reaching distance were evaluated in two consecutive sessions with a short break in between. Abduction and elbow extension strength was measured isometrically using a 2-DOF force and torque sensor. Thresholds of expression of flexion synergy were measured with a spring-pully-based mechatronic device and defined as the highest percent of abduction strength achieved to lift and reach targets at the beginning and end of reaching range of motion. Reaching performance was measured and defined as the distance to a standardized target near the end of reaching range of motion under 3 conditions. Test re-test reliability was evaluated with a one-way random effects model (ICC1,k). Minimal detectable change (MDC) scores were calculated. ICC's were calculated for abduction (0.963) and elbow extension (0.983) strength, flexion synergy takeover (0.919) and emergence (0.949) thresholds, reaching on the table (0.982), reaching against gravity (0.968), and reaching at 50% abduction strength (0.974). The MDC and overall mean and standard deviation were calculated for abduction (5.69Nm, 24.44 ± 10.67Nm) and elbow extension (5.66Nm, 18.53 ± 15.66Nm) strength, takeover (0.14, 0.91 ± 0.17) and emergence (0.17, 0.56 ± 0.27) thresholds, supported reaching (0.07, 0.91 ± 0.20), reaching against gravity (0.15, 0.73 ± 0.30), and reaching at 50% abduction strength (0.13, 0.69 ± 0.29). These data provide evidence of excellent test-retest reliability supporting the application of high-resolution outcomes even when limited to fewer than 10 repetition attempts per outcome due to clinical time constraints. The associated MDCs indicate the amount of change needed to be considered real. Precise measurements are critical to identifying subtle but meaningful responses to novel interventions that are presently being developed in rehabilitation medicine.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.07/W25

Topic: C.09.Stroke

Support: R01NS058784

Title: Estrous Cycle Stages and Cerebral Blood flow: Implications for Ischemic Tolerance in Stroke

Authors: *X. LIANG, H. CHEN, T. BLISS, G. STEINBERG;
Stanford Univ., Palo Alto, CA

Abstract: Background: Previous stroke studies in female rodents have demonstrated that the severity of ischemic damage is differentially impacted by the estrous cycle. Estrogen is considered to be neuroprotective, but it is unclear whether this protection is dependent on the vasodilator properties of estrogen. In this study, we investigated the impact of estrus cycle stage on stroke lesion volume and whether that impact was dependent on cerebral blood flow (CBF) changes during stroke.Method: Ischemia was induced in adult (10 weeks) male and female Sprague-Dawley rats by 30 minutes middle cerebral artery occlusion (MCAO) using the suture model. Prior to the stroke procedure, the phases of the estrous cycle (proestrus(P), estrus(E), metestrus(M) and diestrus (D)) were determined by vaginal smear tests in female rats. Cerebral blood flow (CBF) was measured by laser doppler and infarct volume were measured by TTC staining at d2 post-stroke.Result: Females in proestrus and diestrus (P/D) stages of the estrous cycle, which have reportedly high levels of estrogen, had significantly smaller CBF decreases during ischemia and a trend for smaller infarcts than those in estrous and metestrus stages, which have lower estrogen levels. This suggests that the protective effects of estrogen may be related to effects on blood flow. However, when compared to male rats, females in P/D stages had significantly smaller brain infarcts despite having similar CBF decreases. This implies that additional mechanisms are responsible for the neuroprotection observed in females when compared to males.Conclusion: Estrous cycle stage affects CBF decrease during ischemia, but this does not account for the difference in infarct size observed between male and female rats. Our study indicates that multiple mechanism are responsible for ischemic tolerance in specific stages of the estrous cycle.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.08/W26

Topic: C.09.Stroke

Support: NIH Grant R21NS121589

Title: Employing patient-derived induced pluripotent stem cells as a cellular model for studying Moyamoya Disease

Authors: *T. CHIANG[1], K. TOKAIRIN[2], Z. DEMIRAG[2], S. P. RAO[1], A. PENDHARKAR[1], M. Y. CHENG[2], G. K. STEINBERG[2];
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Abstract: Background: Moyamoya disease (MMD) is a rare, progressive cerebrovascular disease that can lead to ischemic and hemorrhagic stroke. The main histopathological characteristic in MMD is the thickening of the intimal layer that causes narrowing of the vessel, suggesting dysfunctions in the vascular smooth muscle cells (VSMCs) and/or endothelial cells (ECs). The mechanism of MMD is largely unknown, primarily due to the lack of established MMD-specific cellular or animal models. In this study, we established patient-derived cellular co-culture models using MMD iPSC-derived ECs and VSMCs, and generated a 3D vascular organoid model to more closely mimic the MMD vasculature and function. Methods: iPSCs were generated using peripheral blood mononuclear cells (PBMCs) from MMD patients or healthy controls (n=4 per group). Differentiated ECs and VSMCs were validated using cell-type specific markers and their functional characteristics were investigated in single and co-cultures. For co-cultures, ECs and VSMCs were labeled with Vibrant cell labeling dyes (DiO and DiD), followed by either transwell migration assay (ECs on bottom of cell culture plate and VSMCs on top Transwell insert) or in vitro angiogenic tube formation assay. 3D cellular organoids were generated from control and MMD iPSCs, and their cellular characteristics were examined using immunohistochemistry with antibodies labeling ECs and VSMCs. Results: While single cultures of ECs displayed comparable tube formation between control and MMD, co-cultures of ECs with VSMCs showed that MMD ECs failed to stabilize in vitro angiogeneic tubes and formed aggregates at 24hr, when compared to control ECs. Transwell migration assays showed MMD VSMCs migrated faster than control VSMCs. Immunostaining of organoids with EC and VSMC markers showed notable differences in cellular composition, with MMD organoid showing compromised integrity of vascular networks compared with controls. Conclusions: We have successfully generated in vitro co-cultures and 3D organoid cellular models for studying MMD. Our co-culture studies showed functional impairment in MMD EC/VSMC and organoid models revealed distinct appearance in their vascular networks, possibly contributing to MMD pathology. Ongoing investigations include elucidating interactions between MMD EC/VSMC using co-cultures and organoid models, as well as their transcriptome analysis. These results will provide valuable insight into the cellular and molecular mechanisms driving MMD.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

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Topic: C.09.Stroke

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Washington University McDonnell Center for Systems Neuroscience
National Institute of Health Grant R01NS125863
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Title: Pathophysiology of voluntary motor commands in patients with multiple sclerosis identified using reverse engineering of motor unit population discharge

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Abstract: Voluntary motor commands originate cortically and are shaped at all levels of the neuraxis, culminating as three types of inputs to spinal motoneurons: excitatory, inhibitory, and neuromodulatory. These components must be appropriately balanced to produced skilled motor output. Disruption of their balance has deleterious effects, evidenced by work in spinal cord injury, stroke, and aging. Multiple sclerosis (MS) lesions occur throughout the central nervous system and can therefore affect voluntary motor commands at any stage of processing. In MS, we have no knowledge about how they are disrupted, or how they relate to common motor deficits (e.g., weakness, spasms, gait difficulty). In part, this is because MS is so heterogeneous in terms of lesion locations, clinical symptoms, and disease course, impeding systematic research of neurophysiological correlates of motor dysfunction. Using high-density surface EMG decomposition and a novel paradigm for reverse engineering of motor unit population discharge, we can feasibly estimate aspects of excitatory, inhibitory, and neuromodulatory components of the voluntary motor command on a person-specific basis. Our ongoing study is characterizing these components in MS to gain insight into the heterogeneous neural mechanisms of motor deficits. We tested 5 ambulatory patients with MS (female, mean age 48) with mild disability. 4 had weakness, mild-moderate sensory deficits, and spasticity and/or spasms. The fifth had a normal clinical motor exam. We recorded high-density surface EMG from tibialis anterior (TA) and soleus (SOL) during isometric plantarflexion and dorsiflexion, performed as slow triangle contractions to a target of 20% maximum voluntary torque. EMG was decomposed into motor unit spike trains using blind source separation. We calculated a number of motor unit variables, most notably delta-F, which estimates motoneuron excitability and the balance of neuromodulatory and inhibitory inputs. There were consistent differences in MS patients vs. controls. For TA, values were decreased for delta-F (3.9 vs. 5.9 pps), initial firing rate acceleration (5.8 vs. 7.1 pps), firing rate range (9.3 vs. 11.9 pps), and max firing rate (12.3 vs. 15.0 pps). SOL had more modest decreases in delta-F (3.0 vs. 3.8 pps) and firing rate range (4.8 vs. 5.6 pps). Self-sustained firing was longer for MS patients. Within a patient, abnormalities in motor unit variables were not consistent across muscles and legs. Interestingly, there were several abnormalities in the patient with a normal clinical motor exam, indicating that perhaps our measures are sensitive to subclinical changes in processing of voluntary motor commands.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.10/W28

Topic: C.09.Stroke

Support: JSPS KAKENHI Grant Number 20K19393

Title: Distinct recovery processes for quantity and quality of reaching function in people with moderate-to-severe hemiparesis after stroke

Authors: *S. UEHARA[1], A. YUASA[2], K. USHIZAWA[2], Y. OTAKA[2];
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Abstract: Post-stroke individuals with severe upper-limb hemiparesis often have difficulty performing goal-directed functional movements or even gross movements. A classic work has described the time course of motor recovery after stroke, from the acquisition of movement capacity (quantity of movement) to the restoration of dexterity control (quality of movement). However, it remains unclear whether the improvement in dexterity control parallels the improvement in movement capacity during the recovery process. Here, we sought to address this question by examining arm-reaching performance in individuals with moderate-to-severe hemiparesis in the post-stroke subacute phase (< 6 months after onset). We asked post-stroke participants to perform horizontal reaching in eight directions with their affected and unaffected side using an exoskeleton robotic device at the time of hospital admission and discharge. In this task, the participants were required to quickly and accurately make a reaching movement from a centrally located visual target to one of eight peripheral targets distributed uniformly on the circumference of a circle at 10 cm from the central target by moving the shoulder and elbow. We quantified the movement capacity and dexterity control by calculating the total amount of hand position displacement and the proportion of target-directed movements, an index of how accurately the hand position was directed toward a target, during reaching, respectively. We found that the total amount of displacement of reaching, especially the movement toward the anteroposterior direction, increased significantly during the recovery process, while the proportion of target-directed movements did not show substantial changes. These results indicate that, in arm-reaching performance, the improvement in movement capacity is not necessarily

accompanied by the improvement in dexterity control, suggesting distinct recovery processes for the quantity and quality of arm-reaching performance after stroke.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.11/Web Only

Topic: C.09.Stroke

Support: NIDILRR

Title: Multi-joint assessment of proprioception impairments at upper limb post stroke

Authors: D. XU[1], R. BAGHI[1], K. KOH[2], G. OPPIZZI[2], S. RAO[1], G. KEHS[3], R. BRAUN[3], *L.-Q. ZHANG[1];
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Abstract: Lesions of the central nervous system in stroke survivors may cause loss of the somatosensory function but there has been a lack of characterization of upper extremity multi-joint proprioception deficits in stroke survivors. Daily activities of human motion involve multiple joint coordination in the upper extremity, examination and treatment of multiple-joint proprioception are important. **The objective** of this study was to examine proprioception impairments at the shoulder, elbow, and wrist joints of stroke survivors using a multi-joint robotic device. **Methods:** fifty-three stroke survivors, mean (SD): 55.3 (12.9) years old and 24 healthy controls; mean (SD): 47.4 (17.2) years old, participated in this study. Each participant was seated with the initial position of 70 deg shoulder horizontal adduction, 60 deg elbow flexion, and 0 deg wrist flexion. One of the three joints was randomly selected and moved slowly at 0.5 deg/s inward or outward by the robot, with a total of 18 trials (3 joints, 2 directions/joint, 3 repetitions/condition). The participant was asked to close eyes during testing and press a handheld switch as soon as he/she felt movement of a joint and report which joint was moved and in which movement direction. Proprioceptive acuity was measured using the threshold detection of passive motion. **Results**: The results from this study included comparisons of changes in the threshold of proprioceptive acuity, response errors, and deficit of somatic sensation between the two groups. There was significant impairment of proprioceptive acuity with larger threshold angles in the stroke survivors compared to healthy controls across the joints. Specifically, the threshold angles of outward motion were 7.18±5.39, 6.55±5.15, 9.07±7.33 in stroke survivors vs. 2.09±0.77, 1.94±0.4.9, 1.90±0.56 in healthy controls for the shoulder, elbow, and wrist respectively [$F(1, 73) = 15.3241$, $p = 0.001$]. The threshold angles of inward motion were 7.32±4.68, 6.83±5.7; 8.03±5.73 in stroke vs. 1.99±0.63, 1.90±0.56; 2.18±0.69 in healthy controls for the shoulder, elbow, wrist respectively [$F(1,73) = 38.807$, $p =$

0.001]. Stroke survivors had significantly more errors in detecting the joint motion than the healthy group ($P < 0.001$). **Conclusions:** Significant changes of proprioception acuity in stroke survivors were identified across the multiple joints of the upper extremity. It provided a quantitative evaluation of proprioception during multi-joint robot-controlled movement. The quantitative evaluation of deficits in multi-joint proprioception provides guidance for impairment-specific sensory-motor rehabilitation in stroke survivors.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.12/X1

Topic: C.09.Stroke

Support: NIH Award 1 R03 HD099426-01A1
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Title: One joint is not enough: interaction torque between hip and knee explains reduced knee flexion after stroke

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Abstract: Hip circumduction is a typical compensatory post-stroke gait pattern that uses hip abduction to ensure foot clearance during swing. This pathological pattern is required to overcome reduced knee flexion. One possible cause of altered knee kinematics is the weakness of hip flexors. Here, we compared the patterns of torque generation between healthy adults and those after stroke. We collected full-body overground locomotor kinematics in stroke survivors and age-matched controls (Ncontrol=20, Nstroke=20, 603 swing phases). Using a previously validated dynamic model of bilateral limb dynamics (7 DOFs), we solved inverse dynamics to compute inertial, interaction, and gravitational torques at the hip, knee, and ankle joints. We found that knee inertial torque at the onset of swing was not different between the two groups. As expected, hip flexors generating inertial torque were weakened after the stroke. The post-stroke knee pathology was due to the decrease in the intersegmental inertial torque originating at the hip and producing only 66.8 ± 21.9% of the control peak-to-peak torque range. This means

that reduced energy transfer from the hip during swing initiation led to reduced knee flexion, despite normal muscle-generated inertial knee torque. This suggests that rehabilitation focusing on improving intersegmental coordination and enhancing hip force-generating capacity can improve gait in post-stroke individuals.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.13/X2

Topic: C.09.Stroke

Support: NIH grant 5R01HD059783-08

Title: Functional independence is predicted by ipsilesional arm strength and dexterity, and this relationship is not moderated by apraxia, laterality of hemispheric damage, or contralesional impairment in stroke survivors with severe or severe-moderate paresis

Authors: *C. MAENZA[1,3], N. M. KITCHEN[1], T. E. MURPHY[2], C. WINSTEIN[4], L. SHANKAR[1,5], R. SAINBURG[1,3];
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Abstract: We previously reported that chronic unilateral stroke survivors with mild, moderate or severe contralesional impairment had motor deficits in their less-affected, ipsilesional arm that were specific to the hemisphere of damage, and varied with contralesional impairment severity. However, previous research has been unable to disentangle the potentially moderating effects of additional factors - such as apraxia, laterality of hemispheric damage, and severity of contralesional impairment - on this association, specifically in stroke survivors with severe or severe-moderate paresis. We hypothesized that severe or severe-moderate deficits in the contralesional limb would lead to greater reliance on the ipsilesional arm for activities of daily living and, as a result, functional independence should be substantially impacted by ipsilesional arm strength and dexterity. We predicted that functional independence should depend on ipsilesional arm strength and dexterity regardless of 3 potential moderators: (1) apraxia, (2) laterality of hemispheric damage, and (3) severity of contralesional impairment (Upper Extremity Fugl-Meyer < 15= severe; 16-34= severe-moderate). Linear regressions of functional independence (Barthel Index) on either ipsilesional dexterity (Jebsen-Taylor Hand Function Test) or ipsilesional grip strength were performed separately with each of the 3 categorical covariates of interest: (1) apraxia (yes/no), (2) laterality of hemispheric damage (left/right hemisphere), and (3) severity of contralesional impairment (severe/severe-moderate) in 72

chronic stroke survivors. Our current findings confirm that in this population of stroke survivors functional independence is associated with ipsilesional arm strength and dexterity, and that this dependence is not moderated by apraxia, laterality of hemispheric damage, or contralesional impairment among those with severe or severe-moderate impairment. These findings indicate that ipsilesional arm strength and dexterity should be primary targets for evaluation and treatment in interventions based on physical rehabilitation (PT & OT) in patients with severe contralesional paresis.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.14/X3

Topic: C.09.Stroke

Support: JSPS KAKENHI Grant Number JP21K11323

Title: Impact of entopeduncular nucleus on motor dysfunction and recovery in the rat model of brain stroke

Authors: *R. SAKAI[1,2], K. MURATA[2], K. KURODA[2], T. RYOKE[2], A. MAEGAWA[2], A. IDEGUCHI[1], Y. FUKAZAWA[2];
[1]Fukui Hlth. Sci. Univ., Fukui, Japan; [2]Univ. of Fukui, Eiheiji, Japan

Abstract: Intracerebral hemorrhage (ICH) is a prevalent clinical condition and animal models of ICH have been widely used to study its neuropathology and to develop treatments. However, the influence of the size and brain region, especially the entopeduncular nucleus (EP), of the injury on the impairment of motor function and its recovery is still elusive. Thus, we performed experimental ICH in the rat internal capsule (IC) and found the involvement of EP in the severity of motor deficits and recovery. We, then, examined the effect of the EP lesion alone by microinjection of kainic acid (KA) into EP on the motor function and found that the motor dysfunction is mild and recoverable. To induce ICH, collagenase type IV was stereotactically injected into the unilateral IC of 11 Long-Evans rats. We found the hemorrhagic tissue in four regions, IC, dorsomedial and ventrolateral regions adjacent to the IC, and EP by histological investigation with hematoxylin-eosin staining and measured the volume of the damaged regions

in individual rats. We also assessed forelimb motor function using “single pellet reaching task test” before and 2, 7, 14, 21, and 28 days after the enzyme injection. All rats exhibited a significant decline in motor function on postoperative day 2. The extent of recovery varied among rats and 6 rats showed recovery at the postoperative day 28 while 5 rats showed no or slight recovery until day 28. Analysis of the forelimb reaching process revealed that preoperative rats primarily failed at grasping of food pellets, while rats at postoperative day 28 failed by the inappropriate trajectory of the forelimb movement to the food pellets. The success rate of the forelimb reaching task on day 28 was found to be significantly correlated with the residual volume of the EP ($p = .0031$, $r^2 = .6394$, Pearson's correlation coefficient), suggesting the importance of EP for motor recovery. To directly evaluate the impact of the EP damage on the motor dysfunction and recovery, we injected KA into EP unilaterally. The damaged area by KA was histologically confirmed by Nissl staining and immunohistochemical staining for NeuN, confirming selective lesion to the EP. Forelimb movement was assessed as in the ICH experiment. We observed that the EP lesion resulted in motor impairment at postoperative day 2 and 7, followed by recovery of motor function by day 28. These results suggest that EP damage is involved in motor dysfunction in our stroke model. We note that the differences in the recovery process between the EP injury caused by ICH and excitotoxic lesion.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

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Title: Motor unit population behavior of involuntary muscle spasms during voluntary contractions in a patient with multiple sclerosis

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Abstract: People with multiple sclerosis (MS) can have lesions throughout the brain and spinal cord and experience a wide range of sensorimotor deficits. Some people with MS have

sensorimotor features typically found in people with spinal cord injury (SCI) (weakness, spasms, neuropathic pain). Our knowledge about pathophysiological contributors to these symptoms in SCI is growing, but we know little about them in MS. Here we provide insight with a case study of a female in her 50s with MS, as we serendipitously captured the motor unit population discharge of unexpected muscle spasms during voluntary contractions. In the legs, the patient had 5/5 strength, diminished vibratory sense, and absent DTRs at S1-S2. Lesions were in subcortical brain areas, brainstem, cerebellum, and cervical and thoracic spinal cord. She was active, regularly running 5 miles. Her most bothersome symptoms were spasms and burning parasthesias. We recorded high-density surface EMG from tibialis anterior (TA) and soleus during isometric plantarflexion (PF) and dorsiflexion (DF). She performed both steady hold and slow triangle contractions to 20% of maximum. EMG was decomposed into motor unit spike trains using a convolutive blind source separation algorithm, resulting in 30-40 motor units. During steady contractions on the right, spasms occurred in TA during DF and PF. In SCI, spasms are mediated in part by unregulated persistent inward currents (PICs) that markedly increase motoneuron excitability, so we expected to see recruitment of new motor units during the spasm with self-sustained firing after the spasm. Instead, during spasms when TA was the agonist, already recruited motor units fired in an ON/OFF pattern, with 3-5 discharges during ON followed by an OFF of 100-200 ms. With each successive ON/OFF, the ON discharge rate and the OFF duration decreased, suggesting a strong damped oscillatory input to the motoneuron pool. Estimated PIC amplitude during triangle contractions was somewhat decreased in TA vs. controls. When TA was the antagonist, soleus motor units responded to the spasm differentially, some increasing and some decreasing their firing rate. On the left, spasms were in soleus during DF. The patient's relatively intact strength and lack of substantially different PICs is consistent with a reasonably balanced, if a little low, level of neuromodulation. Findings seem most consistent with disruption of spinal inhibitory mechanisms governing afferent feedback to motoneurons, perhaps driven by lesions in corticospinal projections onto spinal GABAergic interneurons gating sensory flow or via spinal lesions directly impacting the balance of spinal excitation and inhibition.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.16/X5

Topic: C.09.Stroke

Support: 5T32HD101395-02
1R01NS105759-01A1

Title: Quantifying the Effect of Trunk Postural Control Deficits on Reaching in Hemiparetic Stroke

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Abstract: The trunk provides a stable base of support to facilitate upper limb interaction with the environment. Post hemiparetic stroke, damage to descending corticospinal pathways prevents performance of activities of daily living including reaching. Yet, the impact of a stroke on coordinated trunk and arm reaching has been largely unexplored, particularly in the context of the flexion synergy (involuntary flexion of the elbow, wrist, and fingers during shoulder abduction). The goal of this study is to examine reaching post stroke when the trunk is unrestrained, more closely resembling daily life. We hypothesize that deficits already present due to the flexion synergy will be exacerbated when the trunk is unrestrained. 9 individuals with hemiparetic stroke (64.11 ± 6.57 years old; Fugl-Meyer Assessment (FMA) 7-42/66; Reaching Performance Scale (RPS) 0-13/36; and Trunk Impairment Scale (TIS) 12-16/23); and 4 age-matched controls (66.25 ± 0.96 years old) participated in the study. The trunk and arm were instrumented with motion capture markers to quantify arm kinematics off line. Individuals sat in a Biodex chair with the trunk either restrained or unrestrained. Participants were asked to reach while their arm was fixed to a robotic device that can generate a frictionless table environment and impose downward forces equal to 25% or 50% of their maximum shoulder abduction force. Reaching distance (RD) was computed as the 3D distance between the 3rd metacarpophalangeal joint and the glenohumeral joint (Meskers, 1998) at the end of the reach and normalized to limb length. A two-way repeated measures ANOVA was used to assess the effect of load and restraint on RD. The relationship between RD and the clinical measures was evaluated using linear regression.For the paretic arm, RD decreased with loading (p=0.01). Trunk restraint only affected the loaded conditions, with a significant reduction in RD (25%: 75.42±17.79 rest. vs 72.57±16.95 unrest. p=0.05 and 50%: 75.12±18.61 rest. vs 72.72±16.95 unrest. p=0.03). Trunk restraint reduced RD for the non-paretic arm (p=0.006). Neither load nor restraint affected RD in controls. RD was correlated with FMA (unrestrained: p=0.02 and restrained: p=0.05) and RPS (only restrained: p = 0.03). Consistent with previous studies, flexion synergy is the major impairment affecting reaching, reflected in reduced reaching distance with increasing shoulder load. The trunk played a significant role in reaching for both the paretic and non-paretic limbs, however only in loaded conditions. Future work will further examine the relationship between the trunk and flexion synergy based on the trunk and arm muscle activation patterns.

Disclosures: K.C. Suvada: None. **J. Deol:** None. **J.P. Dewald:** None. **A. Acosta:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

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Topic: C.09.Stroke

Support: the Research Program funded by Korea Disease Control and Prevention Agency (3300-3334-300-260-00, 2013-E33017-00, 2013E-33017-01, 2013E-33017-02, 2016-E33003-00, 2016-E33003-01, 2016-E33003-02, 2019-E3202-00, 2019-E3202-01, 2019-E3202-02, 2022-11-006

Title: The Effect of Age on Motor Recovery after First-Ever Ischemic Stroke: How and How much

Authors: *H. LEE[1], M. SOHN[3], J. LEE[2], D. KIM[4], Y.-I. SHIN[5], G.-J. OH[6], Y.-S. LEE[7], M. JOO[8], S. LEE[9], M.-K. SONG[10], J. HAN[11], J. AHN[12], Y.-H. LEE[8], Y.-H. KIM[13], W. CHANG[14]; [2]Rehabil., [1]Konkuk Univ. Med. Ctr., Seoul, Korea, Republic of; [3]Rehabil. medicine, Chungnam Natl. Univ. Hosp., Dae-jeon, Korea, Republic of; [4]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [5]Rehabil. Med., Yangsan Pusan Natl. Univ. Hosp., Yangsan, Korea, Republic of; [6]Wonkwang University, Sch. of Med., Iksan, Korea, Republic of; [7]Kyungpook Natl. Univ. Sch. of Med., Daegu, Korea, Republic of; [8]Wonkwang Univ. Sch. of Med., Iksan, Korea, Republic of; [9]Jeju Natl. Univ. Hospital, Jeju Natl. Univ. Sch. of Med., Jeju City, Korea, Republic of; [10]Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of; [11]Hallym Univ., Chuncheon, Korea, Republic of; [12]Ewha Womans Univ., Seoul, Korea, Republic of; [13]Sungkwunkwan Univ., Seoul, Korea, Republic of; [14]Samsung Med. Ctr., Seoul, Korea, Republic of

Abstract: In this study, we aimed to demonstrate the effect size of age on motor function after ischemic stroke by elucidating how age affect motor function through Directed acyclic graph framework.

We reviewed clinical data of patients enrolled in the Korean Stroke Cohort for Functioning and Rehabilitation between August 2012 and January 2021. We identified potential confounders and mediators through a rigorous literature review, which were then incorporated in a directed acyclic graph. To examine the association between age at stroke onset and Fugl–Meyer Assessment of Upper Extremity score (FMAUE) or Fugl–Meyer Assessment of Lower Extremity score (FMALE) at 3 time points (1 week, 3 months, and 6 months), we performed longitudinal analyses with repeated measures of FMAUE (or FMALE) using mixed-effects models with random slopes and intercepts for each individual. For multi-strata of age, we estimated multinomial logistic regression model-based propensity scores, which results in balancing the confounders of other strata with youngest strata. The inverse of the calculated propensity score was used as the weight, the IPTW to estimate the average treatment effect. We used 50 imputed data sets to perform IPTW analyses after performing multiple imputation using iterative chained equations to impute missing covariates and outcomes.

We enrolled 6,282 ischemic stroke patients. Our DAG suggested that minimally sufficient adjustment sets for estimating the total effect of age at stroke onset on motor function are sex, body mass index, premorbid modified Rankin Scale, risk factors for stroke, comorbidities, smoking, alcohol consumption, family history, and level of education as cognitive reserve proxy,

which acts as the confounding variables. In the adjusted model, we found that the age strata with ≥75y have significant differences of FMAUE up to post stroke 6 months comparing the age stratum with <55y. We also found that the age strata with ≥65y have significant difference of FMALE up to post stroke 6 months comparing the age stratum with <55y. After balancing the potential confounders using propensity scores to perform IPTW analyses, all the SMDs of the confounders among the age strata were <0.1. FMAUE and FMALE at 1 week, 3 months, and 6 months post stroke in all the age strata were lower than those of control (age at stroke onset <65) with statistical significance, except for the age strata '65 - 74'.
We developed the novel framework that can identify the effects of specific factors on motor recovery by comprehensively including various variables that are known or estimated to be related to motor recovery after stroke.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.18/X7

Topic: C.09.Stroke

Support: American Heart Association grant no. 17SDG33690002

Title: Association between task-related changes in resting state functional connectivity and motor recovery in chronic post-stroke individuals

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Abstract: Stroke is a heterogeneous disease with significant variability across individuals in response to rehabilitation programs. As such, current clinical measures fail to accurately predict an individual's response to a specific intervention. In this work, we test the hypothesis that one's capacity to form new functional connections in the brain after performing a motor task, measured via changes in resting state functional magnetic resonance imaging (rs-fMRI), is indicative of their potential for motor recovery. Fourteen chronic post-stroke individuals (5 M; aged 66.07 ± 11.31) with a neuroimaging-confirmed diagnosis of subcortical stroke resulting in upper extremity impairment were recruited. All individuals were screened to have residual active range of motion necessary to complete the motor task. The protocol consisted of twelve training sessions carried out over four weeks, where training tasks focused on the upper extremity, such as picking up coins or tying shoelaces. Motor function was assessed via the Upper Extremity Fugl-Meyer (UE-FM) at baseline and after training. Before training, participants underwent a

neuroimaging session, which consisted of an initial rs-fMRI scan, a task fMRI scan, a second rs-fMRI scan and standard anatomical scans. During the task, participants moved an MR-compatible joystick with their affected hand in a center-out reaching pattern to cued targets. Resting-state functional connectivity (rsFC) was quantified between nine regions of interest (ROI) in a cortico-thalamic-cerebellar network, consistent with our previous work on healthy subjects, and computed as the Fisher-transformed correlation coefficient of the average rs-fMRI BOLD signal measured before and after task execution. Linear models were built to analyze the relationship between rsFC in each ROI pair before and after task execution and participants' ΔUE-FM score. The model that best explained the variance in the ΔUE-FM scores included the ipsilesional S1 - CB8 pair (R^2=0.63, p=0.015). We utilized lasso regression to establish a single predictive model accounting for rsFC in all ROI pairs. Change in rsFC between ipsilesional S1 - CB8 was the most predictive term that remained after the penalized regression. However, after accounting for the many predictor variables, this term was not significant. When restricting the analysis to the ipsilesional cortex and the cerebellum, a few significant terms emerge: ipsilesional S1 - CB8, REST2-1 (p=0.002) and ipsilesional M1 - CB6, REST1 (p=0.001). These results suggest that task-induced changes in cortical-cerebellar connectivity may be a relevant predictive factor of upper extremity motor recovery after stroke.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.19/X8

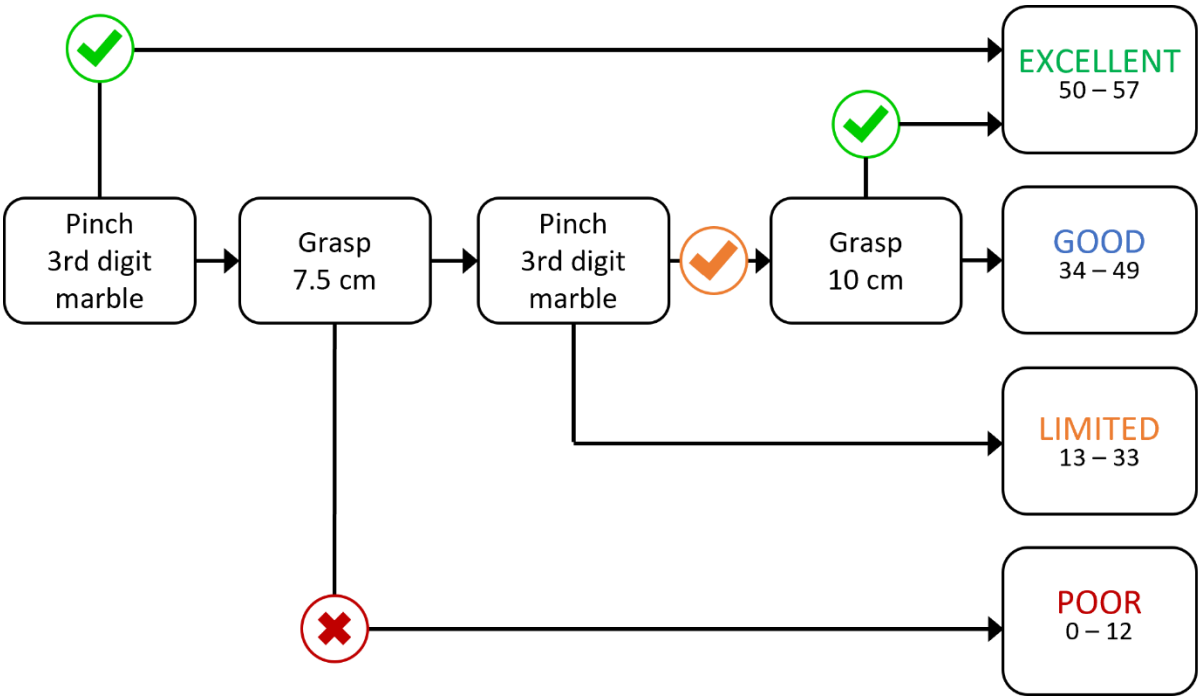
Topic: C.09.Stroke

Title: Accuracy and reliability of remote categorisation of upper limb outcome after stroke

Authors: *H. JORDAN, C. M. STINEAR;
Univ. of Auckland, Auckland, New Zealand

Abstract: Background There is a growing interest in motor assessments after stroke that can be performed quickly and remotely. The Fast Outcome Categorisation of the Upper Limb after Stroke-4 (FOCUS-4) assessment classifies upper limb functional outcome remotely and was developed via a retrospective analysis of Action Research Arm Test (ARAT) scores. The FOCUS-4 assessment includes three tasks from the ARAT that are performed during a videocall, and it classifies upper limb functional capacity into one of four outcome categories that correspond to a range of ARAT scores. The FOCUS-4 assessment has yet to be prospectively validated. **Aims** This study aimed to evaluate the accuracy and reliability of the remote FOCUS-4 assessment for categorizing upper limb functional outcome after stroke when administered and scored during a videocall compared to an in-person ARAT. **Methods** Data were collected from 26 participants at three months post-stroke (3M), 27 participants at six months post-stroke (6M),

and 51 participants at the chronic stage of stroke (> 6M). Participants performed a remote FOCUS-4 assessment during a videocall and an in-person ARAT, and FOCUS-4 assessment accuracy was evaluated by comparing the upper limb outcome from both assessments. Participants at the chronic stage of stroke also performed a second remote FOCUS-4 assessment to assess reliability. Items needed to perform the FOCUS-4 assessment were mailed to participants and the videocalls were not recorded. **Results** Overall accuracy of the remote FOCUS-4 assessment was 88% at 3M, 96% at 6M, and 78% at the chronic stage of stroke. Reliability at the chronic stage of stroke was 84%. The FOCUS-4 assessment was most accurate and reliable for participants with mild or severe upper limb functional impairment. **Conclusions** The FOCUS-4 assessment has potential for categorizing upper limb outcome after stroke but external validation is required. The remote FOCUS-4 assessment could be used as an alternative to the in-person ARAT for classifying upper limb functional capacity or to screen potential participants for stroke trials.



Disclosures: **H. Jordan:** None. **C.M. Stinear:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.20/Web Only

Topic: C.09.Stroke

Title: Modularity organization of dense EEG brain connectivity predicts motor recovery after stroke

Authors: *M. SEDGHIZADEH, H. AGHAJAN;
Sharif Univ. of Technol., Tehran, Iran, Islamic Republic of

Abstract: This study proposes indicators of the brain's functional connectivity measured at hospital admission after stroke as predictors of early motor recovery, and highlights the importance of the integrity of local brain networks in effective motor rehabilitation after stroke.The analysis was conducted on a dataset published in [1]. Three minutes of resting state 256 channel EEG data were recorded at admission time for 27 individuals who had experienced a stroke. The Functional Independence Measurement (FIM) motor score was recorded at admission and discharge and was used as indicator of motor recovery.In our analysis, Phase Locking Values (PLV) were calculated in the alpha band for all pairs of EEG channels as a measure of the brain's functional connectivity, and a graph was constructed for each participant. The average of all PLVs was used as a threshold to build a binary graph. In the resulting graph, each node represents an EEG channel, and the presence of an edge between a pair of nodes indicates a PLV higher than the threshold. Three graph features, namely the average Clustering Coefficient (CC), Small Worldness (SW), and Global Efficiency (GE) were extracted from each participant's graph. The average CC measures the modular organization of the brain's network connectivity as it represents the average number of connected node groups. The SW feature is similar to average CC but it also considers the path length. However, GE only takes the path length into account. Significant correlations were observed between each of the average CC and SW with the rate of improvement in the FIM score, exhibiting correlation coefficients of about 0.61 (both with p-values < 0.01), while no noticeable correlation was found between GE and the FIM score's rate of improvement (Fig. 1).The findings suggest that the connectivity within local networks of the brain may play a more critical role in early motor recovery prognosis than the connectivity between distant regions. **References**[1] Cassidy, Jessica M., et al. "Coherent neural oscillations inform early stroke motor recovery." Human Brain Mapping 42.17 (2021).

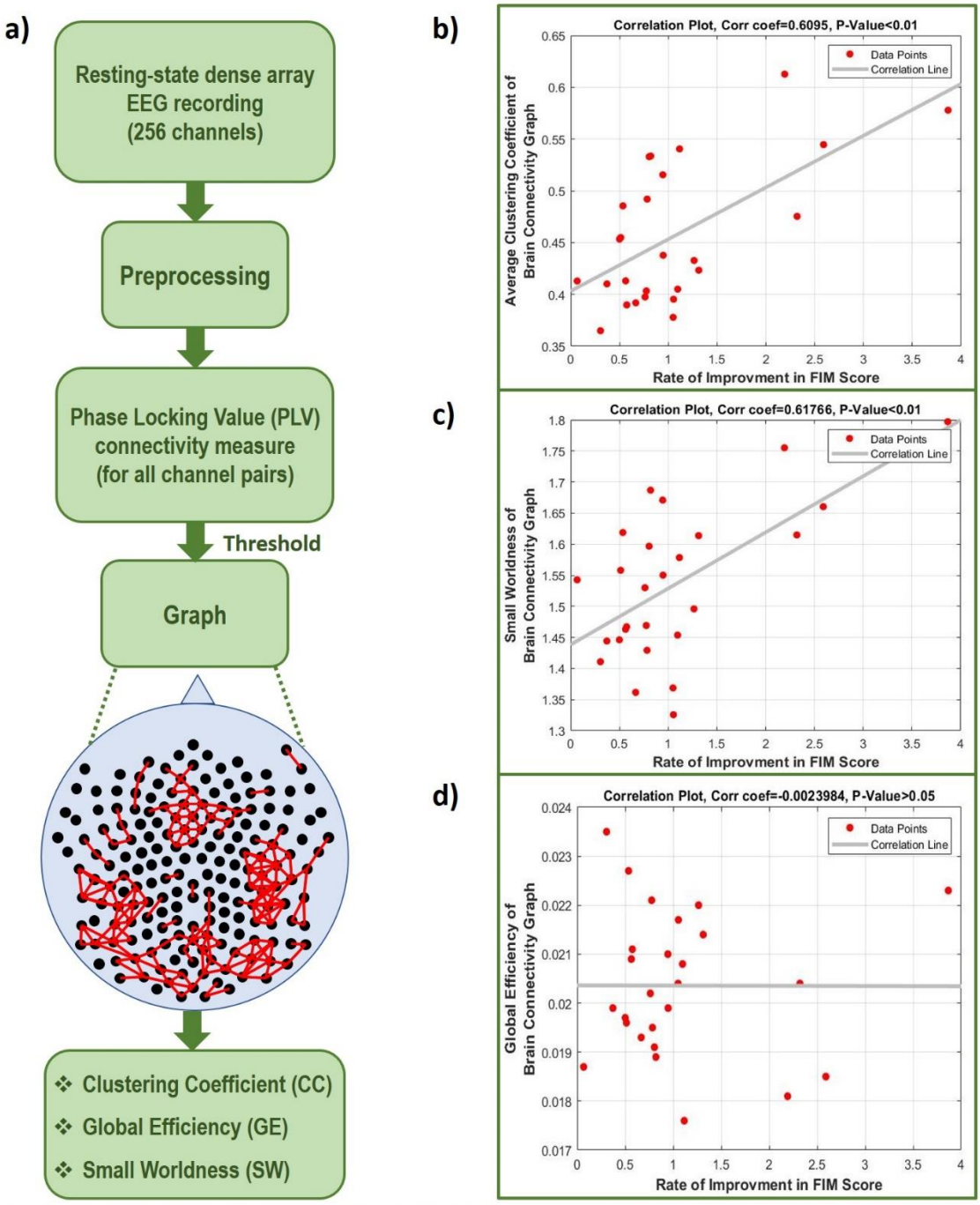


Figure 1: a) Overview of data processing methodology. CC, SW, and GE features are extracted from the brain connectivity graph constructed from PLV analysis of EEG channel pairs. b, c, d) Correlation of average CC, SW, and GE, respectively, with the admission to discharge rate of improvement in the FIM score.

Disclosures: M. Sedghizadeh: None. **H. Aghajan:** None.

Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.21/X9

Topic: C.08. Ischemia

Support: R01NS105646
R01NS123378
R01NS117568
P50HD105353
1R01NS105 646-01A1
RC1MH090912-01
T32GM008692
UL1TR000427
K23NS086852
T32EB011434
R01EB000856-06
R01 EB009103-01
N660 01-12-C-4025
N66001-11-1-4013
1T32EB011434-01A1

Title: Bci-derived clinically relevant post-stroke motor improvement will not be a one-sized fits all model

Authors: *A. B. REMSIK[1], P. L. VAN KAN[2], N. ADLURU[3], V. A. NAIR[4], V. PRABHAKARAN[1];
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Abstract: Introduction: Stroke can cause persistent upper extremity motor impairment limiting quality of life for survivors. Current therapeutics lack solid evidence of usefulness. Novel therapeutic BCIs that utilize operant conditioning and neuroplasticity as a therapeutic mechanism deliver marginally superior clinically meaningful improvements but are not used in standard practice for which clearer evidence of patient fit and dosing evidence are lacking. Research shows a correlation between patient factors - like task acquisition and handedness or age, lesion location and volume, time since stroke, etc. - and recovery potential with or without BCI intervention. This sub analysis of an ongoing study seeks to determine whether patient factors at baseline will impact recovery. **Methods**: All participants in these parallel analyses completed all aspects of the study protocol and up to 30 hours of BCI intervention. Group mean changes in outcome measures following intervention were analyzed using two sample and paired sample t-tests, where appropriate. 34 participants met inclusion criteria (62.082 years, +/- 12.95, 11 R impaired). **Results:** At cohort level, there was a positive improvement in primary outcome measures by end of BCI intervention (those over 60 yrs averaged improvement of 1.33 and those under 0.421). Two sample t-test yielded insignificant differences between group mean changes

(p = 0.579). For the 9HPT, those with non-concordant strokes realized greater motor gains compared to the concordant group (+2.4sec vs. +0.5sec) and the group mean change between conditions was not significant (t(19.45) = -0.904 , p = 0.811). Grip strength improvement was greater for chronic stroke group (+6.879 pounds/inch^2, + 5.567 pounds/inch^2). Both groups grip strength gains over intervention were significantly different (chronic group t(11) = 2.422, p = 0.0359, and non-chronic group t(5) = 1.0697, p = 0.0359), respectively but the differences between these group means were not significant (t(16) = 1.0697, p = 0.345). **Discussion and Conclusion:** In these samples, expectations for motor performance metric changes associated with participant factors violated expected hypotheses. Participant factors will determine clinical efficacy of non-invasive BCIs.

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Poster

PSTR538. Stroke, Damage, or Disease: Mechanisms of Abnormal Movement

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR538.22/X10

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Title: Exploring the Impact of Concurrent Transcranial Direct Current Stimulation (tDCS) and Functional Magnetic Resonance Imaging (fMRI) on Chronic Stroke Survivors: Enhancing Upper Extremity Function and Unveiling Connectivity Patterns

Authors: *C. A. SALAZAR[1], N. C. ROWLAND[2];
[1]Dept. of Neurosurg., Med. Univ. of South Carolina (MU Neurosci. Inst. - Grad., Charleston, SC;
[2]Dept. of Neurosurg., Med. Univ. of South Carolina, Charleston, SC

Abstract: Background: Transcranial direct current stimulation (tDCS) can be an effective therapy for chronic stroke survivors. However, the variability in patient response to tDCS interventions remains a critical concern. Motor fiber connectivity is known to be disrupted in unique patterns following stroke, which might explain response heterogeneity to tDCS. Despite this, most tDCS studies do not involve concurrent neuroimaging that could reveal important changes in motor cortical networks. In this study, we proposed an innovative approach of administering tDCS simultaneously during MRI acquisition. **Study Objective:** By administering a 10-minute tDCS session, we aimed to create a protocol that aligns with common fMRI sequence durations. We also performed the Fugl-Meyer Assessment Upper Extremity (FMA-UE) before and after tDCS stimulation as well as a finger tapping task during stimulation. The impact of a single tDCS session on upper extremity motor function as well as the effect on connectivity patterns were examined. We hypothesized that tDCS motor response can be predicted by observing changes in functional magnetic resonance imaging (fMRI) connectivity patterns. **Methods**: Chronic stroke survivors (n=19) and healthy controls (n=20) were recruited. MRI acquisitions included structural, diffusion, and functional sequences. Anodal tDCS (2mA)

was administered for 10 minutes using a bihemispheric montage. All participants were blinded and 1:1 randomized to tDCS delivery (stim vs sham). **Results**: Chronic stroke survivors randomized to stimulation demonstrated a significant increase in FMA-UE score from pre- to post-stimulation (p=0.0005). This increase was not observed in the stroke sham group. Moreover, when examining seed-to-seed based connectivity in the stroke group post stimulation, a significant increase in connectivity was observed between the left precentral gyrus and bilateral sensorimotor network (SMN). This increase was significantly correlated with improvements in the pre FMA-UE score for the affected limb (left precentral to left SMN: p=0.0316; left precentral to right SMN: p=0.0322). **Implication**: The preliminary findings demonstrate the utility of concurrent fMRI and tDCS to reveal significant changes in connectivity patterns within the precentral gyrus and sensorimotor networks. This novel protocol holds promising implications for the development of more effective stroke rehabilitation protocols by leveraging the understanding of connectivity dynamics influenced by tDCS.

Disclosures: C.A. Salazar: None. **N.C. Rowland:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.01/X11

Topic: C.10. Brain Injury and Trauma

Support: NIH NS043277

Title: Proinflammatory microglial activation leads to ADAM17-mediated cleavage of CSF1R and prevents glial scar formation

Authors: *D. HERNÁNDEZ ESPINOSA[1], A. THATHIAH[2], E. AIZENMAN[2];
[1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Neurobio., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Background: Glial scar formation is a critical component of the repair process of damaged tissue within the nervous system. To achieve morphological and functional recovery, a coordinated response from microglia and astrocytes is required. Although proinflammatory microglia activation has been linked to an inadequate tissue repair response, the precise molecular mechanisms underlying this association remain elusive. Methods: In this study, we explore the impact of proinflammatory microglia activation on glial scar formation using an in vitro model of mechanical injury in mixed rat neuronal-glial cortical cultures. Immediately following mechanical damage, the cultures were treated with lipopolysaccharide (LPS) and interferon-gamma (IFNγ) for 48 hours. After treatment, we assessed glial scar formation using confocal microscopy and Western blot and ELISA analysis of established inflammatory markers. Results: Our findings suggest that proinflammatory activation reduces microglial proliferation and impedes scar formation. Notably, we found that microglial activation leads to an elevation in

levels of a disintegrin and metalloprotease 17 (ADAM17) and triggers the ADAM17-mediated cleavage of colony stimulating factor 1 receptor (CSF1R), which plays a critical role in the proliferation and migration of microglia. The lack of microglial proliferation within the injury ultimately hampers glial scar formation. Interestingly, our results demonstrate that specific ADAM17 inhibition, following treatment with TAPI-0, or a zinc chelator effectively promotes appropriate glial scar formation during inflammation. Moreover, zinc chelation elicits the production of trophic factors to enhance tissue recovery. Conclusions: Our findings show that zinc reduction effectively blocks the ADAM17-mediated cleavage of CSF1R and prevents impaired tissue repair during inflammation.

Disclosures: D. Hernández Espinosa: None. **A. Thathiah:** None. **E. Aizenman:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.02/X12

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS107853

Title: 18 kd translocator protein (tspo) plays a critical regulatory role in microglia/macrophage mediated responses after brain injury.

Authors: *F. BONSACK[1], S. SUKUMARI RAMESH[2];
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Abstract: Intracerebral Hemorrhage (ICH) is the second most common type of stroke, with devastatingly high morbidity and mortality rates. Currently, there are no effective treatment options for ICH, making it imperative to identify and characterize novel molecular targets for therapeutic intervention. 18 kD translocator protein (TSPO) is a mitochondrial protein of enigmatic function. Previous studies in our lab demonstrated profound TSPO expression in microglia and infiltrating macrophages following ICH in the ipsilateral brain striatum, as evidenced by immunohistochemistry and flow cytometry analysis. Moreover, SiRNA-mediated knockdown of TSPO in murine macrophage cells led to an increased release of inflammatory cytokines TNF-a and IL-6. Based on our studies, we hypothesized that TSPO could be a negative regulator of ICH-induced inflammation, a significant contributor to secondary brain injury. To address this, we employed global TSPO knockout mice and generated macrophage-specific and microglia-specific TSPO knockout mice, and ICH was induced in them. In line with our hypothesis, all three male TSPO knockout mice exhibited significantly augmented neurobehavioral outcomes with a concomitant increase in neurodegeneration compared to their respective controls. Interestingly, there was no difference in neurological deficit scores in female TSPO knockout mice compared to their respective controls. Further studies are needed to elucidate whether there is a sex-based difference in the functional role of TSPO following ICH.

Also, the macrophage-specific knockdown of TSPO in mice exhibited a reduction in hematoma resolution at day-3 post-ICH, as evidenced by susceptibility-weighted MRI. A decrease in hematoma resolution was not observed in the microglia-specific knockout mice, highlighting a possible differential role of TSPO in microglia and macrophages. Future studies from our lab will address the mechanistic roles of TSPO contributing to the pathophysiology of ICH. Altogether, the data implicate a novel regulatory role of TSPO in brain damage post-ICH.

Disclosures: F. Bonsack: None. **S. Sukumari Ramesh:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.03/X13

Topic: C.10. Brain Injury and Trauma

Support: USU VPR Intraumural SUR-90-12083

Title: Systemic inflammation induced from polysystem trauma is a critical driver of temporal and spatially resolved gene expression in key regional compartments of the injured brain.

Authors: *C. J. ROWE[1,2,6], J. MANG[2,3], B. HUANG[2,3], K. DOMMARAJU[4], B. K. POTTER[2], S. A. SCHOBEL[2,6,5], E. R. GANN[2,6,5], T. A. DAVIS[2];
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Abstract: Blast exposure can cause devastating life-threatening polysystem trauma. Despite considerable efforts, the role of the systemic inflammation following major trauma on secondary brain injury is largely unknown. In this study, we aimed to identify and delineate altered neuroinflammatory signaling responses in regional compartments of the brain following various traumatic injuries. Adult male rats (400-500g) either received a (1) head-on whole-body blast overpressure exposure only (120kPa; BOP), (2) complex extremity trauma (CET) involving closed femoral fracture and soft-tissue crush injury, 3 hours (h) of prolonged tourniquet-induced limb ischemia and limb amputation through the zone of injury, or (3) BOP+CET. At 6, 24, and 168h post-injury cohorts of rats (n=7 timepoint/model) were euthanized and 8 anatomic regions of the brain were dissected and profiled for neuroinflammatory-neurodegeneration gene expression signatures using a custom low-density RT-qPCR array. We compared the molecular heterogeneity of gene profiles of the brain in the steady state of naïve animals with trauma-induced changes over time. Differential gene expression (DEG) analysis indicated similar expression patterns of genes involved in neurotransmission and transcription across all regions of the naïve brain and known targets involved in perpetuating inflammation to be extremely low or undetectable. Gene expression associated with neuroinflammation and neuropathology were

robustly increased acutely after injury where the magnitude of the response across structural brain regions correlated with injury severity (BOP+CET>CET>BOP). We identified a time dependent injury-associated change following BOP, wherein few DEGs were detected at 6h post-injury, yet excessive significant pathological changes in neuroinflammatory cascades were detected 168h post-injury occurring primarily in the hippocampus, amygdala, and thalamus. In contrast, remote extremity trauma (CET) in the absence of direct brain injury resulted in a much earlier diverse, and aberrant neuroinflammatory response across most brain regions, a majority of which remained elevated at 168h. BOP+CET resulted in a global heightened and prolonged neuroinflammatory response comprising expression of additional functional regulatory networks genes linked to immune cells, immune mediators and immune signaling pathways. These findings provide a foundation for discerning the pathophysiological consequences of acute extremity injury-induced systemic inflammation on promoting remote brain neuroinflammation, which ultimately may contribute to pathological dysfunction and cognitive impairment.

Disclosures: C.J. Rowe: None. **J. Mang:** None. **B. Huang:** None. **K. Dommaraju:** None. **B.K. Potter:** None. **S.A. Schobel:** None. **E.R. Gann:** None. **T.A. Davis:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.04/X14

Topic: C.10. Brain Injury and Trauma

Title: Functional deficits are associated with increased neuroinflammation and axonal injury, and dysregulated AQP4 expression and polarity in a rat model of mild traumatic brain injury

Authors: *Y. ZHANG[1,2], L. CHEN[2], G. DING[3], L. LI[3], H. PANG[2], Q. JIANG[3], M. CHOPP[3,4], Z. ZHANG[3], A. MAHMOOD[2], Y. XIONG[2];
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Abstract: Traumatic brain injury (TBI) is a major health problem worldwide. Glymphatic system for the clearance of brain waste relies on the polarized water channel aquaporin 4 (AQP4) of the astrocytes. This study is aimed to investigate the long-term functional and histological outcomes with a focus on AQP4 expression and polarization after mTBI. Adult male Wistar rats were subjected to a mild closed head injury. Sham rats underwent surgery without injury. Neurological and cognitive functions were assessed up to 90 days after injury. The brains were collected at 1 day, 7 and 90 days after injury for immunohistochemical analyses of Tau, neuroinflammation, axonal injury, AQP4 expression and polarity. Compared to sham animals, mTBI causes long-lasting cognitive memory deficits measured in Morris water maze and novel object recognition tests up to 3-months after injury. The cognitive dysfunction is accompanied by the increased amyloid precursor protein (APP) expression, increased axonal injury (Tau, p-neurofilament heavy chain) and decreased generation of neuroblasts and neurogenesis in the

brain. Moreover, the injury characterized by activation of astrocytes in most measured brain regions is significantly correlated to the observed neurological deficits. With this model, we detected the dysregulation of AQP4 polarization in the injured brain, indicating the impairment of perivascular clearance pathway. The increased accumulation of protein tau was found in the brain, reflecting the inefficiency of waste clearance. Our findings demonstrate that mTBI induces long-term functional deficits which are consistent with our previous data that mTBI leads to the disturbed glymphatic function and reduced cerebral waste clearance.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR539.05/X15

Topic: C.10. Brain Injury and Trauma

Support: NIH grant 1R35NS116852-01
NIH grant 5R37NS077908-08

Title: Cation exchange as a driver of reduced water diffusion in cytotoxic edema

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Abstract: Following traumatic brain injury or ischemic stroke, alongside the onset of seizure activity, cytotoxic edema can cause significant cell death through excessive cellular swelling. This swelling is thought to be due to accumulation of excess sodium inside cells, leading to water uptake through osmosis. We propose that this excess sodium does not add to the existing potassium inside the cells but rather exchanges with it, with the larger hydration shell of sodium essentially squeezing free water out of the cells even as they swell.
We evaluated cell swelling and death in a chronically epileptic in vitro preparation using multiphoton microscopy. Organotypic hippocampal slice cultures were made from CLM1 (Clomeleon) and wild-type C57BL/6J mice on P6 and incubated in vitro. Slices were imaged with transgenic fluorophores such as Clomeleon, TurboRFP, and GFAP-GFP to visualize healthy neurons and astrocytes, and bath-applied fluorophores such as SBFI-AM and fluorescein-dextran to assess apoptotic neurons.
Oxygen-glucose deprivation (OGD) induced slice swelling in both sodium-free (high potassium) and potassium-free (high sodium) solutions. Even in the absence of OGD, exposure of slices to a sodium-free (high potassium) solution caused significant slice swelling, and exposure to a

sodium-free (high lithium) solution less so. Application of the Na/K-ATPase blocker Ouabain caused moderate slice swelling independent from the high potassium or high lithium effects. Application of kynurenic acid to block seizure activity prevented the Ouabain-induced swelling. Application of the selective KCC2 inhibitor VU 046 prevented the high lithium-induced slice swelling. Fluorescent protein (FP) emission was quenched in both neurons and astrocytes during high potassium-induced slice swelling, though partial recovery of emission was possible upon washout. Application of the NKCC1 inhibitor Bumetanide did not prevent any of the swelling or quenching effects.
To our surprise, OGD induced slice swelling in sodium-free solutions, and in fact these sodium-free solutions induced significant swelling even in the absence of OGD. Additional swelling observed after inhibiting Na/K-ATPases could be prevented by blocking seizure activity, suggesting a role for glutamate excitotoxicity. Inhibiting KCC2 prevented swelling, suggesting reversed transport in neurons under sodium-free conditions, but inhibiting NKCC1 had no effect on neuronal or astrocytic swelling. The reversible nature of the swelling and the FP quenching indicate that the swelling, though significant, does not inevitably lead to cell death.

Disclosures: **T. Balena:** None. **K.P. Lillis:** None. **K.P. Normoyle:** None. **K.J. Staley:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR539.06/X16

Topic: C.10. Brain Injury and Trauma

Support: NJCBIR19PIL010

Title: Intercellular adhesion molecule-1 promotes the transmigration of neutrophils and formation of neutrophil extracellular traps in traumatic brain injury.

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Abstract: Traumatic brain injury (TBI) causes the blood-brain barrier (BBB) dysfunction and transmigration of immune cells into the brain, an important mechanism underlying neurovascular damage and neuroinflammation. In TBI, BBB dysfunction promotes leukocyte transmigration and accumulation in the brain. Activation of neutrophils causes the release of nuclear and granular contents to form an extensive web-like structure of DNA called the neutrophil extracellular trap (NET). NETs contain double-stranded DNA, histone, and granule proteins including neutrophil elastase, cathepsin G, and myeloperoxidase (MPO). Intercellular adhesion molecule-1 (ICAM-1) is identified as an initiator of neuroinflammatory responses that leads to neurodegeneration and cognitive and sensory-motor deficits in several pathophysiological conditions. However, the underlying mechanisms of ICAM-1-mediated neuroinflammation, transmigration of leukocytes, and formation of Neutrophil Extracellular Traps (NET) in the brain

and its link with functional deficits following TBI remain elusive. We hypothesize that ICAM-1 has significant roles in the transmigration of leukocytes to the brain, the formation of NET, and impairing sensory-motor and cognitive functions. Deletion of ICAM-1 using CRISPR/Cas9 technology remodels the neurovascular system and promotes functional recovery after TBI. The experimental TBI was induced *in vivo* by fluid percussion injury (25 psi) in wild-type and *ICAM-1*$^{-/-}$ mice and *in vitro* by stretch-injury (3 psi) in human brain microvascular endothelial cells (hBMVECs). We manipulated ICAM-1 pharmacologically and genetically and conducted several biochemical analyses to gain insight into the mechanisms underlying ICAM-1-mediated transmigration of leukocytes and the formation of NET in the brain. We demonstrated that the CRISPR/Cas9-mediated ICAM-1 deletion mitigates leukocyte transmigration and formation of NET by disrupting the paxillin-FAK signaling pathway. We used a cohort of behavioral tests that included sensorimotor and cognitive functions, and psychological stress analyses to test functional outcomes following TBI. Therefore, in this highly significant study, we will uncover novel molecular mechanisms of TBI-induced neurological deficits due to the formation of NET and develop a therapeutic strategy for TBI targeting NET formation. This work was supported by the New Jersey Commission on Brain Injury Research #CBIR19PIL010 to P.M. Abdul-Muneer.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.07/X17

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS076815
NIH Grant R01MH113535
NIH Grant R01AG058621

Title: Single mild closed head injury exacerbates neuropathology of Alzheimer’s disease by promoting expression of TDP-43

Authors: *F. GAO, M. HU, J. ZHANG, J. HASHEM, C. CHEN;
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Abstract: Traumatic brain injury (TBI) is an important risk factor for the development of Alzheimer's disease (AD). However, the molecular mechanisms by which TBI contributes to the development of AD remain unclear. In the present study, we provide evidence that aberrant production of TDP-43 is a key factor in promoting AD neuropathology and synaptic and cognitive deterioration in mouse models of mild closed head injury (CHI). While a single mild CHI does not induce significant changes in neuropathology, synaptic function, or cognitive functions in wild-type (WT) mice, it does result in persistent neuroinflammation and increases in

Aβ plaques, expression of β-secretase (BACE1), TDP-43, and phosphorylated tau (p-tau) in 4-month-old 5xFAD APP transgenic (TG) mice.A single mild CHI also accelerates the deterioration of long-term synaptic plasticity, spatial learning, and memory retention in APP TG mice. However, repeated mild CHI is required in WT animals to induce neuropathology, synaptic and cognitive impairments. Importantly, these changes in APP TG and WT mice exposed to a single or repeated mild CHI are alleviated by silencing of TDP-43 but reverted by rescue of the TDP-43 knockdown. Moreover, we found that overexpression of TDP-43 in the hippocampus aggravates AD neuropathology and provokes cognitive impairment in APP TG mice, mimicking the changes induced by a single mild CHI. We further discovered that neuroinflammation triggered by TBI promotes NF-κB-mediated transcription and expression of TDP-43, which, in turn, facilitates tau phosphorylation and Aβ formation by stimulating the phosphorylation of GSK3β and interacting with BACE1, respectively. Our findings reveal a previously undefined role of TDP-43 in exacerbating AD neuropathology and accelerating synaptic and cognitive declines following TBI. This suggests that TDP-43 could be a therapeutic target for preventing the development of TBI-induced AD neuropathology and dementia.

Disclosures: F. Gao: None. **M. Hu:** None. **J. Zhang:** None. **J. Hashem:** None. **C. Chen:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.08/X18

Topic: C.10. Brain Injury and Trauma

Support: DoD Grant AZ1701452

Title: Pathological characterization and transcriptional profiling of CNS cell populations following mild traumatic brain injury in a mouse model of Alzheimer's disease

Authors: *N. M. BARRINGTON[1], G. E. STUTZMANN[2];
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Abstract: Traumatic brain injury (TBI) affects over 2.8 million people each year, with over 75 percent of those being mild traumatic brain injuries (mTBI). In addition to head injury being a known risk factor for sporadic Alzheimer’s disease (AD), TBI and AD share several pathologic hallmarks including amyloid ß plaque formation, hyperphosphorylation of tau, and increased cellular calcium leading to synaptic deficits. Following mTBI, microglial activation and upregulation of pro-inflammatory cytokines contribute to aberrant neuronal calcium signaling and synaptic deficits, and over time this can lead to AD-like memory and behavioral deficits. Similarly, the pathogenesis of AD has been linked to inflammation mediated by microglial activation. Our research objective is to characterize microglial activation states following mild

TBI and subsequent pathologic changes, including transcriptomic changes that distinguish microglial and other CNS cell populations in the acute and chronic post-injury phases that may contribute to long-term synaptic deficits and progression toward AD. To study tissue pathology and transcriptional profiles of microglial and other cell types following mTBI, we subjected triple transgenic (3xTg) AD mice and non-transgenic (NonTg) control mice to repeat mild TBI (rTBI) consisting of three closed head controlled cortical impacts or corresponding sham control. Seven- and 30-days following injury, brain tissues were harvested and evaluated via immunohistochemical staining for pathological (amyloid beta, tau) and immunological markers (microglia) as well as via single-cell RNA sequencing to evaluate transcriptional profiles. Current data indicate that rTBI-induced pathologic markers increase over time, while microglial markers increase similarly at both the acute and chronic post-injury stage. Single-cell RNA sequencing data suggest several distinct microglial populations characteristic of acute and chronic injury responses as well as potential downstream changes to transcripts related to synaptic function, calcium signaling, and neuronal physiology. These findings support shared pathogenic mechanisms in TBI and AD, particularly those related to neuroinflammation, that may play a role in subsequent neuronal dysfunction and increased vulnerability to neurodegeneration and development of dementia after mild brain injury.

Disclosures: N.M. Barrington: None. **G.E. Stutzmann:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.09/X19

Topic: C.10. Brain Injury and Trauma

Support: Innovative Medicines Initiative 2 under grant agreement no. 821528 (NeuroDeRisk: Neurotoxicity De-Risking in Preclinical Drug Discovery (A.P.))
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European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie agreement No101034307 (Neuro-Innovation PhD Programme)

Title: In vitro non-coding RNA signature of chemoconvulsants

Authors: *M. BANERJEE[1], A. LIPPONEN[1,2], N. KAJEVU[1], T. NATUNEN[1], M. HILTUNEN[1], A. PITKÄNEN[1], N. PUHAKKA[1];

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Abstract: BACKGROUND: Epileptogenic brain insults and seizures in animal models and humans trigger dysregulation of non-coding RNAs (ncRNAs) in brain tissue *in vivo*. Our objective was to assess whether dysregulation of ncRNA could also be induced by epileptogenesis and/or seizure-inducing chemoconvulsants *in vitro* to predict their downstream pathways and determine their potential as treatment targets for epilepsy. **METHODS:** Rat primary cortical cultures were exposed to 11 chemoconvulsants (4-aminopyridine, amoxapine, bicuculline, chlorpromazine, donepezil, kainic acid, pentylenetetrazol, picrotoxin, pilocarpine HCl, SNC80, strychnine HCl) for 24 h. Exposed cells were lysed, and total RNA was extracted for poly-A enriched RNA sequencing. Salmon (version 1.9.0) was used to quantify ncRNA in each sample. Differential expression analysis was done with DESeq2. Quantitative PCR (qPCR) validations for samples exposed to amoxapine, chlorpromazine and SNC80 were conducted on two candidates (ENSRNOT00000076663.2 and ENSRNOT00000080370.2). To assess long non-coding RNA (lncRNA)-microRNA (miRNA) interactions, predicted binding of miRNAs (cut-off score=80) to top candidate lncRNAs (n=3) was acquired from the miRDB (version 6.0).**RESULTS:** Altogether 4,648 different ncRNAs were detected, being present in at least one sample. The number of dysregulated ncRNAs varied from 0 to 814 (FDR<0.05), depending on the chemoconvulsant. Most dysregulated transcripts were long non-coding RNAs (lncRNA). The qPCR analysis confirmed the upregulation of lncRNA-ENSRNOT00000076663.2 in amoxapine (FC=1.9, p=0.016) and chlorpromazine-treated samples (FC=1.5, p=0.016). It also confirmed the upregulation of small nucleolar RNA (snoRNA)-ENSRNOT00000080370.2 in samples treated by amoxapine (FC=3.9, p=0.016), SNC80 (FC=2.2, p=0.016) or chlorpromazine (FC=2.0, p=0.016). The miRDB analysis revealed a high binding potential of lncRNA-ENSRNOT00000076663.2 for 6, lncRNA-ENSRNOT00000019121.5 for 11 miRNAs and ESNRNOT00000097831.1 for 3 miRNAs. **CONCLUSIONS:** Chemoconvulsant-induced dysregulation of ncRNAs was compound specific. Particularly, we found upregulation of snoRNAs and lncRNAs, which were previously found to be regulated by experimental and human epilepsy. Our analysis highlighted miRNA sponging, a prevalent regulatory mechanism of lncRNAs, to be among one of the predicted functions of identified lncRNAs.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.10/X20

Topic: C.10. Brain Injury and Trauma

Support: 2021 10x Genomics Yale Pilot Award

Title: Uncovering the molecular mechanisms of traumatic brain injury at the acute phase

Authors: *F. S. MOHAMMED[1], D. MCGUONE[3], S. B. OMAY[2], Z. ERSON OMAY[2], J. ZHOU[2];
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Abstract: In focal traumatic brain injury (TBI), primary injury stems from direct mechanophysical forces. Secondary injury processes offer a therapeutic window for modulating the physical, cellular, and biochemical processes that contribute to neurodegeneration, yet the spatial patterning of such mechanisms is unknown. Here, we present the spatial transcriptomic profiling of a focal TBI model to better understand the molecular mechanisms at two different post-injury acute time points.
Using the controlled cortical impact model, 2-month-old male C57BL/6 mice received sham treatment or moderate or severe TBIs. At 24- or 48 hours post-surgery, ipsilateral samples were sequenced following the Visium Spatial Gene Expression protocol for fresh-frozen tissue. We employed the Seurat v4 spatial exploration pipeline and extended our analysis by implementing cell/spot characterization integrating publicly available single-cell data from the Allen Brain Atlas and other public spatial datasets to achieve a high-resolution spatiotemporal dataset. BayesSpace computational methods allowed us to achieve subspot spatial resolution.
Unbiased transcriptomic characterization of a cohort of four samples (24hr-sham, 24hr-moderate, 24hr-severe, and 48hr-severe) displayed differential expression patterns across sham and injury models. We established high-resolution cell-type annotation by integrating published single-cell and other spatial transcriptomic mouse brain datasets. This reference-guided analysis and H&E staining exposed structural disruption including focal loss of cortical layer 2/3 in injured animals. We also observed differential spatial co-expression of canonical neuroinflammatory markers including Spp1, Lcn2, Mt1, and Mt2. Both unbiased and reference-guided analysis revealed a strong differential immune response and macrophage marker expression profile in spatiotemporal dimensions. Cell-type matched analysis near the site of injury identified that the Hippo, Liver X-/Retinoid X receptor activation, and Sirtuin signaling pathways were all upregulated 24hrs after impact, reflecting a response to physical blood-brain barrier damage, inflammation, and oxidative stress.
To our knowledge, this is the first exploratory experimental design to elucidate the complex molecular and cellular dynamics of the TBI response at a high spatial resolution. We observed a striking, quantitative, and spatial differential expression in immunomodulatory, inflammatory, and anatomical markers across injury severity and temporal dimensions. Extended cohort studies will provide molecular insight into engineering targeted therapies.

Disclosures: F.S. Mohammed: None. **D. McGuone:** None. **S.B. Omay:** None. **Z. Erson Omay:** None. **J. Zhou:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

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Program #/Poster #: PSTR539.11/X21

Topic: C.10. Brain Injury and Trauma

Support: Department of Defense Technology for the Warfighter Program
The opinions and assertions expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense.

Title: Discovery of biomarkers of blast and impact injury using a novel bioengineered 3D brain-like tissue

Authors: D. SNAPPER[1], V. LIAUDANSKAYA[3], R. VORN[4], Y. KIM[1], I. GEORGAKOUDI[5], J. GILL[4], D. KAPLAN[5], ***A. J. SYMES**[2];
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Abstract: The mechanism through which the brain is injured in a traumatic injury, influences the specific neuropathology and impacts functional outcomes. Biomarkers released into the serum from traumatic brain injury (TBI) can be used to diagnose and assess injury severity, to monitor recovery, predict outcomes, and provide insight into the pathological changes. However, biomarkers may be dependent on the injury modality, and therefore it is necessary to pursue modality-specific biomarkers to be able to eventually address and follow every injury. We have developed a bioengineered 3D brain-like human tissue culture system that provides a completely novel approach to the discovery of serum biomarkers for TBI. The 3D brain-like tissue is composed of hybrid collagen-infused silk donut hydrogels embedded with human IPSC derived neurons, human astrocytes, and a human microglial cell line. The 3D brain-like tissues form mature neural networks by 5 weeks with axons extending across the central region of the donut. The unique advantages of our 3D TBI model includes complete control over cellular composition and microenvironment, with the ability to profile changes in released proteins and microRNAs over time. In addition, functional tissues are generated in much shorter time frames than animal studies and offer the reproducibility and scalability critical for larger scale target and drug screens. We have established this system, and injured the scaffolds by either impact injury with a controlled cortical impact device, or by primary blast injury in an advanced blast simulator. We isolated miRNA and proteins released into the media at different time points after either blast or impact injury in order to discover novel markers of TBI that are shared or differ between injury modalities. Isolated miRNAs were analyzed using Nanostring's nCounter miRNA expression panel. We found a complex pattern of many miRNAs released into media with temporal specificity that differed between blast and impact injury. Proteins were screened by Somalogic aptamer array to find protein biomarkers that were induced at 6 or 48 hours after blast or impact injury. There were many differences between the proteins released after each type of injury, with a greater number and larger induction after impact than blast injury. We are currently verifying some potential biomarkers by orthogonal techniques for each injury type. This system should provide several potential unique blast and impact injury induced biomarkers to diagnose and monitor injuries in a modality specific manner.

Disclosures: D. Snapper: None. **V. Liaudanskaya:** None. **R. Vorn:** None. **Y. Kim:** None. **I. Georgakoudi:** None. **J. Gill:** None. **D. Kaplan:** None. **A.J. Symes:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

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Program #/Poster #: PSTR539.12/X22

Topic: C.10. Brain Injury and Trauma

Title: Elucidating the role of mechanically gated ion channel Piezo1 in neural stem cells differentiation

Authors: *M. KIDD[1], K. JOHNSON[1], A. GRANT[2], K. JOHNSON[2], M.-A. MICCI[2];
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Abstract: The repercussions of traumatic brain injury (TBI) have been linked to later neurodegenerative diseases such as Alzheimer's disease (AD). TBI is most commonly the result of a mechanical force applied to the brain that results in brain damage and neuronal death. Neural stem cells (NSC) are multipotent cells that retain the ability to differentiate into neurons, oligodendrocytes, and astrocytes. NSCs are found in the dentate gyrus region of the hippocampus, which has critical role in memory formation and is most affected by TBI and AD. Here we aimed to better understand the role that the mechanically gated ion channel Piezo1 plays in NSC differentiation. Rat hippocampus NSC (Hipp-NSC) were cultured as neurospheres in proliferating media and divided into three treatment groups, a control group that will not receive a stretch injury, a siRNA mediated Piezo1 knockdown group without stretch injury, a siRNA mediated Piezo1 knockdown group with stretch injury and a siRNA scramble group. For the stretch injury, Hipp-NSC were plated onto polyornithine and laminin-coated dish containing a flexible silicone bottom. Twenty-four hours after plating in proliferating media, Hipp-NSC were stretched using a cell injury controller for 50ms at 30 PSI, allowed to differentiate for 5 days in differentiation media, and then fixed in 4% PF. Immunofluorescence analysis of βIII-tubulin and GFAP was performed to determine the number of neurons and astrocytes respectively. Images were quantified using ImageJ. Piezo1 siRNA knockdown in Hipp-NSC resulted in a significant increase in βIII-tubulin-positive cells compared to the siRNA scramble when no stretch injury was applied. When comparing the number of GFAP-positive cells between the Piezo1 knockdown and the scramble group when no stretch injury was received, there was no significant difference between the two groups. When the Piezo1 knockdown Hipp-NSC received the stretch injury, the significant increase in βIII-tubulin-positive cells was not observed and there was no effect on the number of GFAP-positive cells. Previous work done in the lab has shown that the stretch injury on its own does not have a significant effect on Hipp-NSC differentiation. Here we show a significant increase in neuronal differentiation when Piezo1 is knocked down in rat Hipp-NSC. This increase is reversed when a stretch injury is applied, suggesting that the minimally available Piezo1 or another mechanically gated ion channel is being activated.Since the preservation of hippocampal neurogenesis has been linked to increased cognitive function after TBI and in AD, our data suggests that Piezo1 might represent a potential therapeutic target for these disorders.

Disclosures: **M. Kidd:** None. **K. Johnson:** None. **A. Grant:** None. **K. Johnson:** None. **M. Micci:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.13/X23

Topic: C.10. Brain Injury and Trauma

Support: NSF Grant 2141291

Title: Mechanosensation of human astrocytes following exposure to an applied shock wave

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Abstract: Blast induced traumatic brain injury (bTBI) is a significant health concern as 70% of TBI military patients are diagnosed with blast related injuries. After exposure to a blast wave insult, glia are known to respond, causing neurodegeneration and cognitive impairment over time. Reactive astrocytes are a hallmark sign of brain injury which has been depicted by increased expression of glial fibrillary acidic protein (GFAP). Although increased reactive astrocytes following bTBI has been observed, how astrocytes sense the shock wave energy is understudied. Mechanosensing proteins such as Piezo ion channels and focal adhesion (FA) molecules are known to respond to mechanical forces such as tension, shear, and compression. Piezo 1 protein is one of abundant mechanosensing proteins in human astrocytes (HAs). Upon activation, Piezo 1 initiates a calcium ion influx, and recent studies suggest that this influx is necessary for FA assembly and disassembly. FA proteins sense the external forces via transmembrane integrin receptors and intracellular FA molecules such as integrins β1 and β3, and vinculin. However, how these two different mechanosensing mechanisms interact for HA activation is still unknown. We hypothesize that the applied shock wave will increase the gene expressions of Piezo 1 and FA molecules in accordance with HA activation. For this study, HAs were encapsulated in a mixture of hyaluronic acid and collagen type 1 hydrogel, submerged in a sterile bag filled with astrocyte growth media, and subjected to a shock wave at 16.24 ± 2.35 psi using an exploding bridging wire system. RNAs were collected after 24, 48, and 72 hours for qPCR analysis. qPCR analysis indicated HA reactivity with a significant increase in GFAP at 24 hours (mean 1.000 SEM 0.232 for Sham and 3.297 ± 0.188 for Blast) and 48 hours (1.000 ± 0.128 for Sham and 1.861 ± 0.068 for Blast) post exposure. FA molecules showed significant changes at 48 hours (Vinculin 1.000 ± 0.245 for Sham and 2.361 ± 0.125 for Blast; Integrin β3 1.000 ± 0.156 for Sham and 2.322 ± 0.240 for Blast). Piezo1 gene expression showed a trend of 1.6 times increase in blast samples at all time points. Increased vinculin and integrin β3 expressions imply a potential promotion of cell migration corresponding to the GFAP increase. Also, a trend of increased Piezo 1 expression suggests the activation and recruitment of Piezo 1 along with the activation of FA molecules. This study demonstrated that these mechanosensing

molecules respond to a shock wave with the HA activation, which may imply a potential correlation between mechanosensing proteins and the HA reactivity.

Disclosures: R. Yokosawa: None. **K. Wilson:** None. **P. VandeVord:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.14/X24

Topic: C.10. Brain Injury and Trauma

Title: P17/c18 ceramide mediated mitophagy is an endogenous neuroprotective response in post-traumatic brain injury (tbi)

Authors: *E. KARAKAYA[1], N. OLEINIK[1], J. EDWARDS[1], J. TOMBERLIN[2], A. ERGUL[1], S. BEYAZ[4], B. OGRETMEN[1], O. ALBAYRAM[3];
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Abstract: p17-C18-ceramide-mediated mitophagy is an endogenous neuroprotective response in post-traumatic brain injury (TBI).
E. Karakaya[1], N. Oleinik[2], J. Edwards[1], J. Tomberlin[1], A. Ergul[1], S. Beyaz[3], B. Ogretmen[2], O. Albayram[1,5]; [1]Depart. of Path. & Lab. Medicine, Medical University of South Carolina (MUSC), Charleston, SC, [2]Depart. of Biochem. and Mol. Bio., MUSC, Charleston, SC, [3]Cancer Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, [5]Depart. of Neuroscience, MUSC, Charleston, SC
None of the authors has a disclosure.
Mitochondria are crucial subcellular components that play a significant role in ensuring proper neuronal axon functioning, and recent studies have strongly implicated mitochondrial dysregulation as a major driver of the secondary damage to axons that occurs following TBI. Our recent study showed that the mitochondrial trafficking of CerS1 by the novel p17 transporter induces C18-ceramide synthesis and accumulation in mitochondria undergoing mitophagy in various tissues. To determine how the deletion of p17 affects the long-term recovery and development of secondary axonal degeneration in the novel repetitive less-than-mild (rlmTBI) model, 2-months-old male p17KO and C57BL/6 WT mice were subjected to 7 rlm or sham injury to the dorsal aspect of the skull in 9 days, followed by behavioral testing at 3 and 6 months. Only p17KO male mice developed significant sensorimotor deficits in the secondary injury phase starting at 3 months post-injury. This was followed by cognitive changes, suggesting progressive degeneration. We evaluated the microstructure of white matter components and integrity of myelinated axons using immunostaining for MBP, Luxol Fast Blue staining, and electron microscopy in 6 months after rlmTBI. The progression of axonal degeneration was supported by the myelinated axonopathy and ultrastructural pathologies of axon and myelin degeneration in the neocortex in p17KO mice. Moreover, co-

immunoprecipitation analysis demonstrated that LC3 levels were significantly decreased in the p17-KO mice, compared with WT controls at 6 months post-TBI. Notably, mass spectrometry-based lipidomics analyses revealed reduced mitochondrial C18-ceramide levels in the neocortex compared to WT controls. In conclusion, ablation of p17 in mice results in a loss of stress-induced mitophagy in the brain, contributing to susceptibility to, and recovery from, long-term secondary complications associated with rlmTBI suggesting that p17/C18-ceramide trafficking is an endogenous neuroprotective mitochondrial stress response following rlmTBI.

Disclosures: **E. Karakaya:** None. **N. Oleinik:** None. **J. Edwards:** None. **J. Tomberlin:** None. **A. Ergul:** None. **S. Beyaz:** None. **B. Ogretmen:** None. **O. Albayram:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.15/X25

Topic: C.10. Brain Injury and Trauma

Title: Timely expression of PGAM5 and its cleavage control mitochondrial homeostasis during neurite re-growth after traumatic brain injury

Authors: *Y. WANG, M.-Z. LIANG, L. CHEN;
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Abstract: Patients suffered from severe traumatic brain injury (TBI) have twice the risk of developing into neurodegenerative diseases later in their life. Thus, early intervention is needed not only to treat TBI but also to reduce neurodegenerative diseases in the future. Physiological functions of neurons highly depend on mitochondria. Thus, when mitochondrial integrity is compromised by injury, neurons would initiate a cascade of events to maintain homeostasis of mitochondria. However, what protein senses mitochondrial dysfunction and how mitochondrial homeostasis is maintained during regeneration remains unclear.We found that TBI-increased transcription of a mitochondrial protein, phosphoglycerate mutase 5 (PGAM5), during acute phase was via topological remodeling of a novel enhancer-promoter interaction. This up-regulated PGAM5 correlated with mitophagy, whereas presenilins-associated rhomboid-like protein (PARL)-dependent PGAM5 cleavage at a later stage of TBI enhanced mitochondrial transcription factor A (TFAM) expression and mitochondrial mass. To test whether PGAM5 cleavage and TFAM expression were sufficient for functional recovery, mitochondrial oxidative phosphorylation uncoupler carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) was used to uncouple electron transport chain and reduce mitochondrial function. As a result, FCCP triggered PGAM5 cleavage, TFAM expression and recovery of motor function deficits of CCI mice. Findings from this study implicate that PGAM5 may act as a mitochondrial sensor for brain injury to activate its own transcription at acute phase, serving to remove damaged mitochondria through mitophagy. Subsequently, PGAM5 is cleaved by PARL, and TFAM expression is increased for mitochondrial biogenesis at a later stage after TBI. Taken together,

this study concludes that timely regulation of PGAM5 expression and its own cleavage are required for neurite re-growth and functional recovery.

Disclosures: **Y. Wang:** None. **M. Liang:** None. **L. Chen:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.16/Y1

Topic: C.10. Brain Injury and Trauma

Support: JSPS KAKENHI Grant 21K07274

Title: Involvement of G protein-coupled receptor 3 in retinal ganglion neuron survival and axonal regeneration following optic nerve crush in mice

Authors: S. MASUDA[1], H. SHIRAKI[1], Y. SOTOMARU[2], K. HARADA[1], I. HIDE[1], Y. KIUCHI[1], N. SAKAI[1], ***S. TANAKA**[1];
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Abstract: Glaucoma is an optic neuropathy that affects more than 60 million people worldwide and is currently one of the most common diseases responsible for irreversible blindness. Axonal degeneration, which occurs before retinal ganglion nerve loss, has been implicated in the pathogenesis of glaucoma. In addition to intraocular pressure (IOP), multiple factors are responsible for optic nerve damage associated with glaucoma. One factor contributing to neuronal loss is the reduction of intracellular cAMP following injury, causing retinal ganglion cell (RGC) death because of decreased responsiveness to neurotrophic factors. Notably, the treatment of neurons with depolarizing stimuli or the pharmacological activation of intracellular cAMP restores responsiveness to neurotrophic factors. Moreover, the induction of axonal regeneration by zymosan, oncomodulin, and neurotrophic factors was enhanced by elevated cAMP upon chlorophenylthio-cAMP administration. G protein-coupled receptor 3 (GPR3) is a member of the class A rhodopsin-type GPCR family and is highly expressed in various neurons (Saeki et al., FEBS 1993; Ikawa et al., Brain Res 2021). GPR3 is unique because it constitutively activates the Gαs protein without a ligand and increases the basal intracellular cAMP levels. GPR3 has been previously reported to promote neurite outgrowth (Tanaka et al., JBC2007; Tanaka et al., MCN 2021) and neuronal survival (Tanaka et al., Neurobiol Dis 2014). However, the role of GPR3 in axonal regeneration following neuronal injury has not been adequately investigated. To determine the possible involvement of GPR3 in axonal regeneration following retinal injury, we evaluated retinal GPR3 expression and its involvement in axonal regeneration in mice. GPR3 was relatively highly expressed in RGCs. Surprisingly, RGCs in GPR3 knockout mice were vulnerable to neuronal death under ischemic conditions and during aging without affecting the high IOP. Primary cultured neurons from the retina showed that GPR3 expression

was correlated with neurite outgrowth and neuronal survival. The evaluation of the effect of GPR3 on axonal regeneration using GPR3 knockout mice indicated that GPR3 in RGCs participates in axonal regeneration following optic nerve crush (ONC) under zymosan stimulation. Moreover, regenerating axons were further stimulated upon GPR3 upregulation in RGCs, and this effect was further augmented in combination with zymosan treatment. These results suggest that GPR3 expression in RGCs helps maintain neuronal survival and accelerates axonal regeneration following ONC in mice.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.17/Y2

Topic: C.10. Brain Injury and Trauma

Support: NINDS 1R37NS125632
NINDS R01NS114122
NINDS R01NS115017

Title: Loss of Slc35a2 alters Golgi structure, cortical lamination, and lowers seizure threshold

Authors: *S. ELZINY[1], S. LAPIDUS[2], M. BAYBIS[1], J. BABUS[1], P. IFFLAND, II[1], P. B. CRINO[1];
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Abstract: Somatic loss-of-function *SLC35A2* mutations have been identified in resected epilepsy brain tissue showing focal cortical dysplasia type I (FCDI). *SLC35A2* encodes UGT-1, a transporter of UDP-galactose from the cytosol into the Golgi apparatus. The effects of *SLC35A2* variants on cortical development are not well understood. We hypothesized that *Slc35a2* knockout (KO) altered Golgi structure *in vitro* and results in altered cortical laminar laminar structure in fetal mouse brain *in vivo*, with reduced seizure threshold.**Methods**: We designed a CRISPR/CAS9 plasmid construct targeting *Slc35a2* exons 2/3. Mouse Neuro2a cells were Lipofectamine transfected in serum free media with either the *Slc35a2* KO or a Scramble (Scr) plasmid. *Slc35a2* KO was validated using Western blot (anti-UGT-1; Sigma Aldrich) and qPCR analysis. *In utero electroporation* (IUE) at E14.5 was used to model the effects of somatic *SLC35A2* mutations in the fetal brain. After birth, brains were formalin-fixed, paraffin embedded, and sectioned (8μm). *Slc35a2* KO neurons expressing a reporter eGFP were visualized immunohistochemically (Keyence microscope). Oligodendrocytes (Olig2, Abcam) and cortical laminar structure were assayed; Ctip2 as a marker for layer V neurons (Abcam) and SATB2 (Abcam) for layer II-IV neurons. Sections with eGFP+ neurons were used to calculate a

region of interest at post-natal day 4 (P4). At P60 and P120, IUE KO and control mice were implanted with EEG electrodes (3 mm behind bregma under isoflurane anesthesia). Continuous EEG recordings (72 h) were performed (Pinnacle Technology 3 channel system). Mice from KO, eGFP control, and WT (wildtype) groups were administered the pro-convulsant agent pentylenetetrazol (PTZ) at 55 mg/kg i.p. and recorded for 30 min to capture the time to first electrographic seizure detected by EEG (seizure latency). **Results**: *Slc35a2* KO showed no mRNA expression (qPCR) and no UGT-1 protein expression (WB) in vitro. KO cells exhibited an abnormal Golgi structure, exhibiting an obtuse angular distribution in KO cells in comparison to smaller angles of Golgi distribution in control cells. At P4, *Slc35a2* KO neurons were scattered across cortical layers and within the white matter, whereas the control eGFP+ neurons were appropriately located within layers II/III. There was no difference in Olig2 density across groups. IUE KO mice had a lower seizure threshold at P60 and P120 in comparison to controls, but none exhibited spontaneous seizures within the 72 hour recording period. **Conclusions:** *Slc35a2* KO alters Golgi structure and disrupts cortical lamination. These effects may lead to establishment of an epileptic network and serve as a model of FCDI.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.18/Y3

Topic: C.10. Brain Injury and Trauma

Support: Brain Injury Association of America (BIAA) Dissertation Grant 2023
Cosmos Club Foundation Scholar 2021
Uniformed Services University

Title: Spatial Transcriptomics Identifies Differential Peripheral Immune Cell Modulation of Molecular Responses to Traumatic Brain Injury in Grey Versus White Matter

Authors: *S. K. KOUNELIS-WUILLAUME[1], C. ALBA[2], A. M. FRANK[5], E. GOGUET[3], W. BROOKS[4], G. SUKUMAR[2], M. D. WILKERSON[2], C. L. DALGARD[2], J. T. MCCABE[2], M. L. DOUGHTY[2];
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Abstract: Damage to cortical grey matter (GM) and myelinated white matter (WM) in traumatic brain injury initiates distinct neuroinflammatory and pathophysiological processes in the GM and WM. An early modulator of GM and WM injury are peripheral immune cells (PIC) that infiltrate damaged brain tissue during the acute post-injury period. To test the hypothesis that PICs

modulate GM and WM injury differently, we mapped gene expression changes at 2- and 7-days post-injury (DPI) in an open-skull mild controlled cortical impact (CCI) TBI model in mice using Visium Spatial Gene Expression (10X Genomics). We compared differentially expressed genes (DEGs) in the contused cortex and in two spatially-defined WM regions: the dorsal corpus callosum and external capsule (CC-EC) tract, and in the ventral anterior commissure (AC). We find enhanced expression of genes involved in synapse maintenance and innate/adaptive immunity in the contused cortex at 2DPI compared to sham controls which was attenuated by 7DPI. The WM had a unique injury profile detected in the CC+EC, proximate to the cortical contusion, but not in the more distant AC. CC+EC of CCI mice were characterized by increased expression of markers of astrogliosis, microgliosis, and neurodegeneration at 2DPI which were further enhanced at 7DPI. To examine the effects of PIC infiltration on GM/WM responses post-TBI, we depleted circulating granulocyte receptor-1^+ (GR-1^+) cells, which are comprised of primarily neutrophils and monocytes, using an *in vivo* anti-GR-1 antibody treatment of CCI mice and repeated spatial transcriptomics. Surprisingly, GR-1^+ cell depletion for 2- or 7-DPI had a negligible effect on gene expression in the CC-EC post-injury when compared to isotype-treated CCI controls. In contrast, anti-GR-1 treatment significantly altered the cortical transcriptome at both 2- and 7-DPI timepoints. We identified increased expression of Alzheimer's risk gene *Apoe* along with major histocompatibility genes and key cytokines/chemokines in anti-GR-1 treated CCI mice. Furthermore, interleukin-1 receptor/transforming growth factor beta and Fos/Jun signaling pathways were downregulated along with their immediate early gene targets. These data reveal a single focal TBI acutely activates spatially-dependent transcriptional responses in both GM and WM that are differentially sensitive to GR-1^+ cell depletion. This study extends our understanding of PIC activity in TBI revealing a spatially distinct immunomodulatory role for GR-1^+ cells in GM pathology and the possible risk of neurodegenerative disease later in life.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.19/Y4

Topic: C.10. Brain Injury and Trauma

Support: HHMI
NIA 5R01AG070921
Templeton Foundation
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Title: Somatic genomic alterations in single neurons from brains with chronic traumatic encephalopathy (CTE)

Authors: *C. MA[1,2,3], G. DONG[1,2,3], S. NAIK[4], G. MCDONOUGH[4], A. C. MCKEE[5,6], A. Y. HUANG[2,3], M. B. MILLER[2,4], E. A. LEE[2,3], C. A. WALSH[2,3,7];
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Abstract: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repetitive head trauma. The genetic, molecular, and cellular mechanisms behind the development of CTE are less well understood than in other neurodegenerative diseases such as Alzheimer's disease (AD). The advent of single-cell sequencing technologies allows for the study of molecular perturbations at the individual cell or cell-population level as well as the analysis of contributions of non-germline somatic mutations to disease pathogenesis. Previous studies of single-cell whole genome sequencing (scWGS) on aging and neurodegenerative brains showed that somatic SNVs (sSNVs) increase both with aging and in disease, but present with distinct patterns of mutational signatures, suggesting that genetic, environmental, or disease states might influence this accumulation.
In this study, we applied scWGS to neurons from the prefrontal cortex of CTE brains. Using PTA (Primary Template-directed Amplification), with LiRA (Linked-Read Analysis) and SCAN-SNV (Single Cell ANalysis of SNVs) computational analyses to distinguish sSNVs from amplification artifacts, we compared the rates of sSNV accumulation in CTE and control brains. We found a significant increase of hundreds of sSNVs in CTE as compared with age-matched controls. Additionally, we identified specific mutational signatures more abundant in CTE than in controls, distinct from the composition of mutational signatures in AD, providing insight into potential pathogenic mechanisms of CTE. Since CTE is hypothesized to be caused by exposure to repetitive head trauma, its areas of pathological overlap with other neurodegenerative diseases, such as AD, make CTE a unique model for studying the effects of molecular pathways in neurodegeneration.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.20/Y5

Topic: C.10. Brain Injury and Trauma

Support: R01NS115815-03
R21NS131689-01

Barrow Neurological Foundation
Chuck Knoll Foundation

Title: Glibenclamide treatment restores cerebral blood flow, reduces neuroinflammation and neurodegeneration and ameliorates cognitive impairment post traumatic brain injury in young and aged mice

Authors: *S. RAIKWAR[1], A. RANI[1], W. YOO[4,1], S. AHMAD[2,1], S. CARLSON[5], V. A. VAGNI[6], K. L. FELDMAN[6], S. MIHALJEVIC[1], S. SHAHJOUEI[7], E. NICO[8], A. EBERLE[1], A. GILLESPIE[1], M. WATERS[3,1], A. DUCRUET[2,1], P. M. KOCHANEK[6], R. M. JHA[3,1,2];
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Abstract: Traumatic brain injury (TBI) induced by controlled cortical impact (CCI) causes destruction of cerebrovascular tissue resulting in diminished cerebral blood flow (CBF), development of cerebral edema, hypoxia, neuroinflammation, neurodegeneration and cognitive dysfunction. We hypothesized that Glibenclamide (GLY) treatment post-TBI restores CBF, reduces neuropathological changes and improves cognitive function in an age- and sex-dependent manner. We investigated the effect of GLY treatment in young (8 weeks) and aged (15-19 months) male and female C57BL/6 mice using a clinically relevant CCI TBI model (left parietal cortex, velocity=5.0m/s, dwell time=50ms, depth=1.2mm). Naïve and TBI groups did not receive any treatment, while TBI+Vehicle and TBI+GLY treatment groups were implanted with osmotic minipumps loaded with either vehicle or GLY, (n=6 mice/group). Longitudinal CBF was monitored by laser speckle contrast imaging (LSCI) immediately prior to TBI and at 15 minutes, 24- and 72-hours and on day 21 post-TBI (n=3 mice/group). Cognitive function was monitored post-TBI by novel object recognition test (NORT, day 14) and Morris water maze (MWM, day 21). Mice were euthanized on day 21 post-TBI and their brains were evaluated for neuropathology. LSCI data revealed no differences in pre-TBI CBF values in either ipsilateral or contralateral hemispheres in young or aged mice regardless of sex. In comparison to pre-TBI CBF values (Baseline BL=100%), immediately post-TBI there was a significant reduction (70-78% BL) in the ipsilateral CBF in both sexes in young and aged mice. Ipsilateral CBF started improving 24-72 hours post-TBI in all experimental groups. However, only GLY treated mice displayed significantly improved ipsilateral CBF close to the baseline by 21 days, most pronounced in young females (86-122% BL, $p<0.0001$) and aged males (80-108% BL, $p<0.0001$). Untreated TBI and Vehicle groups failed to improve ipsilateral CBF in any age group or sex (Females: young=64-66% vs old=52-61% BL; Males: young=51-54% vs old=57-63% BL). Quantitative neuropathological assessment revealed that GLY decreased expression of SUR1-TRPM4, neuroinflammatory markers including microglial activation, NLRP3, reactive oxygen species production, and neurodegeneration (amyloid-β, Tau and TDP43) with an increased CD31 immunostaining in the ipsilateral hemisphere in close proximity to the TBI site. GLY treatment improved recognition (NORT) and spatial memory (MWM) in both young and aged male and female mice with no differences noted by sex. Our data suggest that GLY

treatment restores CBF, reduces TBI-induced neuropathology and improves cognitive function post-TBI.

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Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.21/Y6

Topic: C.10. Brain Injury and Trauma

Support: NIH Training Grant 1T32GM142623
NIH Grant 5R25NS107179-02
NIH Grant 5R01NS128096-02
McKnight Foundation Award
Sloan Fellowship
NSF Career Award

Title: Identifying genes responsible for dopaminergic neuron regeneration in the planarian central and peripheral nervous systems

Authors: *K. CLAY, T. MEDLOCK-LANIER, R. ROBERTS-GALBRAITH;
Univ. of Georgia, Athens, GA

Abstract: How does successful nervous system regeneration occur? Although the ability to regenerate tissue after injury exists in many species across the animal kingdom, the ability to regenerate a brain is rare. One organism that can regenerate a brain *de novo* is the planarian. Planarians are flatworms with a robust regenerative capability and a complex nervous system consisting of a brain, ventral nerve cords, and a peripheral nervous system (PNS). Our lab uses the asexual planarian *Schmidtea mediterranea* to elucidate how robust regeneration occurs in nature. To parse out the mechanisms underlying complex neural regeneration, we began with dopaminergic neurons. Dopamine has been linked to locomotive behaviors as well as neurogenesis in many organisms. This makes dopaminergic neurons an attractive candidate for elucidating the journey from stem cell to mature neuron during regenerative neurogenesis. In planarians, dopamine is synthesized through an evolutionarily conserved pathway involving the enzyme *tyrosine hydroxylase (th)* which we can use as a marker for dopaminergic neurons. This gene is expressed throughout the brain and PNS, which allows us to assess multiple populations of neurons within a cell type. With the use of single cell transcriptomic data (Fincher, et al., 2018), we identified 73 candidate genes with enriched expression in *th+* cells. Next, we used RNA interference to knock down candidate genes and then assessed the impact of each gene knockdown on dopaminergic neuron regeneration. We identified seven key genes required for *th+* cells. Knocking down two genes, *amyloid-beta protein precursor* or *lim domain only 3,* significantly decreased the number of *th+* cells in the brain (63.84% and 52.52% respectively compared to controls, $p<0.05$ one-way ANOVA, n=10-12). Knocking down any of five transcription factor-encoding genes - *soxB1-2, friend leukemia integration factor-1 (fli-1), fli-1-like, iroquois-1,* or *iroquois-2* - significantly decreased the number of *th+* cells in the PNS (30.12% and 23.84% respectively compared to controls, $p<0.0001$, n=9-12). We plan to determine if these genes play roles in the regeneration of other neural cell types, if they are regionally specific, or if they regulate each other. Our work will uncover cellular and molecular mechanisms by which genes govern dopaminergic neuron regeneration, which will provide a framework for elucidating the genetic pathway for the regenerative neurogenesis of other neurons in planarians.

Disclosures: K. Clay: None. **T. Medlock-Lanier:** None. **R. Roberts-Galbraith:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.22/Y7

Topic: C.10. Brain Injury and Trauma

Support: NSERC

Title: Molecular mechanisms of spinal cord regeneration: insights from axolotls

Authors: S. WALKER[1], ***K. ECHEVERRI**[2];
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Abstract: Salamanders have the remarkable ability to functionally regenerate after spinal cord transection. Unlike mammals, which form a glial scar after spinal cord injury, axolotl glial cells undergo a pro-regenerative molecular response to promote functional spinal cord regeneration. In response to injury, $GFAP^{+}$ glial cells in the axolotl spinal cord proliferate and migrate to replace the missing neural tube and create a permissive environment for axon regeneration. Molecular pathways that regulate the pro-regenerative axolotl glial cell response are poorly understood. Here we show axolotl glial cells up-regulate $AP\text{-}1^{cFos/JunB}$ after injury, which promotes a pro-regenerative glial cell response. Axolotl glial cells directly repress *c-Jun* expression via up-regulation of miR-200a. Inhibition of miR-200a during regeneration causes defects in axonal regrowth, and transcriptomic analysis revealed that miR-200a inhibition leads to differential regulation of genes involved with reactive gliosis, the glial scar, and axon guidance. This work identifies a novel role for miR-200a in inhibiting reactive gliosis in glial cells in axolotl during spinal cord regeneration.Using RNA-seq analysis, we discovered that the inhibition of miR-200a results in an upregulation of the classical mesodermal marker *brachyury* in spinal cord cells after injury. However, these cells still express the neural stem cell marker *sox2*. *In vivo* lineage tracing allowed us to determine that these cells can give rise to cells of both the neural and mesoderm lineage. Additionally, we found that miR-200a can directly regulate *brachyury* via a seed sequence in the 3'UTR of the gene. Our data indicate that miR-200a represses mesodermal cell fate after a small lesion injury in the spinal cord when only glial cells and neurons need to be replaced.This data suggests that the axolotl spinal cord reacts differently in response to different injury paradigms and may contain different types of stem cells. Here we will present data on transcriptional profiling of axolotl spinal cord cells and the potential to translate findings from axolotl cells into mammalian models of spinal cord injury to promote better outcomes after injury.

Disclosures: S. Walker: None. **K. Echeverri:** None.

Poster

PSTR539. Brain Injury: Molecular and Genetic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR539.23/Y8

Topic: C.10. Brain Injury and Trauma

Support: ERC-2020-SyG SALAMANDRA

Title: Unveiling salamanders' spinal cord regeneration at the functional, structural, cellular and molecular levels

Authors: *A. JOVEN ARAUS[1], Z. TONELLI GOMBALOVA[1], Z. YAO[1], E. LLORENS[1], S. GIATRELLIS[1], P. DAMBERG[1], L. BLANCHÉ[2], I. KHSIME[3], J. SWIEGERS[3], S. PORTILLA[2], D. RYCZKO[3], A. IJSPEERT[4], A. SIMON[1];
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Abstract: Unlike other tetrapods, salamanders restore locomotor functions within a few weeks after full transection. Here, we present our first results on the characterization of the regeneration process at the functional, structural, cellular, and molecular levels. We established a spinal cord transection injury model in the emerging salamander model organism, *Pleurodeles waltl*. We monitored the animal health, bladder function, weight, and locomotor performance of the recovering salamanders. Albeit some inter-individual variations, the recovery process was consistent among injury groups: bladder function was restored from week 3, followed by the abilities to turn around and walk by weeks 4-5, culminating with the reestablishment of propagating swimming waves at weeks 7-10. To assess the extent of spinal cord regeneration, we scanned the salamanders using a Preclinical MRI 9.4T. We obtained high spatial resolution images (voxel size of 0.0375*0.0375*0.2mm) that allowed us to investigate the tissue morphology and delineate accurate segmentations at four different time points after injury. The regenerated spinal cords were slightly compressed dorsoventrally, showing a lack of fluid-filled space between the spinal cord and the vertebra in the ventral side. We found a diversity of tissue morphological variations in the regenerating animals, including thinner spinal cords, fluid-filled cysts, and potentially incomplete axonal recovery. Volumetric and tractography analyses proved decreased volumes as well as decreased number of fibers in the regenerating regions of the spinal cord compared to their uninjured rostral and caudal portions. Nevertheless, consistent with the recovery of locomotor performance, all animals reconnected the rostral and caudal ends of the injured spinal cord. To understand the cellular composition and to gain insights in cellular dynamics during regeneration, we performed single nuclei RNA sequencing and spatially resolved transcriptomics in the same animals in which behavior and tissue had been analyzed longitudinally. The data collectively provide a comprehensive picture of the molecular and cellular landscape involved in locomotion control as well as the injury response that leads to regeneration of tissue and function in salamanders.

Disclosures: A. Joven Araus: None. **Z. Tonelli Gombalova:** None. **Z. Yao:** None. **E. Llorens:** None. **S. Giatrellis:** None. **P. Damberg:** None. **L. Blanché:** None. **I. Khsime:** None. **J. Swiegers:** None. **S. Portilla:** None. **D. Ryczko:** None. **A. Ijspeert:** None. **A. Simon:** None.

Poster

PSTR540. Brain Injury: Human Studies

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.01/Y9

Topic: C.10. Brain Injury and Trauma

Support: NINDS U01NS093334 (JLC, EMR, MES, RAS)
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Black Men's Brain Health Emerging Scholars Fellowship (HA)
Grass Foundations Henry Grass, M.D. Rising Star in Neuroscience Award (HA)

Title: Sulcal Morphological Changes in Former American Football Players: Findings from the DIAGNOSE CTE Research Project

Authors: *H. ARCINIEGA[1,2], L. B. JUNG[3,5], K. BREEDOVE[4], T. L. T. WIEGAND[7], O. JOHN[3], N. KIM[2], Z. H. BAUCOM[8], F. TUZ-ZAHRA[8], Y. TRIPODIS[8], T. BILLAH[2], O. PASTERNAK[2,4,9], M. J. COLEMAN[2], C. H. ADLER[10], C. BERNICK[10], L. BALCER[11,12,13], I. K. KOERTE[3,14,6], A. LIN[15], J. L. CUMMINGS[16], E. M. REIMAN[17], R. STERN[18], M. E. SHENTON[19], S. BOUIX[19,20];
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Abstract: Research Objective and Rationale: Repetitive head impact (RHI) exposure in contact sports, such as American football, has been linked to the possible development of chronic traumatic encephalopathy (CTE). Unfortunately, CTE currently can be diagnosed only at postmortem. Here, we use *in vivo* MRI to detect sulcal morphological changes, one of the earliest signs of CTE in former American football players, and we analyze how age and exposure factors contribute to these effects.**Methods**: Data from the DIAGNOSE CTE Research Project was used to compare the depth and width of the cerebral sulci of former American football players with a history of RHIs (n=170) to unexposed asymptomatic controls (n=58). Sulcal areas of the superior

frontal and occipitotemporal regions were selected for analysis based on published postmortem CTE pathology findings. Their morphological measures were then estimated using CalcSulc, a computational tool used to estimate depth and width. We used a generalized least square model to compare the groups and to evaluate interactions with both age and age of first exposure to tackle football while controlling for age, body mass index, education, race, imaging site, Apolipoprotein E4 carrier status, and total intracranial volume. **Results**: Former American football players showed shallower sulcal depth in the left superior frontal region compared to the unexposed asymptomatic controls. Furthermore, we observed a significant age-by-group interaction in the sulcal depth and width of the left hemisphere occipitotemporal region indicating increased width and shallower depth as age increased in former American football players compared to controls. Finally, we found a significant association between the age of first exposure and left hemisphere occipitotemporal sulcal width.**Conclusion**: These findings suggest sulcal abnormalities observed *in vivo* in individuals with a history of RHI and could possibly indicate the presence of CTE pathology. Faster worsening of sulcal morphology with age in football players as well as an association with age of first exposure to tackle football support these observations as possibly indicative of a progressive condition. These results further suggest that *in vivo* brain morphometry measures show differences between participants with and without RHI and may potentially support the *in vivo* detection of CTE. Further studies with pathological verification of the presence/absence/severity of CTE are warranted.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.02/Y10

Topic: C.10. Brain Injury and Trauma

Support: NIH

Title: Imaging TSPO in the brains of former National Football League players

Authors: *S. SWEENEY[1], R. O'TOOLE[1], C. THOMAS[1], M. YOON[2], J. KILGORE[2], A. SOULE[1], V. KAMATH[2], M. G. POMPER[3], Y. DU[3], L. H. RUBIN[4], J. M. COUGHLIN[1]; [1]Dept. of Mol. Psychiatry, [2]Dept. of Med. Psychology, [3]Dept. of Nuclear Med., [4]Dept. of Neuroimmunology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: National Football League (NFL) players often endure repeated mild traumatic brain injury (mTBI) incurred through the collision sport of American football. The repeated mTBI may precipitate a prolonged, hyperactive glial response that associates with later onset of cognitive deficits. [^{11}C]DPA-713 can be used to track the availability of the translocator protein 18 kDa (TSPO), a marker of glial activation. In this study, we used [^{11}C]DPA-713 with positron emission tomography (DPA PET) to test for the hypothesized high levels of TSPO in a large cohort of former NFL players. DPA PET was used to quantify regional availability (V_T) of TSPO in the brains of 27 NFL players who stepped away from NFL play within the last 10 years, and 27 age-matched, former elite, non-collision sport athletes. All participants were college-educated males. Additional study procedures included brain MRI, neuropsychological testing, clinical interview, and symptom assessments. Longitudinal data collection at two-year follow-up is ongoing. Regional [^{11}C]DPA-713 binding (V_T) was quantified using Logan graphical analysis applied to the time-activity curves with radiometabolite-corrected arterial plasma input function. DPA V_T values were higher in NFL players compared to non-collision sport athletes (P<0.001), with largest differences in hippocampus and cingulate, frontal, and parietal cortices (each P<0.001). Former NFL players performed worse in verbal learning and memory compared to control subjects. At present, there are no changes in V_T over time in players (N=7) or controls (N=5). These new PET data support a persistent glial activation in recently-retired NFL players. Further study of the relationship between the glial response and memory impairment is warranted.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.03/Y12

Topic: C.10. Brain Injury and Trauma

Support: NIH R01NS123374
NIH R01NS082432
The Dana Foundation

Title: Alteration of White Matter Microstructure and Cognitive Performance by Soccer Heading

Authors: *B. DEMESSIE[1], R. FLEYSHER[2], M. L. LIPTON[2];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Columbia Univ., New York, NY

Abstract: Cumulative repetitive head impacts (RHI) in soccer are an area of public health concern due to potential for short and long-term adverse effects on brain function. Adverse effects of RHI due to soccer heading have been identified as alterations of white matter (WM) microstructure and cognitive dysfunction, which are similar to but independent of the effects of concussion. However, the association of WM measures with functional outcomes has not been reported in the context of soccer RHI. Our goal was to assess this relationship and to explore whether measures of abnormal WM microstructure mediate RHI associations with cognitive dysfunction.

Neurite orientation dispersion and density imaging (NODDI) and diffusion tensor imaging (DTI) were used to detect alterations of WM microstructure due to RHI, which are not visible on standard anatomical images. Amateur soccer players (n=309; 18-55 years old, 65% Male, 35% Female) were registered to JHU atlas. To find abnormalities in white matter, a region of interest analysis was done on the player's data in comparison to that of the control group. Fractional anisotropy, mean diffusivity, axial diffusivity, radial diffusivity, orientation dispersion index, intracellular volume fraction, and isotropic volume fraction within 48 JHU atlas regions were measured. Verbal learning and memory capacity were assessed using the CogState's international shopping list (ISL) task. The cumulative number of headers in the past year was estimated using HeadCount, a validated self-administered questionnaire. Age, sex, and history of concussion were included as covariates in the analysis. The significances of the following associations were tested (1) RHI with ISL, (2) RHI with DTI/NODDI metrics, (3) RHI and DTI/NODDI metrics with ISL. To test if DTI/NODDI metrics mediate the relationship between RHI and ISL, a bootstrap analysis with 10,000 simulations was conducted.

Our analysis revealed a significant total effect of RHI on DTI/NODDI metrics and significant direct effects of RHI on ISL across ROIs; however, there were no mediating effects of RHI on ISL through DTI/NODDI metrics. These results suggest that the association of RHI with decreased cognitive performance is not mediated by the substantial variance accounted for by DTI/NODDI metrics, which is correlated with change, and the strength of the association of heading with cognitive performance does not increase when DTI/NODDI metrics are considered. These findings illustrate the complex associations among RHI, white matter microstructure, and verbal memory and will guide future research into the role of WM microstructure changes in the development of cognitive symptoms.

Disclosures: B. DeMessie: None. **R. Fleysher:** None. **M.L. Lipton:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.04/Y13

Topic: C.10. Brain Injury and Trauma

Support: NIH R01NS123374
The Dana Foundation
NIH R01NS082432

Title: Soccer Player Executive Function and Verbal Learning are Adversely Associated with Microstructure Integrity of the Orbitofrontal Gray Matter-White Matter Interface

Authors: *J. Y. SONG[1], R. FLEYSHER[1], M. L. LIPTON[2];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Columbia Univ. Med. Ctr., New York, NY

Abstract: Heading is an integral part of the soccer. However, these repetitive head impacts (RHI) are associated with adverse cognitive performance. We hypothesize microstructural integrity at the orbitofrontal gray matter-white matter interface (GWI), a region susceptible to shear force trauma during head impact, is associated with RHI and with worse cognitive performance. We analyzed RHI (12-month heading from HeadCount), DTI (3.0T; 32 directions; b=800; voxel size 2mm^3), verbal learning (International Shopping List), verbal memory (International Shopping List-Delayed Recall) and executive function (Set Shifting Test) from 353 amateur soccer players (18-53, 27% female). To overcome limitations (e.g., misregistration and partial volume effects) we characterized the transition from low gray matter fractional anisotropy (FA) to high white matter FA by computing the slope of FA along a trajectory orthogonal to the GWI (defined by FreeSurfer) from each white matter voxel across the entire orbitofrontal region, as follows: we binned all orbitofrontal voxels by distance to the GWI and computed average FA within each bin. Average FA vs. distance to the GWI is fit to a 7th order polynomial. FA slope across the GWI is defined as the maximum slope magnitude of the polynomial fit. We fit linear models to test the associations of RHI with orbitofrontal FA slope and of orbitofrontal FA slope with cognitive performance. Greater RHI is associated with lower FA slope in the orbitofrontal region ($p= 0.00745$). Lower FA slope in the orbitofrontal region is associated with poorer verbal learning ($p=0.000422$), verbal memory ($p=0.0073$) and executive function ($p=0.00703$). The orbitofrontal region is a known predilection site for traumatic injury and is implicated in executive function and verbal learning, particularly verbal learning strategies.

Disclosures: J.Y. Song: None. **R. Fleysher:** None. **M.L. Lipton:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.05/Y14

Topic: C.10. Brain Injury and Trauma

Title: A sex-related difference of cognitive functions among college soccer players with and without concussion history during soccer season: pre vs post

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Abstract: Young college athletes can show cognitive deficits after experiencing a concussion earlier in life, and female athletes seem to show longer-lasting symptoms than males. However, whether sex-related differences exist for cognitive functions in NCAA Division III college players across a soccer season is unclear. We examined whether sex-related differences exist in cognitive functions of sustained attention and executive functioning among players with (CH) and without concussion history (NoH) and whether differences depend on the time of the season. We hypothesized sex-related cognitive function differences between CH and NoH players rather at the end of the season.Thirty athletes (M=19.2 yrs.), including 17 CH players (6 females, 11 males) and 13 NoH players (9 females, 4 males), participated in the study. All players performed two cognitive tests on a laptop pre and post-season: i) A Stroop color-word executive function test: Four words (blue, green, red, and yellow) were presented on the screen for 96 trials. In 48 trials, the color and meaning of the word were the same, representing the congruent condition. The other 48 trials were the noncongruent condition where word color and meaning differed. Players were instructed to always select the text color, not the semantic meaning, with a matching key press. ii) A D2 sustained attention test involved nine-count sequences containing varying combinations of the letters d and p presented on the screen. Each letter was framed with a different number of superscripts or subscripts of commas. Players had to press a ‘D2’ button when the letter d was surrounded by two commas and a ‘Not D2’ button otherwise. A new sequence of nine letters appeared once a previous sequence was finished. A block of sequences was terminated after 30 seconds, and 8 blocks were presented overall. Repeated ANOVAs were used to analyze response time (RT; milliseconds), error rate (ER; %), and sustained attention score (CS; D2 test only) in males and females with CH and NoH across a soccer season (Pre/Post). For the Stroop test, there was a significant Time*CH interaction in the congruent condition ($p<0.05$), showing a higher ER in CH post-season and a trend for a Sex*Group*Time interaction in the incongruent condition ($p=0.01$), showing CH females tended to have a higher ER post-season. There were no other significant effects regarding Group, Group*Sex, or Sex*Group*Time on ER (Stroop & D2), RT (Stroop & D2), and CS (D2) (all $p>0.05$).Our results suggest cognitive deficits with decision-making in a Stroop task in CH NCAA Division III soccer players post-season. This deficit may be larger in female athletes when the task requires response inhibition.

Disclosures: P. Acharya: None. **M. Webb:** None. **M.A. Yeomans:** None. **M. Dalecki:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.06/Y15

Topic: C.10. Brain Injury and Trauma

Support: ERA-NET Neuron [01EW1707]

Title: Functional connectivity in the brain at rest over a play season in elite youth soccer players

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Abstract: Repetitive head impacts (RHIs) are often reported in contact-sports athletes. Although RHIs are considered subconcussive, evidence is accumulating that they may impact brain structure (Koerte 2012, 2016, Lipton, 2013) and behavior. Regarding brain function at rest (rs-fMRI), research on the effects of RHIs is limited. In soccer, a few studies report global and local functional connectivity (FC) changes post-season (Dudley 2020; Cassoudesalle 2020). Here, we assess changes in rs-FC within and between motor, cerebellar and default mode networks in young elite soccer players relative to young elite athletes from non-contact sports. Youth athletes are of interest as neck muscle strength, motor control and brain grey and white matter are not yet fully developed. Data from youth athletes (aged 13-16 yrs) were acquired as part of the REPIMPACT study at three sites (Koerte, 2022; includes protocol details). rs-fMRI data were preprocessed and normalized to MNI space. To minimize the effect of head motion on FC strength, 3 thresholds were set: framewise displacement <0.20, time in motion spikes <20%, and DVARS (Derivative of rms VARiance over voxelS) <1.5. This resulted in an overall sample of 215 datapoints; time point 1 (TP1): n=85, TP2: n=66, TP3: 64. No significant differences in head motion between groups and time points were observed. ComBat harmonization was applied to the remaining datapoints to reduce the impact of multi-site variability. Linear Mixed Effects Models were used to analyze (i) group differences in FC strength, (ii) time differences in FC strength, and (iii) interactions between group and time. Covariates were age at TP1, time since TP1, and site. In addition, within the soccer group, we associated (Pearson) FC strength with RHI exposure (using yrs of playing soccer as proxy). There was a main effect of group for FC

strength between the left default mode and right (p = 0.040) and left cerebellar networks (p = 0.044), with higher FC strength for the control relative to the soccer group. However, these findings did not survive a Bonferroni correction (adj. α = 0.002). Within the soccer group, no significant association was observed between FC strength and RHI exposure (all $p_s > 0.05$). In the present cohort of youth elite soccer players, we found no evidence for systematic changes in rs-FC strength over a season of play, when compared to a group of elite athletes from non-contact sports. Note that participants with a concussion history were excluded, which may limit the generalizability of these findings to all contact-sport athletes of this age group. Moreover, these data were not suggestive of an association of RHI exposure and rsFC changes in youth elite soccer players.

Disclosures: **T. Santos Monteiro:** None. **C. Seer:** A. Employment/Salary (full or part-time):; KU Leuven, Department of Movement Sciences. **E.M. Bonke:** None. **E. Yhang:** None. **D. Kaufmann:** None. **S. Kirkerud Sandmo:** None. **A. De Luca:** None. **A. Lin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder BrainSpec. F. Consulting Fees (e.g., advisory boards); Agios Pharmaceuticals, Biomarin Pharmaceuticals, DesignTx, and Moncon MRI. **Y. Tripodis:** A. Employment/Salary (full or part-time):; Boston University School of Public Health. **N. Sochen:** None. **R. Bahr:** None. **A. Leemans:** None. **O. Pasternak:** None. **S.P. Swinnen:** None. **I.K. Koerte:** A. Employment/Salary (full or part-time):; Vice President European Neurotrauma Organization (unpaid), European editor Journal of Neurotrauma (unpaid), Professor at Ludwig-Maximilians-University Munich. 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.; NINDS R01, ERC Starting Grant, ERA-NET Neuron, Schatt Foundation, Abbott Inc, German Ministry of Education and Research, European Research Council, Marie-Sklodowska-Curie Program. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); MRI-related equipment from May Clinic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Siemens AG, Siemens Healthineers, Thieme Publisher. Other; in-kind contribution. China Scholarship Council collaboration with Ludwig-Maximilians-University Munich, Abbott Inc. (paid expert advisor). **J. Gooijers:** A. Employment/Salary (full or part-time):; KU Leuven, Department of Movement Sciences.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.07/Y16

Topic: C.10. Brain Injury and Trauma

Support: CON243227

Title: Examining acetylated-tau levels in student athletes in contact sports

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Abstract: Mild traumatic brain injuries (mTBI) are responsible for 90% of all traumatic brain injuries (TBI) worldwide. Leading causes of TBI from 15-24 years of age are sports and recreational activities. Symptoms are minimal within 1 week of a mTBI, making it difficult to determine when it is safe to resume normal or athletic activities. Importantly, repetitive mTBIs can have long-lasting effects, including chronic neurodegeneration, so early and accurate diagnosis is important for initiation of care. Acetylated tau (ac-tau) is a therapeutic target-based blood biomarker of neurodegeneration in TBI, and blood levels of ac-tau correlate with injury intensity. How long ac-tau remains elevated in the blood after injury, however, is not known. Ac-tau is significantly increased in the blood within 24 hours of TBI in people across a range of injury intensities, before changes in phosphorylated tau or total tau are observed. Here we report our work to track blood levels of ac-tau pre and post mTBI over a sport season in National Collegiate Athletic Association contact sport. Contact sport groups included football, soccer, lacrosse, and wrestling, and low/non-contact sports included basketball, volleyball, cross country/track, and ultimate frisbee. Blood samples were collected from student athletes before their respective sport seasons begin along with their history of any past injuries. During the season, blood samples were collected within 48 hours of a head injury, and again once the player was clinically cleared to return to play. At the end of the season, a sample was collected from each athlete, regardless of whether they had experienced any injury during the season. Analysis of the ac-tau levels in these samples will inform on the trajectory of accumulation of ac-tau with respect to head injury and clinically-determined recovery in student athletes engaged in contact and non-contact sports.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.08/Y17

Topic: C.10. Brain Injury and Trauma

Support: NSF/BCS 1946036
NIH 5 T32 MH103213-08

Title: Episodic Memory Impairment Following Sport-Related Concussion in Indiana University Athletes

Authors: *G. D. NAH[1,2], J. D. CRYSTAL[1,2], N. L. PORT[3,1];
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Abstract: Memory and learning impairment are common cognitive deficits following mild traumatic brain injury (mTBI). Previous research has shown mTBI can cause neuronal inflammation and injury to the hippocampus, which can cause memory impairments. Our previous studies evaluated episodic memory impairment in Sprague-Dawley rats using the Item-in-Context task (Panoz-Brown et al., 2016) and our modified weight drop model. Rats who undergo an mTBI with this injury model have an episodic memory deficit that persists for four days and recovers in eight days compared to the sham (uninjured) group. The sham group did not show any signs of episodic memory impairment. In this study, we developed a computerized version of the Item-in-Context task to evaluate episodic memory function in Indiana University athletes who sustain a sport-related concussion. In our ongoing study, overall memory performance increased modestly (~4%) from an early time point (within 72hrs post-injury) to a late time point (4-6 weeks post-injury). By contrast, episodic memory performance does not change over the same time duration. We also did not observe a meaningful difference of reaction time between the two time points (KStest: p= 0.96). This ongoing study has the potential to be the first study to provide a parallel assessment of episodic memory function in two species.

Disclosures: G.D. Nah: None. **J.D. Crystal:** None. **N.L. Port:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.09/Y18

Topic: C.10. Brain Injury and Trauma

Title: The importance of baseline concussion testing in neurodiverse collegiate student-athletes

Authors: M. DOBSON, *C. J. KETCHAM, E. E. HALL;
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Abstract: Baseline neurocognitive tests are a key component to concussion management protocols and are useful in helping make return-to-play (RTP) decisions. Previous research has shown that neurodiverse populations including attention-deficit/ hyperactivity disorder (ADHD), dyslexia, and autism (ASD) have differences in executive functioning, impulse control, and symptoms that may impact baseline concussion testing measures and frequency of concussions. ADHD impacts inhibition, working memory, and cognitive flexibility resulting in issues with attention, impulse control, and emotional regulation. Previous research indicates that ADHD is a risk factor for concussion frequency. Research also shows that concussions and ASD have many shared symptoms, specifically regarding mental health and cognition. ASD has high rates of comorbidity with ADHD. Dyslexia is a neurodevelopmental disorder related to difficulties learning how to read and write. The purpose of this study was to examine the differences in neurocognitive performance, history of concussions, and symptoms in neurodiverse student-athletes. 5628 (female=2921; 18-24yrs) collegiate student-athletes completed the Immediate Post-Concussion Assessment and Cognitive Testing (ImPACTTM) test. 838 identified as neurodiverse (ADHD=602, dyslexic=161, autistic=8). Concussion history including number of previous concussions and current symptoms, and severity, were collected as part of the ImPACT. Outcome neurocognitive composite measures include visual and verbal memory, reaction time, visuomotor speed, and impulse control. Significant differences were found for neurodiverse athletes for number of previous concussions ($p<0.001$), total symptom score ($p<0.001$), verbal memory ($p<0.001$), visual memory ($p<0.001$), reaction time ($p<0.001$), visuomotor speed ($p<0.001$), and impulse control ($p<0.001$). Further analysis breaks out by diagnosis with differences across memory and symptom measures for all groups ($p<0.05$). These findings suggest that neurodiverse identities are important to know as part of concussion baseline testing to support RTP management. Further discussion will include a more detailed analysis of symptoms at baseline for neurodiverse populations and considerations for concussion education and management.

Disclosures: M. Dobson: None. **C.J. Ketcham:** None. **E.E. Hall:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.10/Y19

Topic: C.10. Brain Injury and Trauma

Support: U.S. Army Medical Research and Materiel Command
Traumatic Brain Injury Center of Excellence
General Dynamics Information Technology for the U.S. Defense Health Agency

Title: Impaired cognitive task performance and elevated resting state global cortex excitability in military mTBI patients

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Abstract: Mild traumatic brain injury (mTBI), one of the most prevalent brain injuries incurred among military service members (SMs), pose a significant challenge to military missions and SMs' quality of life. For SMs who have experienced mTBI, using an ecologically validated military task to assess their cognitive ability is imperative for making return-to-duty decisions. This study examined the effect of mental workload (MW) on the performance of 30 mTBI and 15 control SMs on a cognitive task in an immersive virtual reality environment (iVRE). Additionally, the global cortex excitability and the excitation/inhibition (E/I) balance in the resting state, as measured with the abundance of the periodic alpha oscillations (8-14Hz) and the exponent of the aperiodic component of electroencephalography, were assessed before and after task performance to explore the neural mechanisms underlying impaired cognitive performance. Participants engaged in two identical one-back military patrol tasks with higher MW in one task, where battlefield radio chatter was continuously broadcast and oral responses to specific chatter cues were required. In both one-back tasks, mTBI participants performed worse than the controls as assessed by several measures: significantly slower shooting response time on the one-back task (1441.1 ± 122.9 ms vs 1328.5 ± 137.4 ms) and slightly slower shooting response time on the one-back with chatters task, although not statistically significant (1381.9 ± 110 ms vs 1357 ±140.8 ms); lower percent correct shots (81.8 ± 8.8% vs 88.9 ± 7.2% and 81.4 ± 10% vs 83.5 ± 11.5%), and less percent correct responses to chatter cues (83 ± 11% vs 86.6 ± 6%). Interestingly, the abundance of periodic alpha oscillations decreased significantly (~20%) in mTBI participants but increased significantly in control participants (~30%) after performing the two cognitive tasks, indicating opposite changes in global cortex excitability between mTBI and control participants. The aperiodic component decreased significantly in mTBI participants (~30%) but remained unchanged in control participants, indicating a higher degree of unbalanced E/I ratio toward excitation in mTBI participants. Together, these findings underscore the impact of mTBI on performing cognitive tasks in general, and on coping with increased MW specifically within an iVRE, thereby shedding light on the neural mechanisms underlying impaired cognitive functions in mTBI.

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Poster

PSTR540. Brain Injury: Human Studies

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.11/Y20

Topic: C.10. Brain Injury and Trauma

Title: White Matter Microstructural Changes and Neuropsychiatric Outcomes in Military Service Members with Mild Traumatic Brain Injury: A DTI and NODDI Study

Authors: *S. KIM[1], P.-H. YEH[2], J. OLLINGER[3];
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Abstract: Introduction Mild Traumatic Brain Injury (mTBI) is the signature wound among military service members (SMs) and has been associated with poor neuropsychiatric outcomes. Yet, despite mounting evidence that mTBI can affect long-term outcomes, there is a lack of reliable clinical tools for mTBI diagnosis and prognosis. This study investigated white matter (WM) integrity using diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI) in conjunction with post-injury neuropsychiatric sequalae in military SMs.**Methods**We studied a total of 99 SMs enrolled in a study at the National Intrepid Center of Excellence (NICoE): 65 male subjects (mean age 40.46±4.99) with a history of mTBI and 34 age-matched male controls (mean age 38.94±5.57). All subjects were scanned on a 3T MRI scanner with multi-shell, multiband and in-plane acceleration diffusion MRI (dMRI). Voxel-wise statistical analysis was performed using Tract-Based Spatial Statistics (TBSS) in FSL. Individual maps (fractional anisotropy [FA], mean diffusivity [MD], radial diffusivity [RD] and axial diffusivity [AD], orientation dispersion index [ODI], isotropic volume fraction [ISOVF], intra-cellular volume fraction [ICVF]) were projected onto the mean FA skeleton and voxelwise cross-subject statistics were executed. All subjects completed the PTSD Checklist - Civilian version (PCLC). mTBI subjects completed the Neurobehavioral Symptom Inventory (NSI) for post-concussion symptoms. **Results**Compared to control subjects, mTBI subjects presented with significantly higher PTSD symptoms (PCLC scores: Control=18.97 (3.77), mTBI=41.23 (11.56)). ODI was significantly reduced in mTBI subjects in the right anterior thalamic radiation (Control= 0.63, mTBI=0.51 $p<0.05$, FWE-corrected). General linear models with age as a covariate revealed that ODI values were predictors of PTSD ($\beta=-.278$, $p<0.05$) and cognitive-related post-concussion symptoms ($\beta=.317$, $p<0.05$).**Conclusion**Our results support previous evidence on the neuropsychiatric consequences of military-related mTBI that may be associated with post-injury WM structural disruptions. Specifically, our findings suggest that mTBI may lead to WM changes, as reflected by a lower ODI in the anterior thalamic radiation. This implicates reduced angular variations, loss of structural integrity, and reduced organizational complexity of the axons. Ultimately, understanding how mTBI and its neuropsychiatric sequalae influence WM structure will help inform and refine diagnostic, prognostic, and therapeutic studies, with the ultimate goal of improving outcomes for mTBI survivors.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.12/Z1

Topic: C.10. Brain Injury and Trauma

Support: VA RR&D IK2RX002490
VA RR&D I50RX003000

Title: Functional Fronto-Limbic Connectivity in Post-Traumatic Stress Disorder and Traumatic Brain Injury

Authors: R. CLAAR[1], *D. G. LAMB[2];
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Abstract: Post-traumatic stress disorder (PTSD) and mild traumatic brain injury (mTBI) are heterogeneous, disruptive medical conditions. mTBI often co-occurs or precedes PTSD, particularly in military contexts, and investigations into the underlying neurobiology of PTSD and mTBI often identify overlapping symptom profiles including emotional dysregulation and executive dysfunction and concomitant altered brain activity in frontal and limbic brain regions. This overlap in symptom presentation creates diagnostic ambiguity treatment challenges for clinicians, contributing to reduced quality of life and life participation in impacted individuals. Investigations into the underlying neurobiology of PTSD and mTBI could identify common and differential etiology, improving diagnostic clarity and leading to more personalized treatments for those affected. In a significant portion of patients, current first-line treatments for PTSD do not achieve clinical response, let alone remission, and much is still not understood about the underlying etiology of PTSD and mTBI symptoms. We evaluated a cohort of US Veterans (age 18-45) across four groups: healthy controls (HCs), PTSD and no history of TBI, history of mTBI and no history of PTSD, and history of both PTSD and mTBI. Participants' health records were reviewed to confirm diagnoses and identify any potential confounding factors. All individuals were administered a 2-3-hour battery of neuropsychological, cognitive, and emotional assessments along with structural and functional magnetic resonance imaging. We assessed in activity within and functional connectivity (FC) between frontal and limbic regions during an emotional image evaluation task and the relationship between these variables and assessment scores. Differences in activation to emotionally evocative images were seen between PTSD and mTBI individuals and HCs, particularly in the insula. An ANOVA showed significant differences in FC between our study groups while viewing negative valence images. Follow-up testing revealed FC differences between the medial prefrontal cortex, insular cortices, amygdala, and hippocampus. These results help advance our models of emotional dysregulation in PTSD and mTBI and can serve as foundational information for the development of neurobiologically-based treatments of PTSD and mTBI.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.13/Z2

Topic: C.10. Brain Injury and Trauma

Title: Using magnetoencephalography and magnetic source imaging to track and assess recovery after severe traumatic brain injury

Authors: *N. C. SCHOEDEL[1], A. K. SARMA[2], G. POPLI[2], A. ANZALONE[2], N. CONTILLO[2], C. CORNELL[2], A. NUNN[2], J. A. ROWLAND[4], L. A. FLASHMAN[2], D. COUTURE[2], J. R. STAPLETON-KOTLOSKI[3], D. W. GODWIN[5];
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Abstract: Traumatic brain injuries (TBI) are a leading cause of disability and death globally. As TBI increases in severity, a patient's recovery increases in complexity and their chance of survival decreases. Currently, limited resources exist to track and assess recovery from a severe TBI. Magnetoencephalography (CTF Systems, Inc., MEG 2005) holds promise for evaluating severe TBI patients and identifying electrophysiological biomarkers that are associated with levels of recovery post-injury. This study examined the brain function of patients, (n=7, ages 26-71; IRB 57568; acute Glasgow Coma Scale scores of 3-8) tracking brain activity changes longitudinally at three time points. The first scan occurred at 6.6 +/- 4.3 (mean +/- SD) days post-injury, followed by a second scan 47.6 +/- 20.6 days post-injury followed by a third scan 7.8 +/- 1.5 months post-injury; neuropsychological testing was also conducted at the third scan. Only 4 of 7 participants completed the third scan. MEG data from 30 healthy military veteran participants served as a control group. Using synthetic aperture magnetometry (SAM), we mapped each participant's brain activity at each scan to be used for analysis and comparison. Group-level analysis for the first scan, relative to controls, showed excessive delta neural generators and reduced beta/gamma neural generators, consistent with the comatose state. Alpha/beta power levels were elevated posteriorly and ventrally across all patients, possibly compensating for reduced neural generators in these bands. Patient-level analysis of SAM maps revealed hypofunction zones, islands of preserved activity, and hemispheric asymmetry across bandwidths, with reduced power in the injured hemisphere. At scan 2, brain activity normalized overall, shifting power dorsally and anteriorly, with only increased beta power relative to controls. Individual SAM source maps revealed variability in outcomes, with some exhibiting increased power relative to the first scan, especially in gamma, but continued regions of hypofunction in damaged areas. At the third scan, the patients displayed increased group-wise delta power compared to controls, possibly indicating ongoing secondary neurodegeneration after initial diffuse axonal injury. Individual SAM maps at the third scan continued to vary, with patients exhibiting continued hemispheric asymmetry including diminished power at the injury site. In summary, this pilot study demonstrates MEG's utility in tracking brain function recovery following severe TBI and revealing patient-specific altered activity regions. This represents insight that can be mapped to aspects of functional recovery in future work.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.14/Z3

Topic: C.10. Brain Injury and Trauma

Support: The Sunny Brain Tumor and Brain Disease Research and Development Fund (106-5310-001-400)

Title: Association between IGF-1 and cognitive impairment in mild traumatic brain injury patients evaluating via WCST-64

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Abstract: Traumatic brain injury (TBI) is an important cause of adult injury-related morbidity and mortality. Common acute manifestations of TBI include dizziness, headache, cognitive deficits, and emotional problems. Cognitive deficits are often the most disabling and distressing for the affected persons, family members, and society. Insulin-like growth factor-1 (IGF-1) is a polypeptide closely related to neurons' growth and aging. Previous studies demonstrated that IGF-1 therapy promotes hippocampus neurogenesis and improves spatial memory in rats. This study aims to evaluate the association of IGF-1 and cognitive performance between mTBI and health control groups. 295 mild TBI (mTBI) patients and 200 health control agreed to join and completed all questionnaires. Wisconsin Card Sorting Test 64 (WCST-64) is an instrument to evaluate cognitive impairment. The mean difference between the two groups was compared via student T-tests and Chi-Square tests for continuous and categorical variables. The association of IGF-1 and WCST-64 scores was evaluated via linear regression. The percentage of female participants and the average education years between the two groups were statistically significantly different. However, the average age between the two groups was not significantly different (mTBI:44.3±14.12, control 44.08±14.96). There were 184 (62.37%) female participants in the mTBI group and 136(68%) females in the control group. The average education year of the control group was more than that of the mTBI group(control: 14.20±2.60, mTBI: 12.89±3.18). The average raw scores of five indices in the mTBI group were higher (worse) than those in the health control group. On the other hand, the average standardized scores of these five indices in the mTBI group were lower (worse) than those in the health control group. IGF-1 levels were significantly related with the following scores: total score, correct score, perseverative response, perseverative error, non-perseverative error, and conceptual response. 7 of 10 WCST raw scores significantly differed between brain injury and non-brain injury groups after adjusting by age and education level. 6 of 7 significant WCST raw scores were associated with the IGF-1 levels after adjusting by age and education level. Our finding suggests that IGF-1 was related to cognitive impairment after mTBI, supporting further investigation of its potential as a biomarker.

Disclosures: **Y. Chiang:** None. **K. Chen:** None. **J. Ou:** None. **C. Wu:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.15/Z4

Topic: F.04. Neuroimmunology

Support: Herman Dana Foundation

Title: Decreased mononuclear cell NR3C1 SKA2 and FKPB5 expression levels among adult survivors of suicide bombing terror attacks in childhood are associated with the development of PTSD

Authors: *T. GOLTSER[1,2], A. SHALEV[2], F. BENARROCH[2], L. CANETTI[3], R. MASARWA[2], J. MARTIN[2], D. PEVZNER[1], O. OZ[1], C. SALONER[1], R. AMER[1], M. LAVON[1], A. LOTAN[1], E. GALILI-WEISSTUB[2], R. SEGMAN[1,2];
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Abstract: Life threatening trauma and the development of PTSD during childhood, may each associate with transcriptional perturbation of immune cell glucocorticoid reactivity, yet their separable longer term contributions are less clear. The current study compared resting mononuclear cell gene expression levels of the nuclear receptor, subfamily 3, member 1 (NR3C1) coding the glucocorticoid receptor, its trans-activator spindle and kinetochore-associated protein 2 (SKA2), and its co-chaperon FKBP prolyl isomerase 5 (FKBP5), between a cohort of young adults first seen at the Hadassah Emergency Department after surviving suicide bombing during childhood, and followed longitudinally over the years, and matched healthy controls not exposed to life threatening trauma. While significant reductions in mononuclear cell gene expression levels were observed among young adults for all three transcripts following early trauma exposure, the development of subsequent PTSD beyond trauma exposure, accounted for a small but significant portion of the variance in each of the three transcripts. Long-term perturbation in the expression of immune cell glucocorticoid response transcripts persists among young adults who develop PTSD following life threatening trauma exposure in childhood, denoting chronic dysregulation of immune stress reactivity.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.16/Z5

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant K01HD083459

Title: Behavioral correlates of white matter microstructure of the corpus callosum and bilateral cingulum in children with traumatic brain injury

Authors: F. ONTIVEROS[1,2], W. I. MATTSON[1], K. VANNATTA[1,3], W. CUNNINGHAM[4], E. A. WILDE[5], K. O. YEATES[6], *K. R. HOSKINSON[1,3];
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Abstract: Introduction. The corpus callosum (CC) and cingulum bundles (CB) are important white matter (WM) pathways that enable signaling across cortical brain regions. Traumatic brain injury (TBI) increases risk of disrupted WM microstructure. This study explored variations in WM microstructure along the CC and CB in youth with moderate to severe TBI (msTBI) relative to youth with complicated-mild (cmTBI) or orthopedic injury (OI), and links among WM and emotional and behavioral function. **Methods.** Participants included youth with msTBI (*n*=19, M_{age}=11.46y, 13 male), cmTBI (*n*=13, M_{age}=12.40y, 9 male), and OI (*n*=24, M_{age}=11.60y, 17 male). Parents rated their child's emotional and behavioral function on the Child Behavior Checklist (CBCL). Children underwent MRI in a 3T Siemens scanner, including T1-weighted and 64-direction DTI sequences. FreeSurfer's (v7.3.2) TRACULA performed automated probabilistic tractography reconstruction of the CC and CB, providing mean diffusivity (MD) and fractional anisotropy (FA) values over entire pathways, which were extracted for group comparison. Due to skewed cross-group distribution, nonparametric statistics quantified group differences (Kruskal-Wallis) and cross-domain correlation (Spearman Rho). **Results.** Group differences reflect poorer function in youth with msTBI based on parent-rated social (msTBI<cmTBI) and total competence (msTBI<cmTBI), anxiety/depression (msTBI<cmTBI), withdrawal/depression (msTBI<OI), thought problems (msTBI<OI), sluggish cognitive tempo (msTBI<cmTBI and OI), and obsessive-compulsive problems (msTBI<cmTBI), all *p*s<.05. Reduced FA was noted in the body, genu, and splenium of the CC and bilateral CB (all msTBI<cmTBI), and increased MD in the body, genu, and rostrum of the CC and bilateral dorsal CB (msTBI<cmTBI or OI). Better social and total competence were linked with greater FA in the splenium (*r*=.410) and bilateral CB (*r*s=.375-.383), while worse anxiety/depression was associated with lower FA in the splenium (*r*=-.322) and bilateral CB (*r*s=-.318-.374). Sluggish cognitive tempo and post-traumatic stress were linked with reduced FA in the splenium (*r*=-.342) and left ventral CB (*r*=-.363), respectively. Associations with MD were less consistent. **Discussion.** Difficulties in behavioral function were confirmed in youth with msTBI, with links with WM quality in tracts that provide cross-hemispheric (CC) and anterior-posterior (CB) cortical links. These hint at mechanistic brain-behavior origins of morbidities that could serve as a physiological marker of relative risk (e.g., following shear injury) and response to intervention (e.g., via microstructural improvement).

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.17/Z6

Topic: C.10. Brain Injury and Trauma

Support: CIHR FDN154291
CIHR MOP115172
Doctoral Research Award: Canada Graduate Scholarships

Title: Sleep slow-wave and spindle coupling in chronic moderate to severe traumatic brain injury

Authors: *N. KALANTARI[1,2], J.-M. LINA[3,2], H. BLAIS[2], E. SANCHEZ[4], J. CARRIER[1,2], N. GOSSELIN[1,2];
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Abstract: Electroencephalographic (EEG) slow waves (≤ 4 Hz, > 75 microV) and sleep spindles (bursts of 11-15 Hz EEG activity) are neural oscillations that occur during non-rapid eye movement (NREM) sleep and play significant roles in cognition and brain plasticity. Moreover, slow-wave-spindle coupling -the statistical relationship between the two frequency bands, where the phase of slow waves modulates the amplitude of sleep spindles- is thought to facilitate memory consolidation. Aging impairs this coupling, as evidenced by less precise and weaker slow-wave-spindle coupling among healthy older adults compared to their younger counterparts. Moderate to severe traumatic brain injury (TBI) results in widespread cerebral atrophy, accelerated brain aging, and chronic cognitive deficits. However, whether TBI mirrors the aging effect on slow-wave-spindle coupling remains to be investigated. We hypothesized that TBI patients would demonstrate impaired slow-wave-spindle coupling compared to healthy individuals. In this cross-sectional study, we compared a group of 43 chronic moderate to severe TBI patients (mean age = 32.1 ± 14.0 years, 14 females) one to four years post-injury, with 37 healthy control adults of similar age and sex (mean age = 30.4 ± 12.7 years, 11 females). All subjects underwent overnight in-laboratory polysomnography using a 19-channel EEG montage. EEG artifact rejection was performed automatically, followed by visual inspection. Sleep slow waves and spindles were automatically detected on artifact-free epochs on frontal (F3 and F4) and central (C3 and C4) EEG channels during NREM sleep for all sleep cycles. We computed the strength of slow-wave-spindle coupling for both frontal and central regions and found greater variability in the frontal EEG channel-derived coupling strength in the TBI group compared to

controls. Using repeated measures general linear model in SPSS Statistics, with EEG channel as a within-subject factor, group as a between-subject factor, and age as a covariate, we found no significant interaction between group and regions ($F_{(1, 77)} = 1.31$, $p = 0.26$). Interestingly, and contrary to our hypothesis, TBI patients did not differ significantly from healthy controls in terms of slow-wave-spindle coupling strength ($F_{(1, 77)} = 0.95$, $p = 0.33$). These findings suggest that the injured brain can still orchestrate slow waves and spindles just like the healthy young brain. Alternatively, our findings may hint at the brain's potential to regain its ability to coordinate these neural oscillations during the chronic stage of injury, although further research is needed to confirm this hypothesis.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.18/Z7

Topic: C.10. Brain Injury and Trauma

Title: Anosognosia in Traumatic Brain Injury

Authors: *T. BERESFORD[1], P. J. RONAN[2];
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Abstract: Methods: We conducted a randomized, placebo-controlled, double-blind, clinical trial of valproate in patients, N=48, one year or more following mild and moderate traumatic brain injury (TBI) patients who suffered poorly controlled Irritability one year or more prior to enrollment. Using an Irritability scale, we interviewed both index subjects and their significant other persons, usually family members. The concordance between these two sources of information served as a primary variable of interest In the present comparison. Results: 1) As a group at baseline, the TBI subjects consistently rated their Irritability symptom frequencies as low while, by contrast, their significant others rated them as far more variable and significantly more frequent, p=0.05. 2) Further, the subjects reported no difference in Irritability measures on active drug versus placebo while the significant other persons reported a statistically significant improvement in the subjects' Irritability, p=0.03. Discussion: This study suggests that anosognosia may play a role in the inability of some persons suffering TBI to assess their own mood states and behaviors one or more years after a mild to moderate injury, the time needed for brain healing. For many, the observations of a close observer, such as a family member who witnesses behaviors directly rather than after the fact in self-report, supports the axiom that what people do is more important than what they say. Further, anosognosia itself may obscure effective treatment results and will always require third party observations for greater accuracy as in this study. Conclusion/Implications: Anosognosia, as defined above, must be accounted for

in treatment trials against traumatic brain injury and, likely, other brain impairing conditions. The data from this study do not address involvement of the right hemisphere owing to the non-specificity of the TBI's reported. Further data may do so as knowledge in this field expands.

Disclosures: T. Beresford: None. **P.J. Ronan:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.19/Z8

Topic: C.10. Brain Injury and Trauma

Title: Efficacy of rTMS in improving memory in older adults with TBI

Authors: M. M. ADAMSON[1,3,4], X. KANG[1,3], V. LIOU-JOHNSON[7,9], D. JESTER[8], S. SEENIVASAN[11], D. BALDINI[5,12], *J. W. ASHFORD, Jr.[2,6], M. ZEINEH[10], E. DENNIS[13]; [1]Neurosurg., Stanford Univ., Palo Alto, CA; [2]Stanford Univ., Redwood City, CA; [3]Wriisc-women, [4]Rehabil. Service, [5]WRIISC-WOMEN, [6]WRIISC - War Related Illness & Injury Study Ctr., VA Palo Alto Hlth. Care Syst., Palo Alto, CA; [8]WRIISC-WOMEN, [7]VA Palo Alto HCS, Palo Alto, CA; [9]Clin. Excellence Res. Ctr., [10]Radiology, Stanford Univ. Sch. of Med., Stanford, CA; [11]Uniformed Services Univ. of Hlth. Sci., Washington, DC, DC; [12]Dept. of Psychology, Palo Alto Univ., Palo Alto, CA; [13]Neurol., Univ. of Utah, Salt Lake City, UT

Abstract: Background: Previous studies document the relationship between injury severity, cognitive impairment and functional status. Regardless of injury severity, one of the most frequently reported post-TBI sequelae is cognitive dysfunction including memory problems and executive function. Immediate and delayed recall is often used to examine episodic memory in humans. Paired Associate Learning (PAL) is often used to examine episodic memory in humans. Studies show that hippocampal-whole brain connectivity-behavior relationships were not isolated to single networks, but spanned multiple brain networks and were unique for each behavioral measure. Repetitive TMS delivers therapeutic, non-invasive brain stimulation and is FDA-approved for treatment for major depression. Repetitive TMS has also been used for memory enhancement. To date, no studies have examined rTMS treatment in older adults with a history of TBI. Here we a) assess the efficacy of rTMS to predict improvement in memory performance pre and post rTMS intervention in older patients with TBI, and b) assess hippocampal glucose uptake as a biomarker to detect these changes in memory performance.Method: In a double-blind randomized clinical trial, we used rTMS (10 Hz) treatment on the left dorsolateral prefrontal cortex (LDLPFC) to improve memory problems in older adults with a history of TBI. We enrolled (n=19) Veterans and civilians (mild and moderate TBI; mean age=62.63) in either a placebo or an active trial arm. Participants were enrolled in a PET-MRI, and neuropsychological assessment (including storytelling and visual memory such as PAL) at baseline, after 10 treatments, and six month follow-up. Functional neuronavigation was used to locate DLPFC (using resting state fMRI).Results: Preliminary comparative analysis

indicated significant differences (p<.05) on pre-post assessment in memory measures in the active group. Significant results were observed in immediate (p=0.003) and delayed (p=0.007) story memory recall and also PAL task (p=0.05) in the active group, but not sham. The Active Mean Difference in PET in the hippocampus (DG, subiculum, CA1 but not ERC/PRC) pre-post rTMS was higher than the Sham Mean Difference, but these did not reach significance.Conclusions: Despite the low number of participants, due to COVID-19, a significant effect of rTMS treatment to LDLPFC in improving storytelling and visual paired associate memory tasks. This research highlights the importance of assessing memory paradigms that are directly correlated with hippocampal-mediated cognitive decline and TBI-associated health problems in older adults and the treatments that can be used to mitigate them.

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Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.20/Z9

Topic: C.10. Brain Injury and Trauma

Title: Traumatic brain injury and mechanical ventilation: clinical implications for long-term autonomic dysfunction

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Abstract: In severe cases of traumatic brain injury (TBI: Glasgow Coma Scale (GCS) < 8), patients may be placed on mechanical ventilators to help maintain cardiopulmonary function. While providing life-saving intervention is the primary objective, ventilator use has troublingly been associated with long-term complications in respiratory disease patients including changes in diaphragm function and arterial O_2 regulation indicative of autonomic nervous system (ANS) dysfunction. However, the potential development of ventilator-induced diaphragmatic dysfunction (VIDD) in severe TBI patients following ventilator use contributing to poorer clinical outcomes has not been thoroughly examined. The purpose of this project was to determine the influence of mechanical ventilator utilization on clinical prognoses and outcomes of severe TBI indicative of prolonged diaphragmatic dysfunction and autonomic nervous system impairment. Electronic medical records from a local hospital network pulled between May 2018 and March 2022 identified 58 patients admitted with a moderate or severe TBI; 15 of whom were placed on a ventilator. Variables collected for retrospective analysis included length of hospital stay (days), range of recorded GCS scores, oxygen saturation, and length of time on mechanical

ventilation (hours) if the patient received such treatment. Pearson's coefficient revealed a highly positive correlation between the time spent on ventilation and the length of hospital stay ($r = 0.747$; $p<0.001$). Patients that underwent ventilation also displayed significantly lower GCS scores upon admission (10.33 ± 3.52 vs. 14.69 ± 1.00; $p<0.001$) as well as greater range of GCS scores (8.56 ± 3.14 vs. 1.29 ± 1.92; $p<0.001$) throughout their treatment. In addition, patients placed on ventilators exhibited significantly reduced oxygen saturation levels throughout their stay and beyond the conclusion of administered ventilation (93.94 ± 8.36 vs. 97.14 ± 2.61; $p=0.015$). Medical records, specifically concerning oxygen saturation, suggest that mechanical ventilation may have a detrimental influence on health outcomes in patients with severe TBI. Future efforts directed at examining ANS function following TBI may help shed light on the physiological sequelae that can impair a patient's long-term quality of life.

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Poster

PSTR540. Brain Injury: Human Studies

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Program #/Poster #: PSTR540.21/Z10

Topic: C.10. Brain Injury and Trauma

Support: FRQS Grant 338277

Title: Continuous sedation in disorders of consciousness: impact of demographic and clinical characteristics on connectivity measures in high-density EEG

Authors: *R. JUTRAS[1], C. MASCHKE[3], M. HAN[3], K. DOLHAN[3], L. NORTON[4], A. M. OWEN[4], S. BLAIN-MORAES[3], C. DUCLOS[2];
[1]Univ. de Montréal, Montreal, QC, Canada; [2]Univ. de Montréal, Montréal, QC, Canada; [3]McGill, Montréal, QC, Canada; [4]Western Univ., London, ON, Canada

Abstract: Introduction and Objective Establishing a clear prognosis for unresponsive brain-injured patients is challenging, particularly in the acute phase when behavioral responses are unreliable and confounded by continuous sedation. Our goal is to develop a clinically accessible tool using high-density electroencephalography (hd-EEG) for prognosis by quantifying the reconfiguration of functional connectivity during temporary cessation of sedation. The clinical utility of this tool may be limited by the insufficient understanding of the impact of demographic and clinical variables on resting state connectivity measures under continuous sedation. Therefore, this study aims to investigate the effect of individual characteristics on functional connectivity measures in unresponsive, brain-injured patients under continuous sedation, in the acute phase of brain injury. Methods As of the submission date, we recorded cerebral activity in 20 patients with severe brain injury (14M/6F; 50.8 +/- 18.9 years old) for 5 min under continuous propofol sedation using a 128-electrode EEG system from Electrical Geodesics

(MagstimEGI, Eugene, OR, USA). The directed phase lag index (dPLI) was calculated for each 10-sec window within the alpha frequency band (8-13 Hz). The mean dPLI matrix was used to derive the Feedback Dominance Index (FDI), representing anterior--posterior connectivity. Hub Posteriority Ratio (HPR) was also used, highlighting frontal-posterior hub degree differences (minimally spanning tree). Both features have been found to be modulated by levels of consciousness. We employed mixed model linear regression to explore the link between connectivity measures (FDI, HPR) and demographic and clinical factors, such as sex, age, Glasgow Coma Scale-assessed consciousness level at admission, and infusion dose of propofol. Correlational analyses explored potential interaction effects among individual characteristics. Results No significant associations were found between FDI or HPR and individual characteristics (sex, age, level of consciousness, propofol dose). Moreover, these characteristics did not exhibit any interrelationships ($p>0.05$). Conclusion These preliminary results suggest that anterior-posterior connectivity and hub topology in brain-injured patients under continuous sedation does not correlate to variations in sex, age, level of consciousness, and propofol dose. Our study contributes to the understanding of individual variability in functional connectivity, aiding future research to confidently differentiate modulation due to consciousness levels from those due to intrinsic factors. Recruitment is still ongoing.

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Poster

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Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01AG071228

Title: Astrocyte exosomes isolated from patients with a combined history of COVID-19 and mTBI induce a neuroinflammatory phenotype in primary cultured astrocytes

Authors: *D. LINSEMAN[1], E. ROBSON[1], J. TAVEE[2], C. PRUSMACK[3], A. GROSSBERG[1]; [1]Univ. of Denver, Denver, CO; [2]Natl. Jewish Hlth., Denver, CO; [3]Resilience Code, Englewood, CO

Abstract: Mild traumatic brain injury (mTBI) is a major public health issue worldwide and a subset of patients experience persistent neurological symptoms that last for months to years. Sustained inflammation following mTBI is thought to contribute to impaired astrocyte function, neuronal degeneration, and blood-brain barrier damage which may leave the brain vulnerable to further injury or infection. COVID-19 is known to lead to chronic neurological complications (i.e., neurological long COVID) and may cause similar deleterious brain effects as mTBI. To investigate whether a combined history of COVID-19 and mTBI may lead to worsened

outcomes, we enrolled study participants in one of four study groups: those with history of BOTH COVID-19 and mTBI, history of either COVID-19 or mTBI, and no history of EITHER COVID-19 or mTBI. We examined whether patients with a history of mTBI (in particular, multiple mTBIs) have exacerbated long-term neurological sequelae following COVID-19 infection and whether astrocyte-derived extracellular vesicles (ADEVs) isolated from these patients contain altered cargo capable of inducing an inflammatory phenotype in cultured astrocytes. Our results suggest that individuals with a combined history of COVID-19 and mTBI have worsened severity and frequency of a variety of neurological symptoms (e.g., headache, brain fog, etc). In addition, ADEVs isolated from the plasma of individuals with a combined history of COVID-19 and mTBI, and displaying chronic and severe neurological symptoms, demonstrate an increased propensity to induce an inflammatory phenotype in primary cultures of rat and human astrocytes in vitro. These novel findings indicate that a combined history of brain injury and COVID-19 may be associated with worsened symptomology due to a persistent state of heightened neuroinflammation.

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Poster

PSTR540. Brain Injury: Human Studies

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Topic: C.10. Brain Injury and Trauma

Support: National Natural Science Foundation of China (Nos. 82030050 and 82102170)
China Postdoctoral Science Foundation (No. 2020M681330)

Title: Unveiling the potential of super resolution imaging in neurosurgery: challenging conventional methods in assessing neural/brain functional recovery

Authors: *C. HUA[1,2], X. WANG[3], F. LIANG[1];
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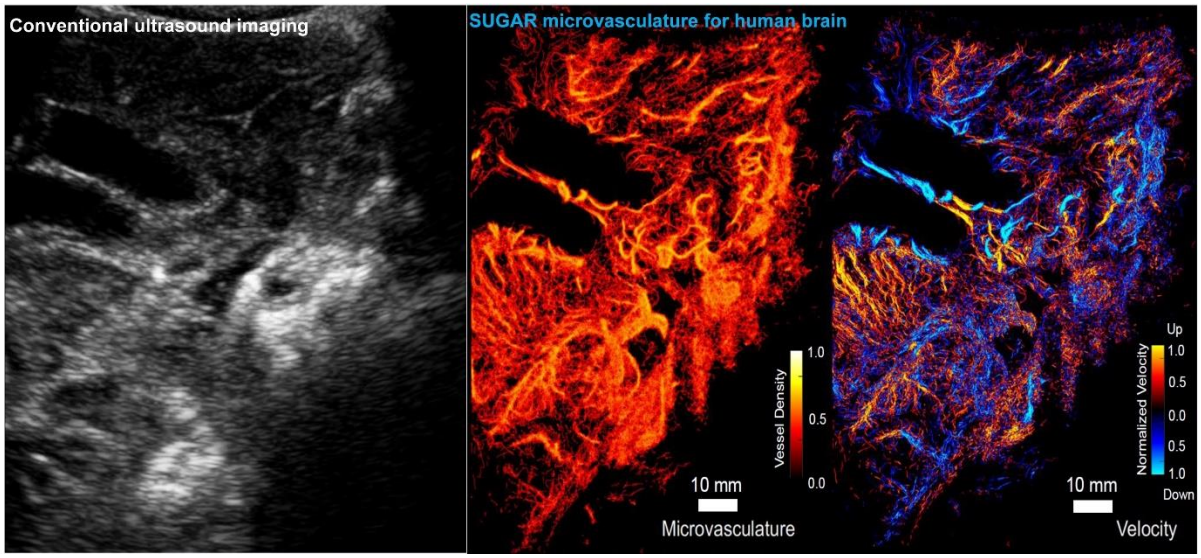
Abstract: Cerebral perfusion and its restoration are critical factors in the prognosis and treatment of neurosurgical disorders. Traditional methods such as CTA, MRA and Intracranial Pressure (ICP) monitoring have been widely employed as gold approaches to assess cerebral perfusion. However, the inability of these methods to detect microscale perfusion has led to increasing discrepancies in recent years. In this study, we propose a novel non-invasive method to assess cerebral microperfusion by employing bedside ultrasonography in conjunction with a super resolution reconstruction imaging method called Super Ultrasound for Greater Accuracy

and Resolution (SUGAR). Our imaging modality allows for the precise visualization of microvasculature distribution in deep cerebral regions with a resolution of 25 μm. Additionally, it enables the evaluation of various microcirculation metrics, including diameter, density, tortuosity, and velocity. A typical result is that the vessels observed in patients with high ICP value possessed lower fractal dimension of 1.46, indicating the vessel's limited ability to branch out. By utilizing SUGAR method, we discovered instances where regular administrations failed to enhance cerebral microperfusion, contrary to the results obtained from gold standard approaches. Our imaging approach combines the advantages of standard ultrasonography, such as safety and portability, with exceptional super resolution capabilities, enabling efficient and accurate diagnosis of cerebral perfusion. Moreover, the high resolution provided by this method offers the potential for early assessment of neurofunctional restoration, thereby avoiding overly optimistic conclusions based on traditional angiography or ICP monitoring. In summary, our study introduces a promising approach for non-invasive visualization of cerebral microperfusion, shedding light on its potential influence on neurofunctional restoration. The application of this imaging modality could lead to improved prognostic and therapeutic strategies in the field of neurosurgery.



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Poster

PSTR540. Brain Injury: Human Studies

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Topic: C.10. Brain Injury and Trauma

Support: National Defense Science and Engineering Graduate Fellowship F#00006027
Commonwealth Neurotrauma Initiative Grant A262-90012

Title: Predicting clinically relevant elevations in intracranial pressure using the circulating peptidome of brain injured patients.

Authors: *K. E. PLATFOOT[1], A. K. ROY[2], A. B. VALADKA[3], A. K. OTTENS[1];
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Abstract: Nearly 1 million brain injuries result in hospitalization in the United States each year from traumatic brain injuries (TBI) and stroke. Of these, approximately 200,000 are fatal while 200,000 - 330,000 result in long-term disability. Often, complications from secondary insults like elevated intracranial pressure (ICP), contribute to poor prognosis and worsened patient cognitive and functional outcomes and increased mortality. Unfortunately, the field lacks predictive tools of secondary insults. For example, high ICP is suspected from diminished responsiveness that is confounded by brain injury and sedative care. Clinicians then reactively, not preemptively, surgically implant pressure transducers, an inherently risky procedure available mainly at level-one trauma centers.
To address this, a predictive diagnostic assay was developed based on injury byproduct peptides. Brain injury drives proteolytic activity in tissue remodeling and cell dynamics, effluxing peptides via waste clearance to the periphery. Serum samples at 24-h from 13 TBI, stroke, and polytrauma patients were collected along with ICP telemetry data. The augmented circulating peptidome was quantified after solid-phase and mass filtration using high-performance mass spectrometry with data-independent acquisition for label-free quantification. A predictive model for high (> 20 mmHg) ICP events within 72 h of injury was built using partial least squares discriminant analysis on a subset of patients and validated with remaining patients.
Patients with high ICP events were differentiated from those without by 227 peptides with variable importance in projection scores > 1.0. A top-10 peptide model produced an AUC of 1 and high predictive accuracy (Q2 = 0.9684). Validation in a separate cohort demonstrated ideal classification of all subjects. Among predictive peptides were fragments of fibrinogen, a primer of the NLRP3 inflammatory response that contributes to ICP elevations; hemicentin-1 which is focused at perivascular spaces in the CNS and involved in regulating fluid homeostasis within the brain; proprotein convertase 1/3, an enzyme that influences activation state of macrophages; and prothrombin, a precursor to the clotting factor thrombin whose dysregulation contributes to changes in ICP.
The peptide biomarker model here aims to provide clinicians with a tool to identify, within 24 h of injury, those at risk for high ICP events in the first several days to guide transfer to a trauma center and subsequent drug and surgical interventions. This study justifies further inquiry of the circulating peptidome for additional secondary sequelae critical to patient outcomes.

Disclosures: K.E. Platfoot: None. **A.K. Roy:** None. **A.B. Valadka:** None. **A.K. Ottens:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.25/Z14

Topic: C.10. Brain Injury and Trauma

Support: CIHR

Title: Post-concussion changes in functional network connectivity relative to the pre-injury brain.

Authors: N. CHURCHILL[1], M. HUTCHISON[2], S. J. GRAHAM[3], ***T. SCHWEIZER**[2];
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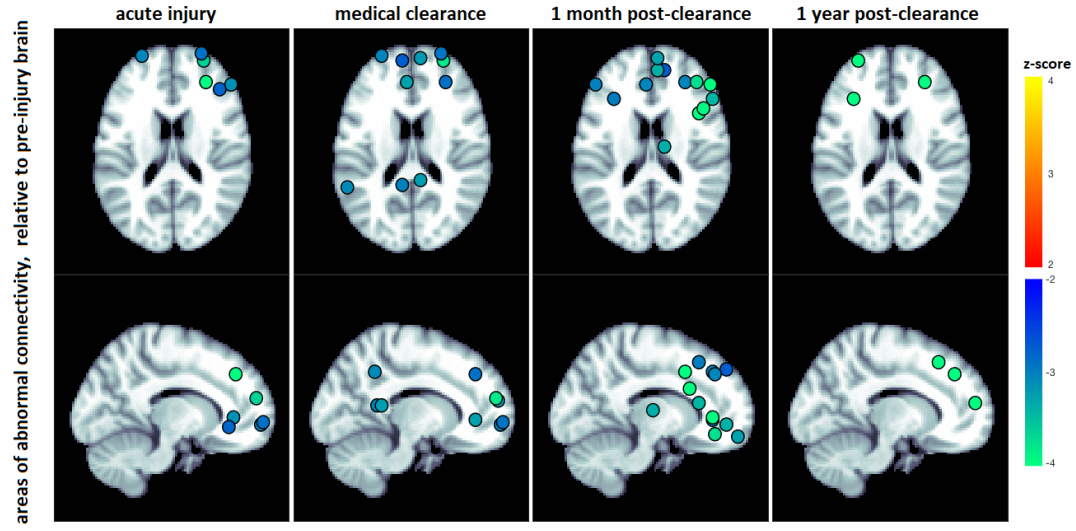
Abstract: Concussion is a major health concern, with an estimated 4 million cases occurring annually in sport and recreation in North America alone. Recent neuroimaging studies have raised concerns about persistent post-concussion brain changes, suggesting that recovery of brain function is incomplete at medical clearance, potentially the increasing risk of subsequent injury. However, these studies are cross-sectional in design, comparing brain networks of concussed individuals to uninjured controls. It is essential that we measure how brain function is altered relative to its "pre-injury" state, in order to determine whether it has truly recovered at medical clearance or shows persistent changes. In this study, a large sample of 167 varsity athletes had resting-state functional magnetic resonance imaging (fMRI) collected at pre-season baseline. Of this cohort, 25 were later concussed, with imaging at acute injury, medical clearance, and up to one year later. An additional 27 athletes without concussion were re-imaged as controls. Concussed athletes showed significant post-concussion declines in anterior brain connectivity lasting beyond medical clearance. This study provides the first characterization of brain function after concussion, relative to the pre-injury brain. The results of this study indicate that disturbances in connectivity are present at and beyond medical clearance, highlighting the complex, long-term nature of recovery after injury.



Disclosures: **N. Churchill:** None. **M. Hutchison:** None. **S.J. Graham:** None. **T. Schweizer:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.26/Z15

Topic: C.10. Brain Injury and Trauma

Support: ALF agreement ALFGBG-976044 and 964972
Wilhelm and Martina Lundgren, Science fund 2019- 3078
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the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862)
the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, and #ADSF-21-831377-C)
Hjärnfonden, Sweden (#FO2022-0270)
the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at UCL (UKDRI-1003

Title: Neuro injury biomarkers GFAP and S100B during surgery in the steep Trendelenburg position

Authors: *R. C. VITHAL[1], A. EL MERHI[1], A. KOSOVIC[1], A. CHANDAN[1], H. ODENSTEDT HERGES[1], H. ZETTERBERG[3], C. BIÖRSERUD[2], M. STARON[4], J. LILJENCRANTZ[1], L. BLOCK[1];
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Abstract: Introduction: Laparoscopic robotic-assisted hysterectomy and prostatectomy require a steep Trendelenburg positioning of the patient. A prolonged Trendelenburg position may cause impaired venous return from the head, resulting in reduced cerebral blood flow. This study investigates whether the neuro biomarkers glial fibrillary acidic protein (GFAP) or S100 calcium-binding protein B (S100B), measured before and after anaesthesia and surgery in the

Trendelenburg position, were elevated to a degree indicative of any harmful effects on the brain.Methods:Data for this prospective observational study were collected at the Sahlgrenska University Hospital, Gothenburg, Sweden, between September and November 2021. Patients scheduled for lower abdominal surgery in either the steep Trendelenburg or the supine position in general anaesthesia were eligible for inclusion. All patients were monitored with near-infrared spectroscopy and continuous blood pressure monitoring beyond standard monitoring. Blood samples were obtained preoperatively and 2 and 24 hours after surgery to analyse concentrations of GFAP and S100B. Results:There was no significant increase in GFAP concentration when comparing preoperative levels with 2-hour samples and 24-hour samples in either group ($p=0.06$ in the Trendelenburg group and $p=0.60$ in the supine group). There was a significant increase in S100B concentration when comparing preoperative levels with 2-hour and 24-hour samples in both groups ($p<0.001$ for the Trendelenburg group and $p=0.006$ for the supine group).Conclusion:The main result of this study is that anaesthesia and surgery in the steep Trendelenburg or supine position does not cause a release of neuro-specific injury biomarker GFAP but cause a serum S100B increase in both groups. This indicates that anaesthesia and surgery in the steep Trendelenburg position do not cause cerebral injury and that the increased levels of S100B are most likely from extracranial sources.

Disclosures: R.C. Vithal: None. **A. El Merhi:** None. **A. Kosovic:** None. **A. Chandan:** None. **H. Odenstedt Herges:** None. **H. Zetterberg:** None. **C. Biörserud:** None. **M. Staron:** None. **J. Liljencrantz:** None. **L. Block:** None.

Poster

PSTR540. Brain Injury: Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR540.27/Z16

Topic: C.10. Brain Injury and Trauma

Support: Gerber Foundation Grant (CME & MBB)
NIH EY027881 (PAR)
NIH HD097327 (MBB)
NIH K12 NS079414 (CME)
Boston Children's Hospital Department of Neurology (CME)
Child Neurology Society Dodge Young Investigator Award (CME)

Title: Longitudinal zinc concentrations in whole blood of preterm infants: associations with brain and body growth

Authors: *C. M. ELITT[1,2], S. CHERKERZIAN[3,2], K. A. BELL[3,2], J. O'BRIEN[3], M. IRAGAVARAPU[3], S. MINGA[3], R. SUTTIN[3], J. WANG[1], M. M. ROSS[1], S. G. REMIS[1], J. SHANAHAN[1], B. I. FEDELES[4], T. E. INDER[5,6], H. CHRISTOU[3,2], M. B. BELFORT[3,2], P. A. ROSENBERG[1,2];
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Women's Hosp., Boston, MA; [4]MIT, Cambridge, MA; [5]Children's Hosp. of Orange County, Orange, CA; [6]Univ. of California, Irvine Sch. of Med., Irvine, CA

Abstract: Dietary zinc is essential for brain development. Preterm infants are at high risk for zinc deficiency due to absent 3rd trimester transfer from the placenta, impaired absorption, and, potentially, inadequate dietary intake. The objectives of this study were twofold: (1) to quantify longitudinal whole blood zinc concentrations among very preterm infants (VPIs) and compare with full term infants (FTIs), and (2) to determine whether zinc status was associated with brain and body size in preterm infants measured at term equivalent age. We studied 43 VPIs [gestational age (GA) range, 24-30 weeks] and 15 FTIs (GA range, 37-39 weeks). We used inductively coupled plasma-mass spectrometry (ICP-MS) to measure zinc isotope concentrations (^{64}Zn, ^{66}Zn, ^{68}Zn, ^{70}Zn) in peripheral whole blood samples collected from VPIs at 4 time points [day of life (DOL) 1, DOL14, DOL30, hospital discharge] and from FTI cord blood. Total zinc concentrations were calculated using ^{64}Zn. Zinc isotope ratios (^{66}Zn/^{64}Zn, ^{68}Zn/^{64}Zn, and ^{70}Zn/^{64}Zn) were also determined and compared to natural abundance ratios. Outcome measures in VPIs at term equivalent age included anthropometric indicators (weight, length, head circumference), fat-free mass determined by air displacement plethysmography, and quantitative brain metrics from magnetic resonance imaging. A high zinc group consisting of infants with 1 or more outlier values [defined as 75th%ile + 1.5*(IQR)] was identified within the VPI population. Among VPIs median zinc concentrations decreased over the first month of the NICU hospitalization as previously reported and then increased by hospital discharge. Compared to FTI cord blood [714 ug/L; interquartile range (IQR): 674-866 ug/L], VPIs had higher zinc concentrations at discharge [943 ug/L; IQR: 736-1176 ug/L; $p<0.05$; Wilcoxon rank-sum test to compare medians]. Infants in the high zinc group had higher median weight z-scores at discharge ($p=0.0271$) and showed a trend towards larger cerebellar diameters ($p=0.0723$). Interestingly, median isotopic ratios (^{66}Zn/^{64}Zn, ^{68}Zn/^{64}Zn) in the FTIs and VPIs were all greater than natural abundance ratios, indicating differential zinc isotopic absorption or cellular transport in both VPIs and FTIs. Our results suggest that higher whole blood zinc concentrations than normally found in the NICU population may be associated with improved somatic and brain growth in the NICU. These studies have important implications for understanding the nutritional basis for optimal neurodevelopment among VPIs and for devising NICU-based zinc supplementation strategies.
Character count: 2229 (2300 limit)

Disclosures: **C.M. Elitt:** F. Consulting Fees (e.g., advisory boards); Akebia Therapeutics. **S. Cherkerzian:** None. **K.A. Bell:** None. **J. O'Brien:** None. **M. Iragavarapu:** None. **S. Minga:** None. **R. Suttin:** None. **J. Wang:** None. **M.M. Ross:** None. **S.G. Remis:** None. **J. Shanahan:** None. **B.I. Fedeles:** None. **T.E. Inder:** None. **H. Christou:** None. **M.B. Belfort:** None. **P.A. Rosenberg:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.01/Z17

Topic: C.10. Brain Injury and Trauma

Support: 2R01NS091617-06/GRANT13161291
Brain Institute Presidential Fellowship University of Virginia
Quantitative Neurobiology of Behavior Fellowship at University of Virginia

Title: Schwann Cell Response to Nerve Injury

Authors: *E. STEPANOVA[1], C. CHO[2], J. LEE[3], S. HUNTER-CHANG[4], J. N. CAMPBELL[3], C. DEPPMANN[1];
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Abstract: Neurodegenerative conditions affect millions of people worldwide. While molecular pathways involved in axonal breakdown, a hallmark of neurodegeneration, have been extensively studied, many questions remain unanswered about the role of glial cell reprogramming in neurodegeneration. Our previous research findings challenge the commonly held belief of an "all or nothing" principle, where axon disintegration and demyelination by Schwann cells (SCs), glial cells of the peripheral nervous system are coupled (as inferred from wild type and Sarm-knockout/KO, phenotypes). Specifically, we observed that deleting the death receptor 6 (DR6) gene preserved axons after injury while still enabling partial demyelination by SCs, suggesting SC in response to injury acquire a previously unknown transitory cell state that occurs when the axon is damaged but still non-degenerating. To molecularly characterize SC states after injury, we performed single nucleus RNA sequencing of ~2000 sciatic nerve SCs (4 biological replicates) in wildtype and DR6 and Sarm1 knockout mice. Surprisingly, SCs lacking DR6 or Sarm1 showed significant molecular changes relative to SCs from wildtype mice. For instance, unlike wildtype SCs, many myelinating SCs from DR6 or Sarm1 knockout females formed a SC cell state marked by expression of Hexb, a gene commonly enriched in immune cells such as microglia and macrophages, and only a few expressed genes that mark myelinating SCs in wild type mice, such as Adamtsl1 and Erbb4. Our data suggest sexual dimorphism in SC maintenance and development as well as divergence of myelinating SCs in mutants vs wild type animals even in the absence of injury. Ongoing work suggests that SCs in DR6 and Sarm1 knockout respond differently to injury by enriching a transitory population of SCs also with genes normally expressed in immune cells. These studies provide insight into transcriptional signature of SC states SCs associated with a preservation of myelination after injury and so may provide new therapeutic targets for rescuing demyelination.

Disclosures: E. Stepanova: None. **C. Cho:** None. **J. Lee:** None. **S. Hunter-Chang:** None. **J.N. Campbell:** None. **C. Deppmann:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.02/Z18

Topic: C.10. Brain Injury and Trauma

Title: Objective Assessment of Functional Recovery Following Nerve Transfer for Brachial Plexus Injury Using Reachable Workspace

Authors: *S. ANDALIB[1], L. GONZALES[2], A. TEDESCO[2], A. GUPTA[2], M. N. HATCH[3], O. STEWARD[4], T. R. JOHNSTON[2], R. GUPTA[5];
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Abstract: Methods for assessing post-operative patient outcomes and upper extremity range of motion (ROM) are limited by observer bias and the inability to accurately characterize the functional utility of affected limbs. Here, we used a novel technique to objectively quantify functional upper extremity ROM changes pre and post-motor nerve transfer surgery for brachial plexus injuries. Our method incorporates a sensor-based and automated image capture technology, i.e. the Reachable Workspace (RWS) system, in addition to clinician-measured medical research council (MRC) muscle strength scores. Pre- and post-operative shoulder ROM was assessed for patients with brachial plexus transection injuries in various spatial locations using the Microsoft Kinect 2.0 sensor (Redmond, WA). Arm movements included a combination of vertical and horizontal sweeping movements with movement trajectories automatically quantified by the RWS system, with the results plotted in four quadrants. This data from the RWS is presented as relative surface area (RSA), which represents the area of space an individual can reach, normalized by the individual's arm length. Our data demonstrate improved shoulder functional ROM as early as 5 months after nerve transfer surgery. Clinical strength measurements also improved over this time, from 0/5 (no visible muscle contraction) at baseline to 3/5 (movement against gravity). All RWS data directly correlated with clinical observations and quantitative functional ROM measurements. This study supports that RWS is an objective, observer-independent clinical assessment tool that can more accurately quantify functional recovery following surgical reconstruction after brachial plexus nerve injury.

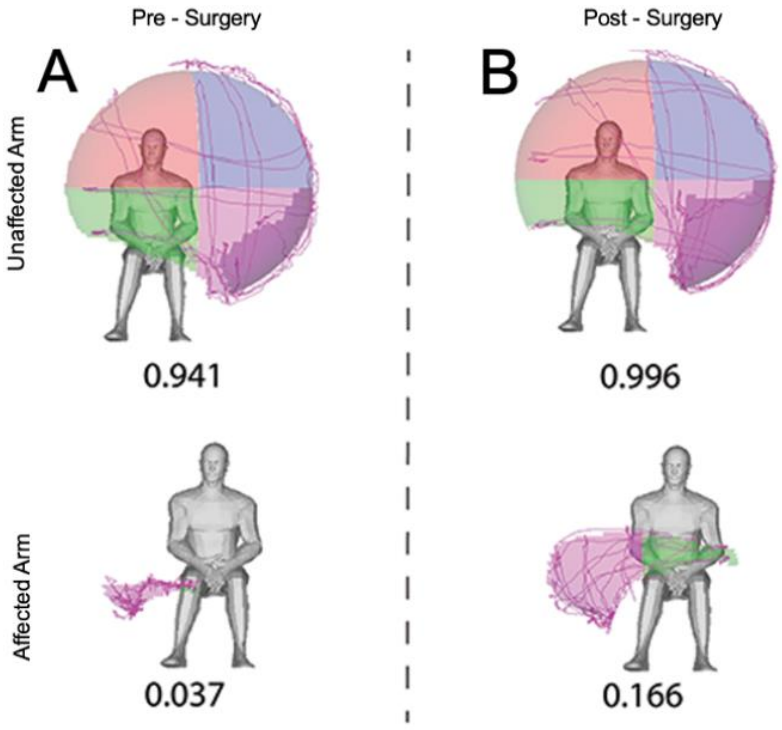

Figure 1. Example of upper extremity function recovery in a radial to axillary nerve transfer patient (Patient 2). (A) Pre-surgery reaching in unaffected (top panels) arm vs. affected arm (bottom panels). Before surgery, only minimal reaching in the lower lateral quadrant (pink) is present. (B) By 6 months post-surgery, there is marked improvement in the lower lateral (pink) quadrant and the appearance of reach into the lower medial quadrant (green).

Disclosures: S. Andalib: None. **L. Gonzales:** None. **A. Tedesco:** None. **A. Gupta:** None. **M.N. Hatch:** None. **O. Steward:** None. **T.R. Johnston:** None. **R. Gupta:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.03/Z19

Topic: C.10. Brain Injury and Trauma

Support: Edward Via College of Osteopathic Medicine- Intramural grant

Title: Evaluation of median nerve fascicular topography using landmark morphometrics

Authors: A. PHAM[1], H. IZADPANAH[1], J. MILLARD[1], *K. C. S. ROBALLO[1,2];
[1]Edward Via Col. of Osteo. Med., Blacksburg, VA; [2]Virginia–Maryland Col. of Vet. Medicine, Virginia Tech., Blacksburg, VA

Abstract: While significant research has been conducted on the overall macro-anatomy of peripheral nerve trunks in the upper extremities, our understanding of the microanatomy remains limited. Specific details such as nerve diameter, number of fascicles, length, and route along their pathways are still relatively unknown. However, this information holds crucial importance in cases of peripheral nerve injury (PNI) where nerve grafting may be necessary. PNI occurs in around 1.64% of individuals affecting their limbs, and only about half of those treated for PNI achieve functional recovery. Hence, this pilot study aims to examine the variability of fascicular arrangement in the median nerve using landmark morphometrics. For that, median nerves were isolated from 20 whole-body human donors preserved in formalin. Donor sex was recorded, and measurements were taken as indicators of body size. The median nerves were divided into nine equally sized segments and cross-sectioned, imaged and analyzed. For this pilot study, we analyzed nine cross sections from nine donors, each containing 10 fascicles. A total of 130 landmarks were collected using semi-landmark techniques. The mean landmark configuration was determined through Generalized Procrustes, and principal component analysis was employed to investigate variations in fascicular topography. Wireframe graphs, lollipop diagrams, and transformation grids were generated to assess and visualize the findings. As result, the median nerve exhibited an average diameter of 2.07 cm on the right side and 1.95 cm on the left side. The average nerve length was 18.91 cm on the right and 18.56 cm on the left. The application of principal component analysis yielded eight principal components, of which the first two accounted for 51.3% of the total shape variation observed. The analysis of PC1 and PC2 indicated that peripheral fascicles exhibited relatively consistent locations. However, the lollipop diagrams revealed significant variability in the relative positions of the more interior fascicles, both in terms of direction and magnitude. In conclusion, the findings of this study revealed the presence of shared patterns among fascicles in the upper limb. Notably, the location of these fascicles exhibited variability, thereby potentially impacting measurements of non-fascicle tissue in nerve trunks. This variability could lead to implications when it comes to nerve grafting, necessitating further research in this area. Lastly, by identifying these common patterns, our aim was to enhance our understanding of fascicle pathways and improve clinical approaches to nerve grafting, surgical repair, and electrode implantation.

Disclosures: **A. Pham:** None. **H. Izadpanah:** None. **J. Millard:** None. **K.C.S. Roballo:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.04/Z20

Topic: C.10. Brain Injury and Trauma

Support: Queen's University, Department of Surgery

Title: P75ntr exon lll null mutant mice do not display deficits in functional recovery or remyelination following median nerve injury but exhibit significantly impaired sensory regeneration

Authors: M. TOPLEY[1], A.-M. CROTTY[3], M. KAWAJA[2], *M. HENDRY[1];
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Abstract: **Introduction** Schwann cells are a key glial cell for neuronal regeneration following peripheral nerve injury. The neurotrophin receptor p75NTR is a known Schwann cell regulator in neuronal regeneration, however, the effects of the p75NTR on functional recovery post injury remains controversial. This study investigates the role of the p75NTR on functional recovery in a mouse median nerve injury model using p75NTR mutant mice compared with wildtype. In addition, we also examine the impact of p75NTR on the number of regenerating motor and sensory neurons as well as remyelination following axotomy. **Methods** 5 C57Bl/6 and 5 p75NTR exon III null mutant ($p75^{-/-}$) mice underwent median nerve transection in the axilla. Forelimb grip strength was conducted every 3 days post operatively using a Bioseb grip strength meter for a 21-day period. Mice then underwent retrograde labeling of the regenerating median nerve 5mm distal to the transection site at 21 days post injury to delineate the sensory neurons in the dorsal root ganglia and motoneurons in the ventral spinal cord.Quantitative histomorphometry was also carried out on 1 mm segments of nerve removed from the retrograde labelling site. This tissue was fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide and myelin characteristics were analysed using a custom macro with Clemex Vision Pro software. **Results** The grip strength revealed that there is no difference in forelimb grip strength recovery following median nerve injury in wildtype mice compared to $p75^{-/-}$ mice. Retrograde labelling revealed that the number of regenerated motor neurons between $p75^{-/-}$ mice and wildtypes was 226 +/- 90 and 266 +/- 76.6, respectively (+/-SEM; $p < 0.05$). The number of regenerated sensory neurons between $p75^{-/-}$ mice and wildtypes was 489 +/- 203 and 1156 +/- 527, respectively (+/-SEM; $p < 0.05$). Histomorphometry revealed that there was no difference in myelin thickness, fibre diameter, axon diameter or G-ratio. **Conclusion** In conclusion, $p75^{-/-}$ mice had impaired sensory neuron regeneration but no impact on motor regeneration following median nerve injury. This is reflected in the functional grip strength test where there were no differences in grip strength between the $p75^{-/-}$ mice and the wildtype mice. Lastly, $p75^{-/-}$ mice did not display and significant differences in remyelination 21-days post injury as compared to the wildtype mice. Collectively, this data supports a limited role for p75NTR in functional recovery following median nerve injury given that the diminished sensory neuron recovery is attributable to already reduced sensory neuron pools in $p75^{-/-}$ mice.

Disclosures: **M. Topley:** None. **A. Crotty:** None. **M. Kawaja:** None. **M. Hendry:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.05/Z21

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS118020

Title: Tfeb/3 govern repair Schwann cell generation and function following PNS injury

Authors: A. PATEL[1], H. KIM[2], J. P. SVAREN[3], Y.-J. SON[2], R. DOBROWOLSKI[1], *H. KIM[1]; [1]Rutgers Univ., Newark, NJ; [2]Temple Univ. Sch. of Med., Temple Univ. Sch. of Med., Philadelphia, PA; [3]Univ. Wisconsin, Univ. Wisconsin, Madison, WI

Abstract: Following PNS injury, distal Schwann cells activate a repair program to generate cells supportive of nerve regeneration. The repair Schwann cells promote axon regrowth and myelin clearance, and remyelinate regenerated axons. Transcription factors such as c-Jun and Sox2 have been shown to regulate Schwann cell repair program, however, only a small subset of injury-induced genes have been attributed their functions, suggesting that there are other regulators. TFEB and TFE3 (TFEB/3) are important regulators of cellular clearance and stem cell pluripotency. Goal of the study is to investigate TFEB/3 function in governing Schwann cell repair program. To examine the combined role of TFEB/3, we generated a mouse line lacking both TFEB and TFE3 in the Schwann lineage ($TFEB^{flox/flox}$:$TFE^{-/-}$:Dhh-Cre^{+}) (TFEB/3 SC-dKO). Control and the mutant mice were subjected to nerve transection/crush injury and the distal nerves were analyzed for repair Schwann cell phenotype and function. While deletion of TFEB/3 in Schwann cells has no effect on developmental myelination, it impairs repair Schwann cell generation and the expansion in the distal nerve following PNS nerve injury. RNA-seq analysis show a significant decrease in repair Schwann cell gene signature (p75, Sox2, Runx2, Olig1) in the mutant distal nerves (5DPI). Immunohistochemical analysis show impairment in repair Schwann cell formation and proliferation, without an impact on c-Jun expression. Chip-seq analysis show that TFEB/3 biding motifs are enriched in injury-enhancers in distal Schwann cells. Ectopic activation of TFEB in cultured myelinating Schwann cells is sufficient down-regulate myelin-associated genes without impacting c-Jun expression. Lastly, TFEB/3 SC-dKO mice exhibit impairment in axon regrowth and re-innervation of denervated muscles following nerve injury, as well as in long-term myelin clearance and sensory/motor function recovery. Altogether, our results show that TFEB/3 transcription factors play an important role in governing repair Schwann cell generation and function necessary for PNS regeneration and repair.

Disclosures: A. Patel: None. **H. Kim:** None. **J.P. Svaren:** None. **Y. Son:** None. **R. Dobrowolski:** None. **H. Kim:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.06/Z22

Topic: C.10. Brain Injury and Trauma

Support: JSPS KAKENHI Grant JP22K19615
JSPS KAKENHI Grant JP22H03287

Title: Possible involvement of synthetic synapse organizer protein in the restoration of injured trigeminal nerve

Authors: *E. SAWADA[1], K. TAKEUCHI[2], Y. SATO-YAMADA[1], M. TERUNUMA[1], H. SASAKURA[2], Y. MORIOKA[2], T. MAEDA[1], K. SEO[1];
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Abstract: A synthetic synaptic organizer termed CPTX, which consists of structural elements cerebellin-1 and neuronal pentraxin-1, is known to restore synaptic function. It interacts with presynaptic neurexins and postsynaptic AMPA-type glutamate receptors, and restores locomotor function in mice with spinal cord injury. However, it remains unknown if CPTX recovers sensory function after peripheral nerve injury in mice. In this study, we aimed to examine whether CPTX-mediated reestablishment of central neuronal circuit facilitates regeneration of sensory function, which was lost by trigeminal nerve injury.Animals and methods: CPTX was injected into the trigeminal subnucleus caudalis (Vc), and its effect on regeneration of the 3rd branch trigeminal nerve was examined by investigating the dye delivered in the trigeminal ganglion after injury. Male C57BL6/J mice (6-8 weeks-old) were divided into three groups; sham group, saline group and CPTX group. Sham group only received inferior alveolar nerve (IAN) exposure. After complete transection of IAN, either CPTX-or saline-administration into the Vc was performed in CPTX and saline groups, respectively. To trace axon regeneration, mice received subcutaneous injection of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) around the mental foramen 5 days after IAN transection, and transcardially perfused post 2 days after injection. Fluorescent microscopy examined DiI expression in the trigeminal ganglion.Results: The DiI-positive cells in the trigeminal ganglion significantly decreased in number in saline group compared with sham group ($p<0.05$, Tukey Test). In contrast, no significant difference existed between CPTX and sham groups ($p>0.05$, one-way ANOVA). Conclusion: CPTX injection in Vc may restore trigeminal sensory function by organizing central nerve circuits. (Supported by JSPS KAKENHI Grant Numbers JP22K19615, JP22H03287)

Disclosures: **E. Sawada:** None. **K. Takeuchi:** None. **Y. Sato-Yamada:** None. **M. Terunuma:** None. **H. Sasakura:** None. **Y. Morioka:** None. **T. Maeda:** None. **K. Seo:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.07/Z23

Topic: C.10. Brain Injury and Trauma

Title: Potential sexual dimorphisms in mitral cell dendritic structure with growth, injury and recovery in adult zebrafish

Authors: *J. ROZOFSKY[1], J. M. POZZUTO[2], C. A. BYRD-JACOBS[1];
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Abstract: Consideration of sex as a biological variable has become increasingly important in scientific research. Prior work has shown that adult females of some species recover more quickly than males following neuronal injury. Little is known about sex differences in recovery of brain structures in adult zebrafish. Mitral cells (MC) of the olfactory bulb serve as the primary relay neurons for transmitting odorant information from the olfactory epithelium to higher order brain structures. Prolonged damage to olfactory sensory neurons causes interruption of afferent innervation of MC. Our lab has developed a novel method to quantify the extent of injury and recovery of MC dendritic arborization as a result of chronic deafferentation. Repeated application of the detergent Triton-X 100 to the olfactory epithelium once every three days over a period of 8 weeks caused chronic deafferentation of the bulb, while the left side was untreated to serve as an internal control. Zebrafish were allowed to recover for 3 or 8 weeks, and MC morphological measures were quantified based on number of major branches, total length of dendritic branches, size of dendritic field, and distribution of fine processes of MC dendritic arborization. We hypothesized that degeneration and regeneration effects on MC dendrites would differ between the sexes in zebrafish. We also examined potential sex differences that may exist with MC dendritic arborization during growth, since zebrafish continued to grow throughout the time course of deafferentation and recovery.Control measurements of MC dendritic arbors showed potential differences in male and female fish, with males possessing fewer numbers of major branches and decreased distribution of fine processes. Combined data of males and females showed that, following 8 weeks of repeated damage, MC dendritic morphology within the deafferented bulb significantly decreased in number of tips, total length of dendritic branches, size of dendritic field, and distribution of fine processes. When fish were allowed to recover for 3 or 8 weeks these significant differences were alleviated, as shown by a return of morphological structures to near-internal control levels. Interestingly, preliminary results appear to show quicker recovery of branch length in males, while the number of tips appears to recover more quickly in females, at the 8-week recovery time point. Additionally, the timelines for these processes of growth and recovery appear to differ between the sexes. This research furthers our understanding of plasticity of neuronal processes in the zebrafish olfactory system and potential differences that may exist between males and females.

Disclosures: J. Rozofsky: None. **J.M. Pozzuto:** None. **C.A. Byrd-Jacobs:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.08/Z24

Topic: C.10. Brain Injury and Trauma

Support: Canadian Institutes of Health Research 202003PJT-436747-NSA-CBBA-102664

Title: Cryab contributes to terminating the presence of pro-inflammatory macrophages in the damaged, aged peripheral nervous system

Authors: K. M. HAGEN[1], ***S. S. OUSMAN**[2];
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Abstract: In humans, regeneration of old peripheral nervous system (PNS) neurons is poor after damage. This is due in part, to the reduced growth capacity of some aged PNS nerve cells. However, growing evidence indicates that non-neuronal cells in the PNS environment play an important role in the regenerative deficit seen in injured, aged mammals. For example, old Schwann cells lose their capacity to differentiate into a repair phenotype. As a consequence, they secrete less chemotaxic cytokines needed for macrophage infiltration which negatively impacts the removal of myelin and axonal debris that contains inhibitors against growth. Moreover, old macrophages are less efficient at phagocytosing debris. We are interested in identifying the molecular factors responsible for the inadequate function of old Schwann cells and macrophages. We discovered that Schwann cells and axons express a small heat shock protein called CRYAB that in young, adult mice, plays a role in the re-differentiation of myelinating Schwann cells, remyelination and functional recovery after PNS crush injury. Further, CRYAB levels reduce with aging and this correlates with thinner myelin sheaths in 12 month old mice. We thus investigated if the small heat shock protein influences the number of myelinating and non-myelinating Schwann cells as well as pro- and anti-inflammatory macrophages. A sciatic nerve crush injury was performed on the right sciatic of 3- and 12-month old female 129S6 and CRYAB null mice. At 7 and 28 days post-injury, sciatic nerves were harvested and frozen sections stained for CD16/32, CD206, GFAP and S100beta. We found that the number of GFAP and S100beta profiles was similar between 3- and 12-month old naïve and injured WT and $CRYAB^{-/-}$ nerves. With respect to macrophages, while the number of CD206 profiles was unchanged between control and null animals in all age and injury groups, significantly more pro-inflammatory macrophages were present in the nerves from 12 month old $CRYAB^{-/-}$ animals at 28 days after injury relative to age-matched controls. These were phagocytic macrophages since they contained more myelin debris that correlated with less myelin in the environment of the null nerves. Thus, CRYAB may play a role in terminating the presence of pro-inflammatory macrophages that can release cytokines such as IL-1beta and TNF that can contribute to pain. However, these immune cells are important for phagocytosing myelin debris, thus a balance is needed whereby pro-inflammatory macrophages can enter the damaged, aged PNS to clear debris but induced to leave the nerve afterwards so as to prevent possible pain issues.*Supported by the Canadian Institutes of Health Research*

Disclosures: K.M. Hagen: None. **S.S. Ousman:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.09/Z25

Topic: C.10. Brain Injury and Trauma

Title: The influence of Schwann Cells and Schwann cell-derived exosomes on functional recovery after repair of a long-segmental peripheral nerve defect in the rat

Authors: *E. L. ERRANTE, M. C. COSTELLO, A. J. KLOEHN, J. YUNGA TIGRE, E. SCHAEFFER, A. KHAN, A. D. LEVI, S. S. BURKS;
Univ. of Miami, The Miami Project, Miami, FL

Abstract: Peripheral nerve injury (PNI) is characterized by a loss of cellular and axonal integrity, often leading to limited functional recovery and pain. Many PNIs are not amenable to repair with traditional techniques; however, cell therapies, particularly Schwann cells (SCs), offer the promise of neural tissue and functional replacement. Exosomes, which can be secreted by SCs, carry cellular signaling molecules that facilitate intercellular communication. They have shown promise in PNI, with studies showing SC-derived exosomes have the appropriate protein markers, associate to axons in high concentrations, and are able to improve nerve regeneration. Our laboratory has had success using SCs in preclinical and clinical treatment settings; however, SCs have their own issues, making it imperative to find a better treatment strategy. To that end, the laboratory has begun to investigate if implanted SCs and SC-derived exosomes in complex conduits comparably improve axonal regeneration, functional recovery, and pain outcomes after repair of a severe PNI. Adult male and female Fischer rats were divided into groups, including empty conduits, reversed autograft, SCs, and SC-derived exosomes, and had surgery to produce a significant gap in the sciatic nerve. Animals underwent sensation and pain assessment once every two weeks for the entirety of the 16-week experiment. At the end of the experiment, biochemical, immunohistochemical, electrophysiological recordings, and electron microscopy were performed on all rats and their nerve grafts. Preliminary data indicates that exosomes can be inserted and visualized in the conduits, a novel observation to the author's knowledge. Further, preliminary data indicates that SCs and SC-derived exosomes are comparable treatments as measured by functional recovery and behavioral pain and sensation assessments ($p>0.05$). Additionally, animals treated with SCs or SC-derived exosomes are functionally comparable to control conditions ($p>0.05$), indicating that both treatment methods are satisfactorily treating the PNI. Dry muscle weights between treatment groups were compared and it was found that there was a significant difference in percent recovery between all groups ($p<0.001$). While muscle weights differ, there were no observed differences in electrophysiological recordings when comparing amplitude and latency in the gastrocnemius and tibialis anterior muscles ($p>0.05$). Although the effect of SC-derived exosomes may not be as robust as SCs, their clinical barrier is significantly lower; thus, exosome use in the treatment of PNI is beneficial and should continue to be assessed.

Disclosures: E.L. Errante: None. **M.C. Costello:** None. **A.J. Kloehn:** None. **J. Yunga Tigre:** None. **E. Schaeffer:** None. **A. Khan:** None. **A.D. Levi:** None. **S.S. Burks:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.10/Z26

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant P30GM145497
Start-up funding from the University of New England

Title: Transection of the Saphenous Nerve Results in Sensory and Sympathetic Denervation of the Mouse Tibia

Authors: T. LIZOTTE[1], G. HENDERSON[1], J. HICKEY[1], P. CARADONNA[1], V. E. EATON[1], G. DEOLIVEIRA[1], D. C. MOLLIVER[1], T. E. KING[2], ***K. BECKER**[1];
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Abstract: The saphenous nerve is a sensory nerve that is commonly injured during ACL repair, varicose vein surgery, and other procedures. Retrograde labeling studies have suggested that the tibia is innervated by the saphenous nerve, but this has not been confirmed with nerve transection. Sensory input to bone is important for bone formation and development, but little is known about the consequences of saphenous nerve injury on tibial innervation and bone mineral density (BMD). We hypothesize that saphenous nerve transection will result in a decrease in sensory nerve fibers in the tibia. A greater understanding of elements regulating tibial innervation will help identify risk factors influencing tibial BMD. To demonstrate that the saphenous nerve innervates the tibia, fast blue dye was injected into the proximal tibia of saphenous nerve transected or sham 8-week-old female C57BL/6J mice (n=3-5). The L2-L5 dorsal root ganglia (DRG) were isolated one-week post-injection and the percent fast blue positive cells were quantified. The highest level of fast blue labeling was observed in cell bodies from the L2 (8.1±1.5%) and L3 (6.9±1.3%) DRG with less staining in the L4 (1.5±0.2%) and L5 (2.0±0.4%) DRG of sham surgery mice. Retrograde labeling from the tibia was reduced in the L2 DRG by 75% (p=0.02) in mice with saphenous nerve transection compared to sham surgery mice, consistent with the paradigm that the saphenous nerve is the primary source of tibial innervation. To determine if the transection resulted in denervation of the tibia, the saphenous nerve was unilaterally transected in 8-week-old female C57BL/6J mice (n=3-4) and the ipsilateral and contralateral control tibiae were isolated two-weeks post-transection. Immunohistochemical analysis of sensory and sympathetic innervation was performed using immunofluorescent staining for calcitonin gene-related peptide (CGRP, sensory fibers), tyrosine hydroxylase (TH, sympathetic fibers) and β_{III}-tubulin (β3T, pan-neuron). Nerve fiber density was reduced by 48% in CGRP (p=0.11), 45% in TH (p=0.02), and 55% in β3T (p=0.03) positive fibers in the tibial periosteum ipsilateral to the saphenous nerve transection and compared to contralateral tibiae. Analysis of saphenous nerve transected animals indicated no long-term hindlimb unloading. These data show that saphenous nerve denervation reduces sensory and sympathetic tibial innervation. Ongoing studies will elucidate the impact of saphenous nerve transection on tibial BMD. Our findings demonstrate that the saphenous nerve is a critical source of tibial innervation making transection of the saphenous nerve a versatile model to study the impact of innervation on bone.

Disclosures: **T. Lizotte:** None. **G. Henderson:** None. **J. Hickey:** None. **P. Caradonna:** None. **V.E. Eaton:** None. **G. DeOliveira:** None. **D.C. Molliver:** None. **T.E. King:** None. **K. Becker:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.11/Z27

Topic: C.10. Brain Injury and Trauma

Support: Lone Star Paralysis Foundation
DoD Grant OR180077 W81XWH-19-2-0054

Title: Polyethylene glycol-fusion repair of transected sciatic nerve produces rapid sensory behavioral recovery in rats

Authors: *L. ZHOU, K. K. VENKUDUSAMY, Y. MONTOYA, A. N. OLIVAREZ, C. Z. YANG, G. D. BITTNER;
Inst. For Neurosci., Univ. Of Texas At Austin, Austin, TX

Abstract: Current best protocols to repair transection peripheral nerve injuries (PNIs) involve microsuturing the severed nerve ends to increase the probability of axonal regeneration across the lesion sites. However, behavioral recovery following this neurorrhaphy is typically poor to none due to slow and inaccurate axonal regeneration that do not prevent Wallerian Degeneration (WD) of severed distal nerve axons or atrophy of denervated targets. In contrast, polyethylene glycol fusion (PEG-fusion) repair of PNIs rapidly (within minutes) restores cytoplasmic and electrophysiological continuity, prevents WD of 40-60% of severed axons, immediately reinnervates many neuromuscular junctions, and produces faster and better voluntary behavioral recovery within weeks as assessed by the Sciatic Functional Index (SFI). Using rat sciatic nerves as a model system, we now address another unanswered, clinically important question about PEG-fusion regarding sensory functional recovery. Although SFI testing has a sensory component, it predominantly assesses motor-driven behaviors. In this study, we employed a novel, modified Von Frey filament (VF) testing method to assay the mechanical pain withdrawal threshold in rats following transection PNIs. This weekly testing method did not affect the baseline threshold in either sex over 6 wk post-operation (PO). Sensory areas innervated by the sciatic nerve were denervated immediately following PNIs and gradually recovered to the baseline threshold by 6 wk PO. Successfully PEG-fused animals displayed significantly faster recovery compared to animals receiving only microsutures (Negative control, NC). We observed no hypersensitivity in the sciatic-innervated areas in either group up to 12 wk PO. Finally, we discovered a compensatory mechanism for pain sensation recovery mediated by the saphenous nerve collateral branching, probably accounting for partial return of function in both PEG-fused and NC animals at earlier time points. Five PEG-fusion clinical trials are now underway. Our

results not only suggest additional clinical benefits of PEG-fusion technology, but also further validate its safety by demonstrating the absence of allodynic neuropathic pain.

Disclosures: L. Zhou: None. **K.K. Venkudusamy:** None. **Y. Montoya:** None. **A.N. Olivarez:** None. **C.Z. Yang:** None. **G.D. Bittner:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.12/Z28

Topic: C.10. Brain Injury and Trauma

Support: NIH 5R01NS108189-04

Title: Aging Accelerates Degradation of Human Neuromuscular Junction Following Peripheral Nerve Injury

Authors: *L. P. GONZALES[1], V. CHEN[3], A. TEDESCO[4], S. ANDALIB[5], T. R. JOHNSTON[6], O. STEWARD[7], R. GUPTA[2];
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Abstract: While it is widely recognized that there is a difference in how adult and pediatric patients respond and recover after traumatic nerve injuries, the etiology of this variability in outcomes between young adults and elderly adults remains unclear. While previous studies have emphasized the detrimental impact of increased age in neural regeneration, no study has focused on the effects of aging on human motor endplate (MEP) stability and target end organ innervation following peripheral nerve injury (PNI). Here, we present the analysis of human MEPs from PNI patients ranging from 22 to 77 years old. Denervated muscle biopsies were collected under an approved IRB during standard of care surgical procedures for patients post-PNI. The samples underwent tissue clearing with CUBIC R1 for 2 weeks before immunostaining with acetylcholine receptor-α, neurofilament, and synaptophysin. Z-stack images of motor endplates were collected using the Keyence BZ-X810 inverted fluorescence phase contrast microscope at 20x. The data was divided into young (< 60 years old) and elderly (> 60 years old) groups. MEP morphology (healthy = pretzel; unhealthy = intermediate or plaque) and innervation status (innervated or denervated) of the MEPs was also assessed. Preliminary analysis of the MEPs revealed no significant difference in the percentage of healthy (pretzel) and unhealthy (intermediate and plaque) morphology between the two groups. The samples obtained from the young group showed an average of 13.50% healthy MEPs and 86.50% unhealthy MEPs while the samples from the elderly group revealed an average of 18.33% healthy MEPs and 81.67% unhealthy MEPs. Remarkably, there was an observed two-fold increase in the

percentage of innervated MEPs in the young group (57.75%) compared to the elderly group (29.17%), indicating an increased likelihood of prolonged MEP survivability in the young group. Aging has been linked to the decreased rate of neural regeneration; however, our study suggests that it also plays a role in accelerating the rate of neuromuscular junction degradation following injury.

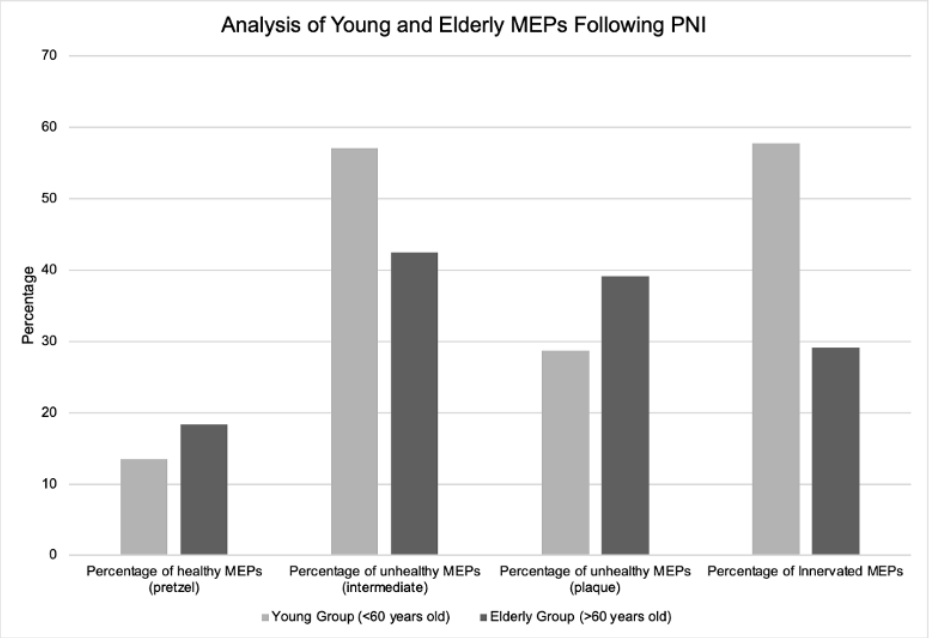

Figure 1. Average percentage of healthy, unhealthy, and innervated MEPs in young and elderly PNI patients. No significant differences were found in the average percentage of healthy and unhealthy MEPs between the two groups. The average percentage of innervated MEPs in the young group is double the average percentage of innervated MEPs found in the elderly group.

Disclosures: L.P. Gonzales: None. **V. Chen:** None. **A. Tedesco:** None. **S. Andalib:** None. **T.R. Johnston:** None. **O. Steward:** None. **R. Gupta:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.13/AA1

Topic: C.10. Brain Injury and Trauma

Support: NIH NIA Grant AG081739

Title: Human Deltoid Muscle Time-Dependent Response to Axillary Nerve Injury: Fatty Atrophy, Satellite Cell Abundance, and Motor Endplate Degeneration

Authors: *A. TEDESCO[1], L. GONZALEZ[1], S. ANDALIB[1], V. CHEN[2], M. HICKS[3], T. R. JOHNSTON[2], O. STEWARD[4], R. GUPTA[2];
[2]Orthopaedic Surgery, [1]Univ. of California Irvine Sch. of Med., Irvine, CA; [3]Physiol. & Biophysics, Univ. of California Irvine, Irvine, CA; [4]Anat. & Neurobio., Univ. of California Irvine, Irvine CA, CA

Abstract: Axillary nerve injury and resultant deltoid muscle denervation produces profound functional disability. Time to reconstructive surgery is a critical factor affecting patient outcomes, but the timeline of neuromuscular deterioration studied in animals has limited extrapolation to the human condition. We characterized muscle degenerative changes with respect to fatty infiltration, myofiber atrophy, satellite cell (SC) density, and motor endplate (MEP) morphology across time post-denervation. Human denervated and control deltoid muscle biopsies were collected and stained with Oil Red O to visualize lipid accumulation; Pax7, MF20, and laminin to quantify SC density and myofiber cross-sectional area (CSA); and acetylcholine receptor-α, neurofilament, and synaptophysin for 3D-characterization of MEP morphology. Denervated biopsies ranged from 4 days-10 years post-injury and were compared to control. Fat content was similar between control and denervated deltoids up to 8 months from injury but was significantly increased at later time points. Although mean myofiber CSA was decreased at all time points after injury, denervated muscle SC density was comparable to controls until the last time points. There is a distinct time-dependent degeneration of MEPs at all time points post-injury. Taken together, human deltoid muscle denervation produces early MEP degeneration and myofiber atrophy, followed by perimysial fatty infiltration and a late increase in SC density and myofiber size variability.

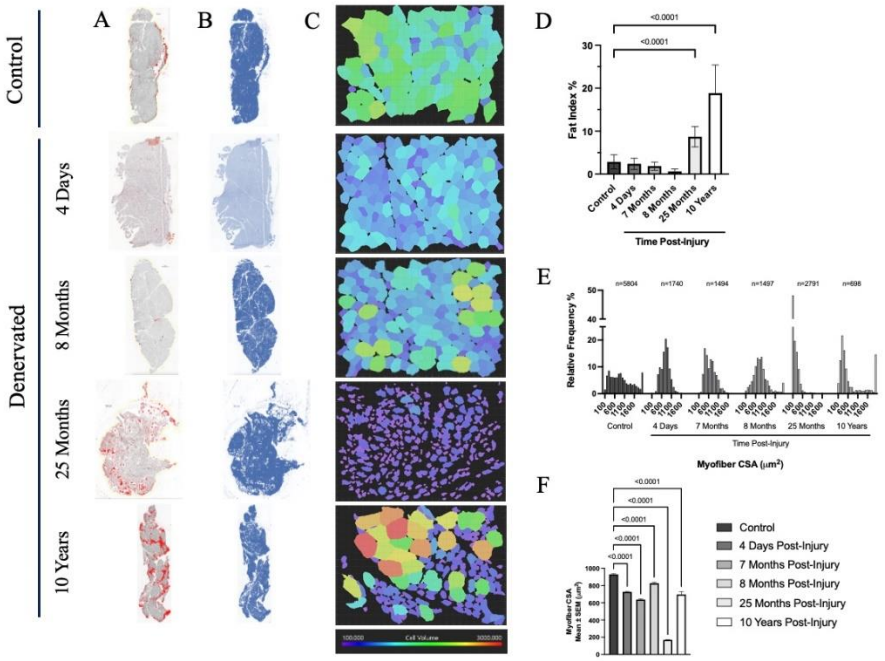

Figure 1. Representative images of Oil Red O-stained fat (A) as a proportion of total sample area (B), shown graphically as fat index percentage (D). Myofiber CSA quantification shown as representative color-scale images at 10X magnification (C), myofiber CSA relative frequency distributions (E), and mean myofiber CSA comparisons (F).

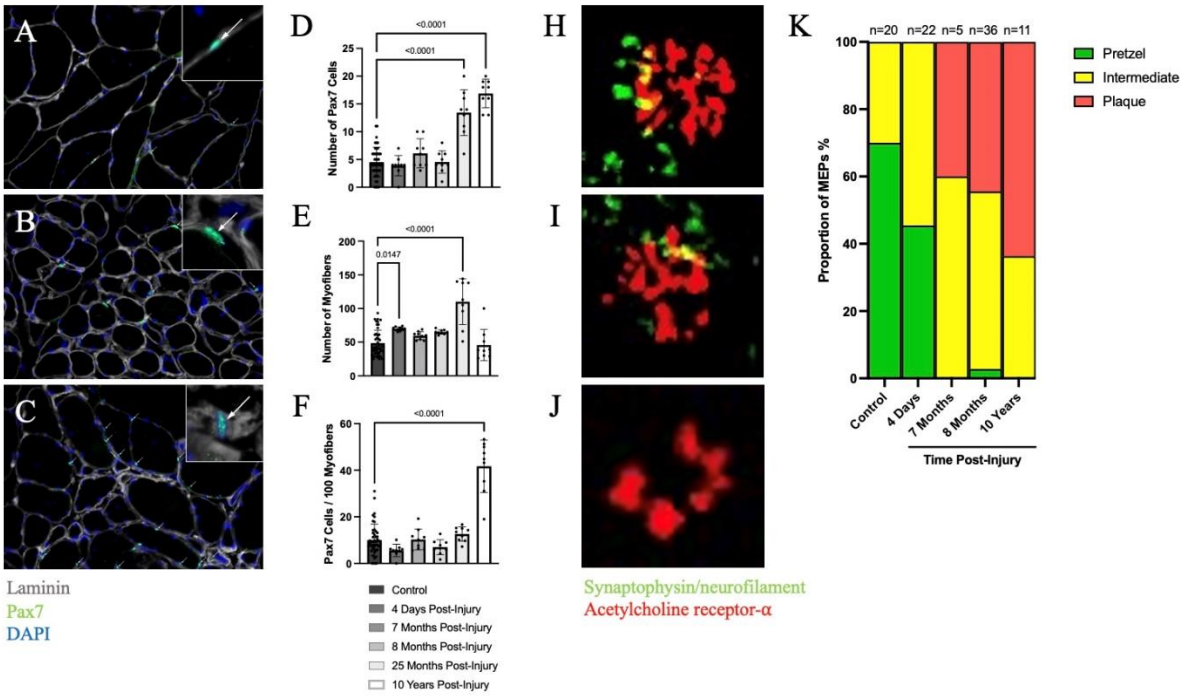

Figure 2. Representative images of control (A), 7 months post-injury (B), and 10 years post-injury (C) deltoid muscle at 20X magnification, with image insets demonstrating Pax7+ DAPI+ SCs. Number of Pax7 cells (D) normalized to number of myofibers (E) in an imaging field to yield Pax7 cell density (F). Classification of MEP morphology as high complexity pretzel (H), intermediate (I), or low complexity plaque (J). Quantification of MEP morphology as a proportion of total MEPs observed (K).

Disclosures: A. Tedesco: None. **L. Gonzalez:** None. **S. Andalib:** None. **V. Chen:** None. **M. Hicks:** None. **T.R. Johnston:** None. **O. Steward:** None. **R. Gupta:** None.

Poster

PSTR541. Peripheral Nerve Trauma, Crush, and Toxic Injury

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR541.14/AA2

Topic: C.06. Neuromuscular Diseases

Title: Transection of Facial Nerve's Digastric Branch as a Model for Inducing Denervation Related Atrophy in the Posterior Digastric Muscle of Rats

Authors: *T. CHOTIRUNGSAN[1,2], Y. TSUTSUI[1], S. KAWADA[1], N. DEWA[1], J. MAGARA[1], T. TSUJIMURA[1], M. INOUE[1];
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Abstract: Background: Recently, we found that the posterior belly of the digastric muscle (post-Dig) is activated during swallowing. However, the functional role of this muscle in functions has not yet been elucidated. **Aims:** We induced post-Dig atrophy and evaluated effects

of atrophy on feeding behaviors in rats. **Methods:** In this study, male SD rats were utilized to assess both morphological and functional changes subsequent to denervation of the post-Dig motor nerve through physiological and histological examinations. Nerve transection was confirmed by EMG responses as well as retrograde tracing using Fluoro-Gold. In the acute experiment, swallow responses induced by mechanical stimulation applied to the vocal folds were recorded in the thyrohyoid, sternohyoid (SH), and post-Dig muscles. For the chronic experiment, rats were allowed to move freely. EMGs of the masseter, temporalis, anterior belly of the digastric muscle (ant-Dig), post-Dig, and SH muscles were recorded before and after denervation to evaluate changes of feeding behaviors. Finally, in the histological study, the post-Dig muscle was harvested immediately and at intervals of one to four weeks after denervation. EMGs, muscle weight and size, as well as muscle fibers were compared between pre- and post-denervation periods. **Results**: After denervation, the post-Dig EMG activity vanished immediately. Additionally, in the acute experiment, the EMG peak and area of the ipsilateral SH and ant-Dig muscles displayed a tendency to decrease during swallowing. In conscious rats, although the frequency of water swallowing notably increased, nothing of EMG activities in other muscles was affected during chewing. The post-Dig muscle's size and weight experienced a significant reduction two weeks after denervation. **Conclusions:** The silence in post-Dig EMG activity and remarkable reduction of post-Dig size and weight indicate the successfulness of atrophy model development. The depression of peak and area of SH and ant-Dig EMGs in the acute experiment suggests that performance of post-Dig muscle may be associated with other suprahyoid and infrahyoid muscles. A single alteration in frequency of liquid swallow reflects post-Dig is predominantly important muscle for fluid swallow in conscious rats. However, other muscles may compensate for atrophy of post-Dig during chewing.

Disclosures: T. Chotirungsan: None. **Y. Tsutsui:** None. **S. Kawada:** None. **N. Dewa:** None. **J. Magara:** None. **T. Tsujimura:** None. **M. Inoue:** None.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.01/AA3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Praxis Spinal Cord Institute
Wings for Life

Title: The acute effects of transcutaneous spinal cord stimulation on spinal excitability in people with spinal cord injury

Authors: *A. M. M. WILLIAMS[1,2], S. SAMEJIMA[1,2], C. SHACKLETON[1,2], R. N. MALIK[1,2], C. T. MORITZ[3], A. KRASSIOUKOV[1,2,4], T. LAM[1,2];
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Abstract: Recent research has established the potential benefits of transcutaneous spinal cord stimulation (TSCS) on different motor outcomes in people with spinal cord injury (SCI), including reduced spasticity, improvements in voluntary control, and performance of functional motor tasks. The Hoffmann reflex (H-reflex) is a neurophysiological assessment that can be used to probe changes in spinal motor excitability, and may be used to reflect changes in spasticity following SCI. Both the input-output characteristics of the H-reflex response and the effect of different stimulation frequencies on H-reflex amplitude (rate-dependent depression) have been used to characterize spinal motor circuitry following SCI. Considering the putative effects of TSCS on spinal cord circuitry, the purpose of this study was to characterize the acute effects of lumbosacral TSCS on H-reflex responses in individuals with chronic SCI. In this cross-sectional study, we recruited adults with a chronic (≥1 year post injury) SCI at or above T6. We recorded EMG signals from the soleus and applied electrical stimulation (monophasic waveform, 1ms pulse) over the tibial nerve in the popliteal fossa. Stimulation began at low levels of intensity, and increased in graduated steps until we observed a plateau in the M-wave amplitude. We used these data to generate recruitment curves and compute the Hwave/Mwave ratio. We also investigated rate-dependent depression by delivering trains of 15 stimuli at different frequencies (0.2Hz, 0.5Hz, 1Hz, or 2Hz). We used this data to compute the average the peak-to-peak amplitude of the last eight H-waves at each frequency. We then repeated the same protocol while the participant received continuous TSCS between the T11-T12 and L1-L2 vertebrae (30Hz, monophasic waveform, 1ms pulses with 10kHz carrier frequency, 25-100mA). In the presence of TSCS, most participants had a decrease in the Hwave/Mwave ratio. Rate-dependent depression was generally weaker in the presence of TSCS. Our results suggest that the application of TSCS may reduce (normalize) spinal cord motor circuit excitability in people with chronic SCI. As many individuals with supra-sacral injuries experience hyperreflexia, these results provide further support towards the potential effects of TSCS on resting spinal cord hyperexcitability.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.02/AA4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Postdoc Fellowship, Faculty of Rehabilitation Medicine, University of Alberta
Internal funds from Faculty of Rehabilitation Medicine, University of Alberta

Title: Spinal reflexes reflecting the putative Ia-motoneuronal pathway after incomplete spinal cord injury in humans - variability and potential mechanisms

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Abstract: Background: People with spinal cord injury (SCI) often develop exaggerated spinal reflexes, commonly studied in the soleus muscle. Compared to the uninjured controls, these commonly manifest as altered H-reflexes, reduced post-activation depression as reflected by reduced rate-dependent depression (RDD) of the H-reflex, and increased clonus. These measures putatively reflect the excitability of the Ia-motoneuronal pathway among other unknown pathways, and are often used to gauge effectiveness of interventions. If the Ia-motoneuronal pathway dominates these reflexive responses, then their excitability should vary in the same way within a person (Aim 1), and from day-to-day (Aim 2). The reliability of these reflexes could also inform the need for averaging when examining the effectiveness of interventions. Methods: Thirteen participants with chronic motor incomplete SCI were compared with age-matched controls. Soleus H-reflex and M-wave were measured on the more affected leg for SCI participants, and on the right leg of the controls. Hmax/Mmax ratio was obtained from the recruitment curve of H and M responses. RDD was evoked from a train of 15 stimuli, applied at frequencies of 2Hz, 1Hz, 0.5Hz, and 0.2 Hz. Clonus was measured while participants walked on a treadmill at a self-selected speed. The same measurements were repeated on a different day about one week apart. Data analysis: RDD was estimated as the average peak-to-peak amplitude of the H-reflex from the last 8 stimuli at each frequency, then normalized to the average obtained at 0.2 Hz. Because the deepest depression was around 2 Hz, the ratio at 2 Hz/0.2 Hz was used to represent RDD. Clonus was quantified as the proportion of signal power contained in the frequencies between 4-10 Hz in the soleus EMG. Relationship between each of the measurements were determined using Pearson's P-M correlation between the measures obtained on Day 1. Intraclass Correlation Coefficient estimated the reliability of each measure between days. Differences in reflex excitability between those with and without SCI were compared for Day 1 using a standard t-test. Results and conclusion: No significant correlations were seen among the measures of reflex excitability (Hmax/Mmax, RDD and clonus). Further, compared to people without SCI, those with SCI had less RDD and more clonus, but similar Hmax/Mmax. Thus, it is likely that the Ia-motoneuronal pathway does not dominate responses in the same way. Day-to-day variability of the reflexes was larger in people with SCI compared to those without, so averaging data from more than a single session is recommended.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.03/AA5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: a grant of the Korea Health Technology R&D Project (HI21C0572) through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare
the Pioneer Research Center Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT & Future Planning (2022M3C1A3090851)
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Title: Effects of frequency-dependent epidural spinal cord stimulation on neuropathic pain and spasticity via inhibiting activated microglia after spinal cord injury

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Abstract: Neuropathic pain and spasticity following spinal cord injury (SCI) affect not only patients' physical and psychological health, but also place a heavy burden on the individuals. Spinal cord stimulation (SCS) is an alternative neuromodulation technique for treating neuropathic pain. While used in clinical trials, the underlying mechanisms concerned with stimulating parameters and the relief of neuropathic pain or spasticity are still unclear. In the present study, we investigated the effect of wireless epidural SCS on neuropathic pain or spasticity, and the alternation of microglia phenotype depending on stimulation parameters. A contusive SCI was made at T11 segment using Infinite Horizons (IH) impactor in adult male SD-rats. Two weeks after SCI, an epidural electrode and device for SCS were implanted. For measuring the alternation of mechanical sensitivity and spasticity, paw withdrawal threshold (PWT) and Modified Ashworth Score (MAS) were assessed before and after SCS at different frequencies and intensity (0, 0.5, 1, 1.5, 2, and 3 h after SCI; 50 Hz, 1,000 Hz; motor threshold MT 40 % and MT 80 %). After SCS, morphological changes associated with microglial activation were also assessed in the dorsal and ventral horn in lumber spinal segments. The therapeutic effects of SCS on neuropathic pain and spasticity were shown in four different groups (50 or 1,000 Hz and MT 40% or 80%) immediately after SCS, but the effects decreased until 3 h after SCS. The accumulative effects for 3 h after SCI on PWT were the highest at 1,000 Hz with MT 80%, and the lowest at 50 Hz with MT 40%. Accumulative effects for 3 h after SCI on spasticity were also lowest at 50 Hz with MT 40%, but the other three groups were similar. The total number of microglia was not changed after SCS in all groups. However, the activations of microglia after SCS significantly decreased at 50 Hz with MT 80% and at 1,000 Hz with MT 80% in both dorsal and ventral horns. The present data demonstrated the therapeutic effects of SCS on neuropathic pain and spasticity after SCI depending on the differently applied frequency or intensity. The present result related to the alternation of activated microglia by SCS suggests

the potential underlying mechanisms of SCS effects. Further study to investigate repetitive effects for several times or tolerance of SCS is needed.

Disclosures: **J. Lee:** None. **H. Ok:** None. **J. Kim:** None.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.04/AA6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: K12 Neurorehabilitation and Restorative Neuroscience Training Network (K12HD093426)
Craig H Neilsen Foundation (FP00235923)

Title: Movement-related cortical stimulation for enhancing corticospinal excitability below the level of incomplete spinal cord injury: A Proof-of-Concept Case Study

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Abstract: After sustaining an incomplete spinal cord injury (iSCI), not only is the transmission of signals at the site of the injury affected, there is evidence that higher brain structures are impacted as well. Studies have shown that the excitability of intracortical circuits within the primary motor cortex is decreased and delayed, leading to decreased ability for initiating movement and recruiting residual spinal motor neurons. TMS has been studied as a modality for enhancing corticospinal excitability and facilitating muscle activation below the level of injury in individuals with iSCI. One approach to target intracortical circuits is to pair TMS with motor intention; also known as movement-related cortical stimulation. In able-bodied, movement-related cortical stimulation has been shown to modulate corticospinal excitability (CE) in a spike-time dependent plasticity manner. We hypothesize that delivering TMS during motor intention will enhance corticospinal excitability and thus improve activation of muscles below the level of injury. One person with chronic incomplete tetraplegia participated first in a crossover study, where we investigated the impact of TMS timing on corticospinal excitability when delivered 50 ms prior to or after movement (1-week washout), based on muscle activity of the abductor halluces. In a follow-up experiment, the participant received 5-consecutive treatment days of movement-related cortical stimulation where active TMS was delivered 50 ms prior to movement onset for 15-20 minutes (120 total stimuli per session). Experiment 1: CE was assessed before and immediately following: (a) Sham TMS delivered 50 ms prior to movement onset; b) Active TMS delivered 50 ms prior to movement onset; c) Active TMS delivered 50 ms after movement onset. We found an increase CE when TMS was delivered prior to movement

initiation, but not following sham stimulation and when active TMS was delivered after movement onset. Experiment 2: We assessed CE and volitionally controlled motor unit recruitment at baseline, the beginning of each treatment session, post 3-days treatment, and post 7-days treatment. Five consecutive treatment sessions of movement-related cortical stimulation resulted in increased CE as well as improved volitionally controlled motor unit recruitment up to 3-days post treatment, where outcome measures returned to baseline by 7-days post treatment. Future studies will extend these findings to a larger sample population and investigate whether movement-related cortical stimulation can be used to prime the motor circuitry prior to receiving therapy in order to impact motor recovery in the iSCI population.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

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Program #/Poster #: PSTR542.05/AA7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: PREP2GO: Eurostars E!113969
Defitech Foundation
Swiss National Science Foundation (NCCR Robotics)
Swiss Commission of Technology and Innovation Innosuisse (STIMO Bridge)
Defense Advanced Research Projects Agency (DARPA), Contract No. N66001-20-2-4046

Title: Automated generation of spinal cord models for pre-operative planning of epidural electrical stimulation surgical interventions

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Abstract: Spinal cord injuries (SCI) and neurological disorders like Parkinson's disease (PD) afflict numerous individuals globally, causing paralysis and diminished quality of life. Epidural Electrical Stimulation (EES) offers a promising avenue to restore movement and hemodynamic stability. However, precise electrode placement is challenging due to individual anatomical differences. Recent studies have demonstrated that building personalized computational models using patient-specific Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) scans can facilitate optimal EES lead placement for selective muscle recruitment. However, these models take an average of 60 human hours to construct, limiting their scalability for widespread clinical use.

We present a transformative solution: an AI-based computational pipeline that streamlines spinal tissue reconstruction. Using a manually labeled data set of subject-specific MRI volumes and publicly available tools, our pipeline automates the reconstruction of white matter, cerebrospinal fluid, vertebral bodies, and intervertebral discs, and the co-registration of CT scans in the MRI space while semi-automating the reconstruction of spinal roots. This approach cuts model-building time by 94%, while maintaining anatomical accuracy across cervical, thoracic, and lumbosacral regions. To enhance robustness, we adapted our pipeline to overcome challenges including MR artifacts, presence of scoliosis, and varying MR vendors and sequences.

Thus far, our pipeline has been used to generate 17 personalized models for different EES-based clinical studies, facilitating 17 surgeries (7 thoracic, 6 lumbar-thoracic, and 4 lumbar), all successful with positive outcomes for the studies participants. Our work enhances the scalability of EES-based neuroprosthetic therapies, potentially restoring walking capabilities and blood pressure stability for SCI patients, and improving gait deficits in PD.

This research was developed with funding from the Defense Advanced Research Projects Agency (DARPA), Contract No. N66001-20-2-4046. The views, opinions and/or findings expressed are those of the author and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

Disclosures: S. Hernandez Charpak: None. **E. Paoles:** A. Employment/Salary (full or part-time):; ONWARD Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ONWARD Medical. **T. Newton:** None. **E. Meliadò:** None. **A. Rowald:** None. **T. Zhou:** None. **J. García Ordoñez:** None. **J. Ledoux:** None. **F. Becce:** None. **V. Spagnolo:** None. **V. Delattre:** A. Employment/Salary (full or part-time):; ONWARD Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ONWARD Medical. **H. Lorach:** None. **E. Martin Moraud:** None. **L. Asboth:** None. **R. Demesmaeker:** None. **T. Milekovic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EPFL. **E. Konukoglu:** None. **S. Mandija:** None. **N. van den Berg:** None. **N. Kuster:** None. **E. Neufeld:** None. **G. Dumont:** None. **J. Bloch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EPFL. F. Consulting Fees (e.g., advisory boards); ONWARD Medical. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.06/AA8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 310030_215668Mechanisms through which noninvasive spinal cord stimulation improves walking and reaching after spinal cord injury

Title: Mechanisms underlying neurological improvements following rehabilitation augmented by externally applied electrical stimulation of the spinal cord

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Abstract: Electrical stimulation of the spinal cord applied during rehabilitation has enhanced upper and lower limb function in numerous preclinical models, from rodents to primates, as well as a number of clinical case studies. Stimulation of the spinal cord can be achieved using non-invasive methodology whereby electrical current is delivered to the spinal cord through surface electrodes (ARC^{EX} Therapy). However, the mechanisms responsible for neurological recovery in response to ARC^{EX} Therapy remain poorly understood. To fill this knowledge gap, we studied the neural pathways that are directly recruited by ARC^{EX} Therapy, and the subsequent modulation of specific neuronal subpopulations mediating the observed neurological recovery in response to this treatment. To support this study, we developed a preclinical model in mice and rats. We developed a wearable, soft jacket wherein electrodes are inserted to deliver ARC^{EX} Therapy to the spinal cord of rodents within a freely moving environment. We then used a judicious combination of computational, anatomical, molecular, and causal methodologies to investigate the mechanisms of ARC^{EX} Therapy. Using RNAscope we detected transcriptionally active neurons following ARC^{EX} Therapy application, allowing the identification of the neuronal subpopulations that are specifically activated by ARC^{EX} Therapy. With a uniquely designed

neurorobotic platform for rats, we quantified the effect of this stimulation on motor recovery of the upper limbs. Furthermore, we conducted electrophysiological studies in conjunction with highly realistic computational rodent models to expose how the electrical fields elicited by ARC^{EX} Therapy propagate throughout the body and show that this stimulation activates neuronal subpopulations embedded in the spinal cord. Combining these cutting-edge techniques, we aim to unravel the intricate mechanisms by which ARC^{EX} Therapy facilitates the recovery of autonomic and sensorimotor functions. We anticipate these results may improve the methodology to deliver ARC^{EX} Therapy, and thereby enhance neurological recovery for individuals living with SCI.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.07/AA9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Sino Swiss: ZLCZ0_206073 Neuroprosthetic management of orthostatic hypotension in multiple system atrophy
MSA Coalition

Title: Neurodegenerative topography dictates the efficacy of spinal cord stimulation to restore blood pressure stability

Authors: *S. AMIR[1,2,3,4], R. HUDELLE[2,3,4], J. SORIANO[5,2,3,4], L. MAHE[2,3,4], N. HANKOV[2,3,4], L. ASBOTH[2,3,4], R. DEMESMAEKER[2,3,4], V. AURELI[2,3,4,6], E. MARTIN-MORAUD[2,3,4], J. BALLY[7], Q. BARRAUD[2,3,4], B. SCHNEIDER[8], E. BEZARD[9], S. P. LACOUR[3], M. A. ANDERSON[2,3,4,10], A. A. PHILLIPS[5,11], J. BLOCH[2,3,4,6], J. SQUAIR[2,3,4,6], G. COURTINE[2,3,4,6];

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Abstract: Neurodegenerative disorders such as Multiple System Atrophy (MSA), Parkinson's disease, Lewy Body Dementia, and Pure Autonomic Failure lead to a severe dysfunction in the autonomic nervous system. This dysfunction causes neurogenic orthostatic hypotension, leaving individuals bedridden and incapable of participating in daily activities. The treatment options for orthostatic hypotension in these populations are limited, and other disease-modifying therapies, like dopamine replacement, can exacerbate the symptoms. Our recent work revealed the mechanisms through which epidural electrical stimulation (EES) of the thoracic spinal cord regulates blood pressure. We leveraged this understanding to develop a neuroprosthetic baroreflex that prevented orthostatic hypotension in various species, including mice, rats, pigs, non-human primates, and humans with spinal cord injury (SCI). Both SCI and neurodegenerative conditions disrupt the communication between brainstem cardiovascular regulatory centers and sympathetic pre-ganglionic neurons in the spinal cord. Thus, we hypothesized that EES applied over the lower thoracic spinal cord could also prevent orthostatic hypotension in patients with MSA. As predicted, EES reduced the severity of orthostatic hypotension and eliminated pre-syncope episodes in this one participant. However, it remains unknown which neurodegenerative populations will benefit from this therapy, how EES controls sympathetic pre-ganglionic neurons in these conditions, and what the effects of long-term stimulation on hemodynamic stability are. To address these questions, we established several rodent models mimicking the diversity of neurodegenerative conditions that cause severe orthostatic hypotension. These models include targeted virally mediated alpha synuclein expression, cell type-specific ablations, constitutive overexpression of alpha synuclein in relevant cell types, and models that simulate mitochondrial function knockdown. Our findings suggest that orthostatic hypotension arises following the degeneration or ablation of any neuron in the natural baroreflex. However, EES could only restore hemodynamic stability under specific topological patterns of neuronal loss. Future studies will explore the correlation between these constraints and patient populations targeted for clinical trials. Furthermore, we will assess the long-term effects of targeted EES over the lower thoracic spinal cord to restore hemodynamic stability.

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consultant of ONWARD medical. **J. Bloch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds patents related to this work. F. Consulting Fees (e.g., advisory boards); Is a consultant of ONWARD medical. **J. Squair:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds patents related to this work. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds patents related to this work. F. Consulting Fees (e.g., advisory boards); Is a consultant of ONWARD medical.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.08/AA10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Defitech Fundation

Title: Epidural electrical stimulation of the lower thoracic spinal cord reduces orthostatic hypotension in people with spinal cord injury

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Abstract: Spinal cord injury (SCI) disconnects brainstem cardiovascular regulatory centers from the sympathetic circuits that regulate blood pressure, leaving affected individuals with daily debilitating episodes of orthostatic hypotension that restrict participation in rehabilitation, and engagement in social activities, exacerbate cardiovascular risk, and dramatically reduce quality of life.Here, we developed ARC^{IM} Therapy, which comprises epidural electrical stimulation (EES) applied over the lower thoracic spinal cord to alleviate hemodynamic instability and is the first purpose-built implantable neuromodulation technology for people living with SCI.As we previously demonstrated in rats, non-human primates, and in one human with SCI, the location of the hemodynamic hotspot was confirmed to be at the lower thoracic level. Indeed, a direct human intra-subject comparison leveraging repurposed technologies revealed that EES applied over the lower thoracic spinal cord led to substantially more efficacious pressor responses compared to stimulation of the lumbosacral spinal cord. In total, we applied ARC^{IM} Therapy in 11 participants across multiple clinical studies, wherein all participants presented with severe orthostatic hypotension. Immediately after turning ARC^{IM} Therapy on, the severity of orthostatic hypotension was robustly reduced in 11 out of 11 participants. We quantified this as a reduction of more than 20mmHg systolic in the drop in arterial blood pressure, as well as an increased tolerance, during formal 10 minutes tilt tests. By modulating and increasing arterial pressure to stabilize blood pressure, cerebral blood flow was also enhanced to healthy levels in the seated position, thereby preventing the occurrence of syncope. All participants use the stimulation multiple hours a day and reported multi-faceted benefits of this novel therapy. Moreover, ARC^{IM} Therapy also enables the prosthetic recruitment of trunk musculature, leading to enhanced trunk stability, rehabilitation, and recovery. These results demonstrate that ARC^{IM} Therapy has the potential to reduce the severity of orthostatic hypotension, increase trunk stability, and significantly improve participants' rehabilitation, social involvement, and quality of life.

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related to this work. F. Consulting Fees (e.g., advisory boards); is a consultant of ONWARD medical.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.09/AA11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Defitech Foundation

Title: Automated configuration of epidural electrical spinal cord stimulation for neurological disorders

Authors: *G. CARPARELLI[1,2,3], P. ABRANCHES[2,1,3,4], S. A. KOMI[2], X. YANG[2,1,3], N. MACELLARI[2,1,3], C. HARTE[2,1,3], G. DUMONT[2,1,3], H. LORACH[2,1,3], F. B. WAGNER[2], V. CEVHER[4], L. ASBOTH[2,1,3], R. DEMESMAEKER[2,1,3], J. BLOCH[2,1,3,5], G. COURTINE[2,1,3,5]; [1]NeuroX Institute, Sch. of Life Sciences, EPFL, Lausanne, Switzerland; [2]Defitech Ctr. for Interventional Neurotherapies (.NeuroRestore), Swiss Federal Inst. of Technol. (EPFL), of clinical neuroscience, Lausanne Univ. Hosp. (CHUV) and Univ. of Lausanne (UNIL), Lausanne, Switzerland; [3]Dept. of Clin. Neuroscience, CHUV and UNIL, Lausanne, Switzerland; [4]Lab. for Information and Inference Systems, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; [5]Dept. of Neurosurgery, CHUV, Lausanne, Switzerland

Abstract: Targeted epidural electrical stimulation (EES) has emerged as a promising intervention to alleviate motor deficits in spinal cord injury (SCI) and Parkinson's disease (PD). However, the scalability of these therapies is hampered by the complexity and duration of the personalization procedures required to achieve the desired results. Optimal parameters are dependent on the specific neurological disorder, the participant-specific deficits and the neurostimulation platform itself. Here, we introduce a new approach that utilizes a semi-automated algorithm based on Gaussian Process Bayesian Optimization to rapidly personalize targeted EES with minimal human resources. This algorithmic approach automates the optimisation of electrical stimulation parameters including the choice of cathodes and anodes, pulse patterns, and frequencies, for each target movement. The framework furthermore includes a short calibration of approximately 5 minutes that maximizes the initial algorithmic knowledge, as well as a smart ramping of the stimulation amplitude to increase patient comfort while remaining time-efficient. The algorithm monitors the motor output through electromyography and motion sensor data, which collectively contribute to establishing a reward measure. Compared to the human-driven approach employed by highly trained experts, our method showcases a significant reduction of the time required to achieve satisfying stimulation personalization. Specifically, it typically elicits the desired target movements by stimulating the correct motor hotspots in less than 15 minutes per target. Furthermore, the movements obtained with the resulting stimulation parameters are functionally comparable to those optimized by the

team of experts. These results establish a clear path towards facilitated wide-spread clinical integration of targeted EES.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.10/AA12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Defitech Foundation
Rolex Award for Enterprise
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Institut Carnot Leti. ETH domain PHRT grant 2022-279

Title: Noninvasive and invasive methodologies of stimulating the cervical spinal cord in order to improve upper-limb functions after tetraplegia

Authors: *T. COLLIN[1,2,3,4], M. BERNY[2,3,4], C. HARTE[2,3,4], I. SAKR[2,3,4], V. SPAGNOLO[2,3,4], N. INTERING[2,4], A. GALVEZ[2,3,4], P. ABRANCHES[2,3,5,4], G. CARPARELLI[2,3,4], S. HERNANDEZ[2,3,4], G. DUMONT[2,3,4], P. BESSOT[6], A. WATRIN[6], L. BOLE FEYSOT[2,3,4], N. HANKOV[2,3,4,7], C. POULIN-KELLA[2,4,8], F. BECCE[8], J.-B. LEDOUX[8,9], C.-D. SASPORTES[2,3,4], C. VARESCON[2,3,4], C. PICQ[6], J. DEDELLEY[6], R. DEMESMAEKER[2,3,4], L. ASBOTH[2,3,4], E. ROSS[6], J. MURPHY[6], J. BLOCH[2,3,4,10], H. LORACH[2,3,4], G. COURTINE[2,4,3,7]; [1]EPFL, Geneva, Switzerland; [2]Defitech Ctr. for Interventional Neurotherapies (.NeuroRestore), Swiss Federal Inst. of Technol. (EPFL), of clinical neuroscience, Lausanne Univ. Hosp. (CHUV) and Univ. of Lausanne (UNIL), Lausanne, Switzerland; [3]NeuroX Institute, Sch. of Life Sciences, EPFL, Lausanne, Switzerland; [4]Dept. of Clin. Neuroscience, CHUV and UNIL, Lausanne, Switzerland; [5]Lab. for Information and Inference Systems, EPFL, Lausanne, Switzerland; [6]ONWARD Med., Lausanne, Switzerland; [7]Fac. of Biol. and Medicine, Univ. of Lausanne, Lausanne, Switzerland; [8]Dept. of Radiology, CHUV, Lausanne, Switzerland; [9]Ctr. for BioMedical Imaging (CIBM), Lausanne, Switzerland; [10]Dept. of Neurosurgery, CHUV, Lausanne, Lausanne, Switzerland

Abstract: Spinal cord injury (SCI) alters the communication between the brain and the regions of the spinal cord that produce upper-limb movements. The consequence is permanent deficits in upper-limb functions. Both non-invasive and invasive methodologies are under investigation to engage the cervical spinal cord in order to improve upper-limb functions after SCI. The noninvasive methodology consists of applying electrical currents through externally-placed electrodes that target broad regions of the cervical spinal cord. The invasive methodology leverages epidural electrical stimulation to target the larger diameter afferent fibers where they enter the cervical spinal cord through the dorsal root entry zones. The recruitment of these fibers leads to the direct and indirect activation of upper-limb motor neurons. Here, we sought to compare the responses from these noninvasive and invasive methodologies in participants without neurological deficits of the upper limbs and in participants with tetraplegia. We recorded electromyographic activity of the upper limbs in response to a wide variety of stimulation parameters including electrode configurations, frequency and amplitudes. We found evidence that both methodologies recruit the afferent fibers to elicit muscle response patterns, and that the specific spatial distributions of these patterns depended on the location of the stimulation. However, only the invasive methodology enabled the recruitment of specific ensembles of motor neurons. We concluded that externally-applied electrical stimulation of the cervical spinal cord is effective for potentiating the activity of cervical neurons during rehabilitation, but only epidural electrical stimulation enables spatially and temporally selective control over specific ensemble of motor neurons, as required to promote the immediate restoration of functional movements after paralysis.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.11/AA13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Defitech Foundation
Defense Advanced Research Projects Agency (N66001-20-2-4046)

Title: Closed-loop control of blood pressure after spinal cord injury

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Abstract: Cervical and high-thoracic spinal cord injuries (SCI) disrupt the communication between cardiovascular regulatory centers in the brain stem and sympathetic preganglionic neurons in the lower thoracic spinal cord, resulting in orthostatic hypotension (OH), increased risk of cardiovascular disease, and reduced quality of life. Here, we present a closed-loop neuroprosthetic system that stabilizes blood pressure during severe orthostatic challenges. We leveraged the ONWARD ARCIM Therapy, which delivers epidural electrical stimulation (EES) over the lower thoracic spinal cord, to modulate blood pressure. Within the HemON clinical trial (NCT05111093), we optimized spatial and temporal features of biomimetic EES in eight individuals with chronic SCI who were implanted with the ARCIM system. We then extended our previous work to implement a proportional-integral controller that modulates EES

amplitudes based on blood pressure measurements acquired from the Finapres Nova, a non-invasive, non-portable blood pressure monitor. The ARCIM Therapy combined with closed-loop prevented orthostatic hypotension in all participants during verticalization. However, this approach relies on a non-portable blood pressure monitor that prevents continuous monitoring in ambulatory settings.
Consequently, we developed a novel, portable, invasive blood pressure sensor system to enable the ARCIM Therapy to operate in closed-loop outside the clinic. We then combined this new, implantable sensor with the ARCIM system in two Yucatan minipigs with SCI. Closed-loop control of EES based on real-time measurements of blood pressure using the implanted sensor mitigated orthostatic hypotension despite simulated orthostatic challenges and environmental changes inducing hypotension. Together, these results demonstrate the efficacy of closed-loop control via the ARCIM Therapy to prevent orthostatic hypotension, and establish a technological path towards continuous closed-loop control of blood pressure in ecological settings.
Acknowledgments: This research is partly funded by the Defitech Foundation. This research was developed with funding from the Defense Advanced Research Projects Agency (DARPA), Contract No. N66001-20-2-4046. The views, opinions and/or findings expressed are those of the author and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.12/AA14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SNSF grant 310030_185214
Defitech Fundation

Title: Deep brain stimulation of the lateral hypothalamus improves neurological recovery after spinal cord injury

Authors: *L. BOLE-FEYSOT[1,2,3], N. CHO[1,2,3], V. AURELI[1,2,3], N. JAMES[1,2,3], K.-A. BARTHOLDI[1,2,3], I. DEWANY[1,2,3], M. GOUBRAN[4], N. INTERING[1,2,3], C. VARESCON[1,2,3], N. HANKOV[1,2,3], L. DUGUET[5], A. WATRIN[5], S. CARDA[3], B. DRAGANSKI[3], J.-B. LEDOUX[6,7], N. BERARD[3], F. BECCE[6], K. GALAN[1,2,3], L. BAUD[1,2,3], Q. BARRAUD[1,2,3], R. DEMESMAEKER[1,2,3], L. ASBOTH[1,2,3], J. SQUAIR[1,2,3], G. COURTINE[1,2,3], J. BLOCH[1,2,3]; [1]Defitech Ctr. for Interventional Neurotherapies (.NeuroRestore), Swiss Federal Inst. of Technol. (EPFL), of clinical neuroscience, Lausanne Univ. Hosp. (CHUV) and Univ. of Lausanne (UNIL), Lausanne, Switzerland; [2]NeuroX Institute, Sch. of Life Sciences, EPFL, Lausanne, Switzerland; [3]Dept. of Clin. Neuroscience, CHUV and UNIL, Lausanne, Switzerland; [4]Dept. of medical biophysics, Fac. of medicine, Univ. of Toronto, Toronto, ON, Canada; [5]ONWARD Med., Lausanne, Switzerland; [6]Dept. of Diagnos. and Interventional Radiology, CHUV, Lausanne, Switzerland; [7]Ctr. for BioMedical Imaging (CIBM), Lausanne, Switzerland, Lausanne, Switzerland

Abstract: Over the last decade, multiple neurostimulation strategies have been proposed to improve locomotion after spinal cord injury (SCI), most of them using electrical stimulation of the spinal cord. Here, we describe the discovery of a new therapeutic target in the brain to improve walking after SCI. A whole brain survey in a preclinical model of SCI revealed the critical role of the lateral hypothalamus (LH) in the recovery of walking, and the delivery of Deep Brain Stimulation in the Lateral Hypothalamus (LH-DBS) in rodents immediately and durably improved walking. Consequently, we investigated the safety and preliminary efficacy of bilateral LH-DBS to improve walking in two individuals with incomplete SCI. Pre-operative imaging, including functional, structural and diffusion tensor magnetic resonance, determined the optimal target region in the lateral hypothalamus. As early as the first session post-operatively, LH-DBS augmented the vigor of muscle activity, which translated into facilitation of voluntary leg movement and walking in both participants. LH-DBS also improved sensory perception over impaired dermatomes. Rehabilitation augmented by LH-DBS (3x3 hours of training per week) improved lower extremity motor scores, even when LH-DBS was turned off. This neurological recovery led to improvements of the 10-meter walk test and 6-minute walk test. These preliminary preclinical and clinical data suggest that DBS of the LH may be an efficacious strategy to improve the recovery of lower limb functions after SCI, and expose a previously unknown involvement of the lateral hypothalamus in the production of walking in humans.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.13/AA15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life Project Grant - Elucidating mechanisms underlying functional repair following spatiotemporal stimulation of the injured cervical spinal cord
Swiss National Science Foundation (310030_185214) - Targeted amplification of the motor recovery engram following spinal cord injury

Title: Whole-brain and single-cell mechanisms underlying arm and hand function recovery in response to electrical spinal cord stimulation

Authors: *I. DEWANY[1,2,3], N. D. JAMES[1,2,3], M. PASQUINI[4], F. VALÉRY-COEN[2], N. CHO[1,2,3], M. GAUTIER[1,2,3], A. Y. TEO[1,2,3], C. KATHE[1,2,3], T. H. HUTSON[5], M. A. SKINNIDER[1,2,3,6], Q. BARRAUD[1,2,3], S. P. LACOUR[2], S. MICERA[2,4], J. W. SQUAIR[1,2,3,7], J. BLOCH[1,2,3,7], G. COURTINE[1,2,3,7];
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Abstract: Contusion at the cervical level is the most common form of spinal cord injury (SCI). Patient surveys have identified improvements in upper limb function as a top priority for individuals that have suffered this type of injury, but no clinical solution is available for improving its recovery. Electrical neuromodulation therapies of the spinal cord have enhanced lower limb function in numerous preclinical models as well as a number of clinical case studies. Despite this success, and the high priority of improved upper limb function for SCI patients, efforts to translate this promising technique to the cervical spinal cord have so far been limited. Given the complex patterns of muscle activation required for the execution of skilled arm movements, adaptation of epidural electrical stimulation (EES) to the cervical spinal cord necessitates a thorough understanding of the functional specificity that can be achieved using this technique, as well as the neuronal circuitry that underlies its effect. Here we leverage our conceptual framework to gain a mechanistic understanding of natural and targeted circuit reorganization by identifying the neuronal subpopulations that orchestrate spontaneous and neuromodulation-induced recovery from cervical SCI. We first designed a stimulation paradigm in which cervical EES is delivered in a spatially selective manner and is temporally patterned in accordance with the real time activity of selected upper-limb muscles. In order to assess the efficacy of this stimulation in a well-controlled, multimodal recording environment, we employed our recently-developed neurorobotic platform designed specifically for upper limb therapy development, functional assessment, and rehabilitation. In order to then enhance our mechanistic understanding of the neuronal circuitry key to upper limb functional repair, we conducted advanced 3D imaging and single-nuclei RNA sequencing studies to establish a molecular cartography of the neuronal subpopulations that steer the natural recovery of upper-limb function. Further studies will now leverage this framework to uncover and compare the neuronal populations engaged by EES, utilizing this information to maximize functionally-useful circuit reorganization induced by neuromodulation.

Disclosures: I. Dewany: None. **N.D. James:** None. **M. Pasquini:** None. **F. Valéry-Coen:** None. **N. Cho:** None. **M. Gautier:** None. **A.Y. Teo:** None. **C. Kathe:** None. **T.H. Hutson:** None. **M.A. Skinnider:** None. **Q. Barraud:** None. **S.P. Lacour:** None. **S. Micera:** None. **J.W. Squair:** None. **J. Bloch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds patents related to this work. F. Consulting Fees (e.g., advisory boards); Is a consultant of ONWARD medical. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds patents related to this work. F. Consulting Fees (e.g., advisory boards); Is a consultant of ONWARD medical.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.14/AA16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Kessler Foundation
Helmsley Charitable Trust

Title: Modulation of spinal cord excitability and motor performance in individuals with chronic cervical SCI following a single spinal cord transcutaneous stimulation training session

Authors: *N. GILLIS[1], P. SHARMA[2], K. JONES[2], G. FORREST[3], S. HARKEMA[2];
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Abstract: Introduction: The majority of spinal cord injuries (SCI) occur at the cervical cord level, and regaining arm and hand function remains the top rehabilitation priority of this population. We have shown that multisite non-invasive spinal cord transcutaneous stimulation (scTS) targeting cervical and thoracolumbar sites combined with motor recovery training facilitates hand function recovery post-cervical SCI. To understand the neurophysiological mechanisms contributing to the observed motor recovery, we investigated the effects of multisite stimulation on spinal network excitability and execution of motor tasks following a single training session using multisite scTS. We hypothesized that a single training session will increase the spinal cord network excitability and improve motor execution capabilities.
Methods: To test the hypothesis, 1) spinal evoked responses in response to single pulse scTS targeting individual stimulation sites, and 2) electromyography (EMG) from selected upper extremity muscles while performing various motor tasks were recorded from ten participants with chronic cervical SCI (C2-C7, AIS A-C) before and after a typical training. A typical training session consisted of 60 minutes of scTS targeting C3-C4, C5-C6, C7-T1, and T11-T12 spinal levels (30-70 Hz subthreshold stimulation with a 5-10 kHz carrier frequency) combined with upper extremity motor recovery training. In addition, EMG from upper extremity muscles during similar motor tasks was collected from neurologically intact participants (N = 6) to obtain normal activation profiles. Results: Preliminary data from four SCI participants demonstrate increased peak-peak amplitudes, reduced activation threshold, and increased recruitment curve slope for most recorded muscles. In addition, motor performance analysis revealed that some participants were able to perform tasks more efficiently compared to their before scTS performance levels. Moreover, the relative contribution of different upper extremity muscles in a motor task demonstrated similar profiles as neurologically intact participants after a single scTS training session. Interestingly, the effects of a single scTS training session on spinal evoked responses and motor performance differed considerably between the right and left sides.
Conclusion: Overall, findings from the present work suggest that multisite scTS facilitated motor recovery in humans with chronic cervical SCI is potentially mediated via alteration in the spinal cord excitability.

Disclosures: **N. Gillis:** None. **P. Sharma:** None. **K. Jones:** None. **G. Forrest:** None. **S. Harkema:** None.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.15/AA17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: KSCHIRT Grant OGMB210076
Kosair Grant G3435

Title: Multimodal spinal neuromodulation enables stepping in children with motor-complete spinal cord injuries

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Abstract: Objective: Experimental epidural spinal stimulation (scES) combined with activity-based locomotor training (AB-LT) and intent enabled two adults with chronic motor-complete SCI to step. Transcutaneous spinal stimulation (scTS) is a nonsurgical neuromodulatory intervention, which similarly alters the functional state of the spinal cord and is safe for use in children. We report the results of the first four children of an ongoing pilot study that assesses the use of a multimodal neuromodulatory approach (using scTS, AB-LT, and cognitive intent) to enable non-ambulatory children with chronic motor complete SCI to intentionally step. **Methods:** A proprietary 5-channel scTS delivering rectangular waveform current was used to deliver spinal stimulation at T11-12, L1-2, and the coccyx to promote a stepping pattern/response. Training began with 20 sessions in a gravity neutral (GN) position with the child sidelying and legs supported, followed by 40 sessions that added upright load bearing with partial body weight support on a treadmill and overground. Children practiced stepping with and without scTS as well as combining the child's cognitive intent ("tell your legs to step") during training. **Results:** Currently, 4/6 children (4-12 years old) with motor-complete SCI above T10 have completed a minimum of 60 sessions of training. Prior to training, all four children had undergone over 100 sessions of AB-LT and could not initiate a step or move their legs intentionally. After 20 sessions of multimodal neuromodulation training, all four children demonstrated the ability to initiate and maintain reciprocal hip and knee excursions in the GN position during testing without stimulation. After 60 sessions, all four children displayed increased muscular activity in the lower extremity (rectus femoris and medial hamstrings) in the GN position and increased muscular activity in the lower extremity when intentionally stepping on the treadmill without stimulation. All children initiated alternating right and left steps on the treadmill and overground, completing the swing phase of gait. Extension during load bearing required manual assistance. **Conclusion:** This is the first report to support that multimodal spinal neuromodulation (integrating cognitive intent to step, increased excitability of the spinal cord via scTS, and afferent input via AB-LT) may enable voluntary stepping in children with chronic, motor-complete SCI.

Disclosures: K. Lucas: None. **G. Singh:** None. **N. Stepp:** None. **M. King:** None. **P. Parikh:** None. **B. Ugiliweneza:** None. **Y. Gerasimenko:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroRecovery Technologies, Cosyma. **A. Behrman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

funds); Royalties as a co-author of a text, Locomotor Training: Principles and Practice from Oxford University Press.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.16/AA18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation Grant: 732918
JHFEA Grant: GN190574A

Title: Multimodal training combining activity based training and transcutaneous spinal cord stimulation to improve motor function: impact on autonomic nervous system in children with spinal cord injury

Authors: *G. SINGH[1], P. PARIKH[2], M. KING[2], K. LUCAS[2], Y. GERASIMENKO[2], A. L. BEHRMAN[2];
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Abstract: Objective: Children with severe cervical spinal cord injury (SCI) experience debilitating limb and trunk muscle paralysis, limiting their ability to sit, stand, walk, reach, and grasp. Neurotherapeutics approaches like activity-based locomotor training (ABLT) and transcutaneous spinal cord stimulation (scTS) aim to target neural networks by combining stimulation with sensory feedback, promoting motor recovery below injury site. While previous research has mainly focused on motor function, adults with SCI undergoing these interventions have also reported improved autonomic function. Similarly, children with SCI (T6≥) can experience cardiovascular complications, including orthostatic hypotension (OH) and autonomic dysreflexia (AD), which significantly impede their engagement in daily activities and training. Thus, the objective of this observational study is to examine the acute effects of cervical-thoracolumbar scTS in children with SCI undergoing multimodal training to improve trunk control and hand function, on blood pressure (BP) and frequency of AD and OH events. Methods: BP data was recorded in sitting and averaged for two conditions: No-scTS and with-scTS at the start of every training session. Incidences of AD and OH were documented. 5 children (6 ± 3 years), with chronic, cervical or thoracic SCI (T6≥) SCI completing 60 sessions of ABLT + scTS (T11 & L1 sites) participated and 3 children (14 ± 4 years), with chronic cervical SCI completing 40 sessions of activity based training (ABT) + scTS (C4, C6, T11 sites). Trunk and arm function were tested pre and post-training completion. Results: In the ABLT +scTS group, all 5 participants improved their upright sitting posture (Segmental assessment of Trunk Control score increased by 27 points). In 4/5 participants, acute application of scTS increased BP to the normative range on training days when their BP was below normative level for their age and height. In the ABUET+ scTS group, all 3 participants improved their hand grip

strength (mean increase by 12 Newton). 2/3 participants showed an increase in BP to the normative range with scTS on training days when their BP was below the normative level. scTS had minimal to no effects on days when BP was high or within normative level. Additionally, in 2/3 participants who initially experienced OH, the frequency of OH episodes decreased over time with ABT +scTS. Conclusions: These preliminary findings suggest that cervical and thoracolumbar scTS may facilitate regulation of autonomic-function in children with chronic SCI. Further studies are warranted to explore the potential impact of scTS on multiple integrated systems: motor and autonomic nervous system.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.17/AA19

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Kessler Foundation
Helmsley Charitable Trust

Title: Selective activation of upper extremity muscles with spinal cord transcutaneous stimulation in humans with chronic cervical spinal cord injury

Authors: *P. SHARMA[1], G. FORREST[3], S. HARKEMA[2];
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Abstract: Introduction: Recent findings demonstrate that the spinal cord transcutaneous stimulation (scTS) targeting the rostro-caudal axis of the cervical spinal cord results in selective activation of the proximal and distal upper extremity muscles depending on the stimulation site. However, the reported findings mostly involve neurologically intact (NI) individuals. As spinal cord injury (SCI) induces anatomical and physiological changes at and around the injured spinal cord, we hypothesize that the injury will alter the selective activation and recruitment properties of upper extremity muscles post-injury in response to scTS. **Methods:** To test the hypothesis, multi-segmental motor responses (MMR) in response to single pulse stimulation targeting

different spinal cord segments along the rostro-caudal axis of the cervical spinal cord were collected from ten adult NI and motor complete cervical SCI participants (C2-C7, AIS A-B). Different stimulus intensities from 5 to 120 mA, in steps of 5 mA, were delivered to C3-C4, C5-C6, and C7-T1 spinal levels. Recruitment curve characteristics, including motor threshold, normalized peak-peak amplitudes, selectivity index, mean activation index, motor activation probability, and post-activation depression, were characterized for the obtained data. **Results:** In NI participants, normalized peak-peak amplitude, selectivity index, and mean activation index of proximal and distal upper extremity muscles were higher for scTS targeting C3-C4 and C7-T1 spinal levels, respectively. In contrast, for SCI participants, we did not observe considerable differences in the recruitment curve characteristics for proximal and distal upper extremity muscles depending on the stimulation site. The muscles with lower activation thresholds demonstrated greater activation irrespective of the stimulation sites. Moreover, SCI participants demonstrated greater differences in the recruitment curve characteristics between the right and left side muscles than NI participants. **Conclusion:** Overall, findings from the present work suggest limited selective activation of the proximal and distal upper extremity muscles during scTS targeting proximal and distal cervical spinal cord in individuals with cervical SCI. The findings may guide the development of future targeted scTS strategies for humans with cervical SCI. Moreover, longitudinal profiling of selective activation may explain the neurophysiological basis of motor recovery during natural or scTS facilitated motor recovery.

Disclosures: P. Sharma: None. **G. Forrest:** None. **S. Harkema:** None.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.18/AA20

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Defense W81XWH-19-1-0734
Christopher and Dana Reeve Foundation

Title: New Support Network experienced by individuals with Spinal Cord Injury enrolled in a Spinal Cord Epidural Stimulation study

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Abstract: Functional impairment resulting from spinal cord injury (SCI), can significantly impact social life. It has been demonstrated that support networks play a crucial role in emotional, social, and daily life, critical to well-being and quality of life. Spinal cord epidural

stimulation (scES) is a cutting-edge procedure that has shown promise to improve cardiovascular, motor, bowel, and bladder function in SCI. This study aimed to explore the perceptions of individuals with SCI and their caregivers on their experience with new support-network while enrolled and after a scES study. Thematic analysis of semi-structured interviews has been conducted with 30 adults with SCI (cervical level; AIS Grade A-C 17 males, 13 females) enrolled in a scES study at the Kentucky Spinal Cord Injury Research Center (KSCIRC) at the University of Louisville (UofL) and 9 caregivers. Codes and transcripts were loaded into Dedoose Software for qualitative analysis. A total of 98 interviews were transcribed across 5 time points (Baseline to 12-month follow up) and 68 excerpts were extracted under the sub-theme "New Support Network." Participants expressed that following injury, the loss of friendships and lack of understanding from people around them have made them feel isolated. During the study, at different timepoints, a common topic mentioned was the importance of being surrounded by other individuals with SCI, and how sharing experiences have made them feel more connected and understood. Witnessing the progress of others, was perceived as inspirational and increased their enthusiasm to continue. Words of encouragement from other participants and advocates were mentioned as a reason to increase their drive and motivation during their training sessions. Prior to the study, bowel, and bladder impairments were a reason to avoid social interactions. During the study, as they started to improve motor skills, sensitivity, and were encouraged by their new support network (other participants and advocates), they started trying new things such as self-catheterization providing them more independence and the opportunity to socialize more. Caregivers repeatedly mentioned that participants were more involved in the community after being part of the study and that even 12 months after, they had maintained networking support they had found. In conclusion, having a support network through participation in the scES study has been beneficial for individuals with SCI. Alongside functional improvements from the program, participants learn from each other and incorporate new activities in their daily lives expanding their social life, improving their well-being, and integration in the community.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.19/AA21

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation Award #733935

Title: Impact of spinal cord epidural stimulation for lower urinary tract function and reduction of non-voiding contractions in spinal cord injured rats

Authors: *K. M. BEASLEY[1,2], D. MEDINA-AGUIÑAGA[1,2], N. L. WILKINS[1,2], C. HUBSCHER[1,2];
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Abstract: Spinal cord injury (SCI) is a devastating event often resulting in severe disruption of multiple body systems. Lower urinary tract (LUT) dysfunction, frequently ranked as a top priority for recovery among the SCI population, affects approximately 80% of all individuals with SCI and is the source of most clinical conditions and hospital readmissions. Urological dysfunction following SCI frequently manifests as detrusor sphincter dyssynergia (DSD), or the discoordination between contraction of the detrusor muscle and the opening of the external urethral sphincter (EUS). DSD leads to inefficient emptying and high bladder pressures which commonly results in the life-threatening reflexive sympathetic response known as autonomic dysreflexia as well as retrograde urine flow through the ureters which can damage the kidneys and lead to renal failure. Spinal cord epidural stimulation (scES) is a novel therapy that has been shown to promote changes in LUT function in both humans and pre-clinical experimental models. It is hypothesized that targeting the thoracolumbar or lumbosacral spinal cord nuclei with scES can not only trigger appropriate reflex responses in the detrusor muscle to achieve efficient voids but also reduce the number of non-voiding contractions (NVCs) during bladder filling. Using spinally intact or severe T3 contused male and female Wistar rats, L6/S1 scES and T13/L1 scES was applied at a range of intensities and frequencies under acute terminal urethane-anesthetized preparations during cystometry-electromyography (CMG-EMG). T13/L1 scES, but not L6/S1 scES, reduced the number of NVCs in both spinally intact and T3 contused male and female rats. Additionally, L6/S1 scES, but not T13/L1 scES, improved the voiding efficiency in spinally intact and T3 contused female rats but not male rats. Our results together indicate that scES is a promising therapeutic strategy for improving LUT function following SCI, with differing effects at the T13/L1 and L6/S1 spinal levels.

Disclosures: K.M. Beasley: None. **D. Medina-Aguiñaga:** None. **N.L. Wilkins:** None. **C. Hubscher:** None.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.20/AA22

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DoD Grant W81XWH-19-SCIRP-TRA

Title: Early bowel targeted spinal cord stimulation facilitates standing in spinal cord injured minipigs

Authors: *R. U. AHMED[1], D. MEDINA AGUINAGA[7], L. M. KONAN[2], C. KNIBBE[3], D. GIBSON[4], S. DAVISON[5], C. HUBSCHER[8], M. BOAKYE[6];
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Abstract: Epidural spinal cord stimulation is a promising neuromodulation tool to restore spinal cord injured individuals' neuronal function. Bowel-targeted spinal cord stimulation (B-scES) facilitates the relaxation of the anal sphincter by modulating the autonomic nervous system. Moreover, B-scES often facilitates stand- and step recovery in the paralyzed. To investigate the B-scES effect on standings in six female Yucatan minipigs were used in this study. Following laminectomy, an epidural stimulator was implanted and mapped in the lumbosacral region, targeting the colorectal and locomotor networks. To induce a severe spinal cord injury (SCI) at T10 level, an impactor was dropped from 20 cm height followed by 5 min compression. After two weeks of recovery, animals were divided into three groups (2/group). Group-1 animals received the first 4 weeks of B-scES (40 sessions) followed by the last 4 weeks of stand-and-step targeted spinal cord stimulation (SS-scES) (40 sessions). Group-2 animals did not receive any stimulation for 8 weeks and then 4 weeks of B-scES. Group-3 animals received first 4 weeks of SS-scES (40 sessions) followed by the last 4 weeks of B- (40 sessions). To activate the neural network for bowel movement and standing, stimulations were provided at L6 and S1 levels. For stepping stimulation was provided at the more rostral level. Kinematics and EMG data were recorded at weeks 2, 8 - and 12 post-injured (p. i.) conditions. A custom-made designed harness attached with a lite gait was used to quantify the average body weight support during standing. To evaluate the locomotion, porcine thoracic injury behavioral scale (PTIBS) was used for a continuous period from recovery to week 12 (p.i.) Following 8 weeks of stimulation, Group-1 animals' average weight support during stand was less compared to Group-3 animals ($p=0.0332$). Moreover, at week 12, Group-1 animals used less body weight support than the other animal groups. The kinematics of angular responses also improved in Group-3 compared to Group-1 and 2. EMG responses increased in all groups of animals immediately after injury and reversed at week-12 only in Group-1 animals. Our preliminary results suggest that early bowel-targeted epidural stimulation has potential to improve standing in minipigs after SCI.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.21/AA23

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Kentucky Spinal Cord and Head Injury Research Trust (KSCHIRT) Grant 21-5

Title: Epidural stimulation for male reproductive function in spinal cord injured rats

Authors: *N. WILKINS[1], D. MEDINA AGUIÑAGA[2], K. M. BEASLEY[3], C. HUBSCHER[4]; [1]Univ. of Louisville Sch. of Med., Louisville, KY; [2]Anatom. Sci. and neurobiology, [3]Anatom. Sci. & Neurobio., [4]Univ. of Louisville Anatom. Sci. & Neurobio., Louisville, KY

Abstract: A vast majority of the spinal cord injury (SCI) population experience reproductive dysfunction, with issues in males related to erection, ejaculation, and sperm quality. Current interventions for ejaculatory dysfunction assist with the retrieval of sperm for in-vitro fertilization. However, the techniques used pose a risk of triggering autonomic dysreflexia (a potentially life-threatening hypertensive episode) and do not improve intimacy or sexual satisfaction. The Hubscher lab group has contributed to a breadth of pre-clinical and clinical research that shows the benefits of spinal cord epidural stimulation (scES) for improving autonomic functions in individuals with SCI, including cardiovascular regulation and restoration of lower urinary tract function. As scES is multifaceted, targeting reproductive function may provide a solution for ejaculatory dysfunction as well. The current pre-clinical study involved terminal scES using a custom 15-electrode Micro-Leads array to target the well-documented spinal generator for ejaculation located at L3 in urethane-anesthetized male Wistar rats without and with T9 transections. The impact of injury chronicity was examined as it relates to efficacy of L3 scES, changes in sperm health, and testicular atrophy. Through the collection of electromyography from the bulbospongiosus muscle (BSM) and the external urethral sphincter as well as pressure measurements from the seminal vesicles, it was found that animals at 3 days post-injury (dpi) were less responsive to scES for ejaculation than animals with more chronic injuries as 7 out of the 10 animals tested only displayed the urethro-genital reflex (the muscular reflex that is associated with the expulsion phase of ejaculation). Animals at 14 dpi and 70 dpi were more responsive to targeted scES, with several animals displaying both the urethro-genital reflex and glans-vasal reflex (the glandular reflex associated with the emission phase of ejaculation). Notable group differences included latency to ejaculation (longer at 3 and 14 than 70 dpi), increased sensitivity to scES detected in the BSM (greater response at 14 and 70 dpi when compared to 3 dpi) and large differences in sperm production (lower terminal sperm counts at 14 and 70 dpi compared to 3 dpi and shams). These pre-clinical findings illustrate the potential benefit of scES as an intervention to target male reproductive organ dysfunction post-SCI, with particular promise in individuals with chronic injury.

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Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.22/AA24

Topic: E.05. Brain-Machine Interface

Support: RS-2023-00208315
RS-2023-00254156
2019R1A6A1A11034536

Title: Harnessing Electroceuticals for Enhanced Recovery in Spinal Cord Injury Models

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Abstract: Spinal cord injury is one of major devastating lesions that result in motor and sensory impairments, often accompanied by complications such as neuropathic pain, decubitus ulcers, and bladder dysfunctions. Electroceuticals, which use electrical signals to manipulate the body's neural circuits, are currently employed for managing neuropathic pain and spasticity through epidural stimulation. However, existing epidural electrodes, being bar-type, primarily stimulate the dorsal part of the spinal cord, making it challenging to deliver appropriate electrical signals to the corticospinal tract located in the deep dorsal or lateral areas of the spinal cord. Moreover, current electrical stimulators, which require connection to external equipment, are difficult to control and maintain, and may increase infection risk.In this study, we developed an innovative electroceutical comprising a neural interface electrode, fabricated from a carbon black and ecoflex composite, and a wireless electrical stimulator. This design allows for effective full coverage of the spinal cord, minimizes spinal cord compression due to its low Young's modulus, and delivers appropriate electrical signals remotely. In vitro studies revealed a significant increase in viability and neurite length of primary cultured cortical neurons following electrical stimulation on the carbon black/ecoflex electrode compared to controls without electrical stimulation. We applied this electroceutical device to contused spinal cords in rats, stimulated the electrode for three days, and conducted evaluations eight weeks post-implantation. Our findings indicated a smaller lesion cavity and reduced number of CD68-positive macrophages following electrical stimulation compared to controls. Additionally, locomotor functions, as assessed by the Basso, Beattie, and Bresnahan locomotor rating scale and the horizontal ladder walk test, showed greater recovery post-stimulation. No complications or spinal cord compression were observed due to the electrode.In conclusion, our specially designed electroceutical, featuring a full-coverage spinal cord electrode and wireless stimulator, effectively promotes functional recovery in rat models of SCI. This promising approach has potential for translation into clinical practice.

Disclosures: **J. Hyun:** None. **B. Khoroldulam:** None. **J. Kim:** None. **S. Kang:** None.

Poster

PSTR542. Spinal Cord Injury: Neuromodulation Therapies III

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR542.23/AA25

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Neilsen Postdoctoral Fellowship Research Grant 733935

Title: A Novel Model to Examine the Impact of Epidural Stimulation on Urinary and Cardiovascular Dynamics in Spinal Cord Injured Rats

Authors: *D. MEDINA AGUINAGA[1], K. BEASLEY[2], N. WILKINS[4], C. HUBSCHER[3]; [1]Univ. of Louisville Anatom. Sci. & Neurobio., Louisville, KY; [2]Anatom. Sci. & Neurobio., [3]Anatom. Sci. & Neurobiol., Univ. of Louisville, Louisville, KY; [4]Univ. of Louisville Sch. of Med., Louisville, KY

Abstract: Spinal cord injury (SCI) is a devastating event often leading to severe impairment of multiple body systems, greatly impacting quality of life. Urological dysfunction after SCI may include detrusor-sphincter dyssynergia (DSD), which is characterized by uncoordinated bladder and external urethral sphincter contractions, causing inefficient emptying and smooth muscle hypertrophy. High intravesical pressures generated during these non-void contractions (NVC) may trigger the anomalous cardiovascular response known as autonomic dysreflexia, which is a rapid onset increase in blood pressure triggered by stimuli arising below the lesion level that can cause myocardial ischemia, brain hemorrhage, or even death. Spinal cord epidural stimulation (scES) is a novel therapy that has been shown to promote changes in both the urinary and cardiovascular systems in humans and pre-clinical experimental models. In order to determine the interrelationship between NVC's and hemodynamic parameters during bladder filling in awake conditions as well as the effect of scES upon both blood pressure and heart rate under acute terminal urethane-anesthetized preparations we have developed a novel model using cystometry-electromyography (CMG-EMG) with intra-arterial blood pressure measurement using intact or chronic T3 contused (mild, moderate and severe) male and female rats. Early data in intact animals suggests a simultaneous increase in blood pressure with bladder contraction as well as a scES-induced modulation of the blood pressure range.

Disclosures: D. Medina Aguinaga: None. **K. Beasley:** None. **N. Wilkins:** None. **C. Hubscher:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.01/AA26

Topic: D.02. Somatosensation – Pain

Support: DoD grant W81XWH2110597
NIH grant NS128080

Title: Low-dose interleukin-2 as a treatment for traumatic brain injury-induced cognitive deficit and pain in mice

Authors: K. CZERPANIAK[1], ***L. FLORES DO NASCIMENTO**[1], T. GUO[1], X. LIU[1], D. WOZNIAK[2], Y.-Q. CAO[1];
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Abstract: Nearly 50 million people experience mild traumatic brain injury (mTBI) every year. Many of them develop post-traumatic headache (PTH) and cognitive impairment, severely affecting productivity and life quality. Despite the high prevalence, mTBI-induced chronic headache and cognitive deficit are poorly understood and lack effective treatments. In mice, low-dose interleukin-2 (LD-IL-2) treatment during the first 2 weeks post-mTBI accelerates the resolution of acute PTH-related behaviors through expansion and activation of regulatory T (Treg) cells. Over-expressing IL-2 in brain astrocytes prior to injury protected mice against cognitive impairment. However, whether LD-IL-2 treatment long after the initial injury is effective for mTBI-induced chronic PTH and cognitive deficit remains unclear. We induced mTBI in mice using a non-invasive closed-head weight-drop method. After the resolution of acute PTH-related behaviors, daily injection of nitroglycerin (NTG, a reliable headache trigger in humans) re-established the persistent facial mechanical hypersensitivity, revealing mTBI-induced hyperalgesic priming that is mechanistically related to chronic PTH. NTG effect was abolished by daily LD-IL-2 treatment starting 6 weeks post-mTBI, suggesting that LD-IL-2 can reverse chronic PTH. Novel object recognition and object location tests were used to assess mTBI-induced impairment in recognition and spatial memory. One week of LD-IL-2 treatment starting 6-days post-mTBI prevented the development of cognitive deficit for at least 3 weeks. Delaying LD-IL-2 treatment till 4 weeks post-mTBI completely reversed the memory impairment in both male and female mice, suggesting that LD-IL-2 is effective for mTBI-induced cognitive deficit. Mechanistically, depletion of endogenous Treg cells not only prolonged acute and chronic PTH-related behaviors, but also abolished the effects of LD-IL-2, indicating that Treg cells facilitate the resolution of PTH and mediate the therapeutic benefit of LD-IL-2. Interestingly, although LD-IL-2 treatment inhibited mTBI-induced astroglial and microglial activation in the cortex and hippocampus, it increased the number of Treg cells in the dura but not in CNS, suggesting that the therapeutic effects of LD-IL-2 are mediated through expansion of Treg cells in peripheral tissues. Collectively, our study indicates that endogenous Treg cells facilitate the resolution of PTH. Importantly, this work identify peripheral Treg as a cellular target and LD-IL-2 as potential therapy for both chronic PTH and mTBI-induced cognitive impairment with a wide treatment time window and long-lasting therapeutic benefit.

Disclosures: K. Czerpaniak: None. **L. Flores do Nascimento:** None. **T. Guo:** None. **X. Liu:** None. **D. Wozniak:** None. **Y. Cao:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.02/AA27

Topic: D.02. Somatosensation – Pain

Support: National Institute of Neurological Disorders and Stroke: R01NS099292
Arizona Biomedical Research Consortium AHDS18-45952

Title: Extracellular alterations in pH and K+ modify the murine brain endothelial cell total and phospho-proteome

Authors: *J. R. WAHL[1,2], A. VIVEK[2], S. M. PALOMINO[2], M. ALMUSLIM[2], K. E. COTTIER[4], P. R. LANGLAIS[3], T. W. VANDERAH[2], E. LIKTOR-BUSA[2], J. M. STREICHER[2], T. M. LARGENT-MILNES[2];
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Abstract: Pathologies of the blood brain barrier (BBB) have been linked to a multitude of central nervous system (CNS) disorders whose pathology is poorly understood. Cortical spreading depression (CSD) has long been postulated to be involved in the underlying mechanisms of these disease states, yet full understanding remains elusive. This study sought to utilize an *in vitro* model of the blood brain barrier (BBB) with brain endothelial cell (b.End3) murine endothelioma cells to investigate the role of CSD in BBB pathology by characterizing effects of the release of major pronociceptive substances into the extracellular space of the CNS. Application of trans endothelial electrical resistance (TEER) screening, transcellular uptake, and immunoreactive methods were used in concert with global proteome and phospho-proteomic approaches to assess the effect of modeled CSD events on the modeled BBB *in vitro.* Findings demonstrated relocalization and functional alteration to proteins associated with the actin cytoskeleton and endothelial tight junctions. Additionally, unique pathologic mechanisms induced by individual substances released during CSD were found to have unique phosphorylation signatures in phospho-proteome analysis, identifying Zona Occludins 1 (ZO-1) as a possible pathologic “checkpoint” of the BBB. Utilizing these phosphorylation signatures, possible novel diagnostic methods may be developed for CSD and warrants further investigation.

Disclosures: J.R. Wahl: None. **A. Vivek:** None. **S.M. Palomino:** None. **M. Almuslim:** None. **K.E. Cottier:** None. **P.R. Langlais:** None. **T.W. Vanderah:** None. **E. Liktor-Busa:** None. **J.M. Streicher:** None. **T.M. Largent-Milnes:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.03/AA28

Topic: D.02. Somatosensation – Pain

Support: PAPIIT UNAM Grant IN218122 to AGH
PAPIIT UNAM Grant IN202222 to MCL
CONACyT-Mexico Grant A1-S-23631 to AGH

Title: The role of oxytocinergic transmission modulating nociception at the trigeminocervical complex

Authors: *A. GONZÁLEZ-HERNÁNDEZ[1], G. MARTINEZ-LORENZANA[1], S. FLORES BOJORQUEZ[1], A. CÓRDOVA QUIROGA[1], A. ESPINOSA DE LOS MONTEROS ZÚÑIGA[1], M. CONDES-LARA[1], *A. GONZALEZ-HERNANDEZ[2];
[1]Inst. de Neurobiología, UNAM, Queretaro, Mexico; [2]Inst. De Neurobiología, UNAM, Queretaro, Mexico

Abstract: Current data support the notion that oxytocinergic transmission inhibits nociception at peripheral, spinal, and supraspinal levels via oxytocin receptor (OTR). Indeed, a neuronal projection from the hypothalamic paraventricular nucleus (PVN) to the spinal cord and trigeminal nucleus *caudallis* (Sp5c) has been described. Certainly, although the trigeminocervical complex (TCC), an anatomical area spanning the Sp5c, C1, and C2 region, plays a relevant role in some pathophysiological pain disorders associated with the craniofacial structures (*e.g.*, migraine), the role of oxytocinergic transmission at this level has been less explored. Hence, electrophysiological in vivo unitary recordings of second-order WDR TCC cells sensitive to peripheral stimulation of the periorbital or meningeal region was performed in anesthetized male Wistar rats. Electrical stimulation of the PVN transiently suppresses the periorbital evoked nociception of WDR cells, *i.e.*, the activity associated with the neuronal firing of Aδ- and C-fibers was inhibited. This PVN-induced inhibition of WDR cell activity was reversed by a selective oxytocin receptor (OTR) antagonist (OVT) given locally. Furthermore, PVN stimulation blocks the neuronal firing of convergent WDR trigeminal cells receiving concomitant input from the meningeal (V1 branch of trigeminal ganglion) and infraorbital region (V2 branch of trigeminal ganglion). Indeed, we found that a single ganglion trigeminal cell could send bifurcated projections to the meningeal and infraorbital area since injecting retrograde tracers near to transverse sinus (V1; true-blue) and infraorbital nerve (V2; fluoro-gold) stain trigeminal cells suggesting a colocalization. In some recorded cells, neurobiotin was injected, and using confocal microscopy, a colocalization between OTR+ and CGRP+ or OTR+ and GABA+ near the neurobiotin-filled cell was found. Together, this data suggest that oxytocinergic transmission inhibits the nociceptive activity of second-order neurons by activating OTR in CGRPergic (primary afferent fibers) or GABAergic (interneurons) cells.

Disclosures: A. González-Hernández: None. **G. Martinez-Lorenzana:** None. **S. Flores Bojorquez:** None. **A. Córdova Quiroga:** None. **A. Espinosa de los Monteros Zúñiga:** None. **M. Condes-lara:** None. **A. Gonzalez-Hernandez:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.04/BB1

Topic: D.02. Somatosensation – Pain

Support: UDEM-UIN21522

Title: Ebf2 is expressed in components of the trigeminal system and plays a role in nociception

Authors: *M. CEPEDA VARELA[1,2], L. E. TÉLLEZ-CÁZAREZ[1], A. N. SALAS-AMADOR[1], S. CÁRDENAS-LOZANO[1], A. M. ORTIZ-MARROQUÍN[1], M. ROBLES-RAMÍREZ[1], K. VAZQUEZ-JASSO[1], G. R. LUÉVANO-ARANDA[1], C. TAVERA[1], I. MEESTER[1], A. C. MARTÍNEZ-TORRES[2], V. ZOMOSA-SIGNORET[1], **R. VIDALTAMAYO**[1];
[1]Univ. De Monterrey, San Pedro Garza Garcia, Mexico; [2]Sch. of Biol. Sci., Univ. Autónoma de Nuevo León, San Nicolas de los Garza, Mexico

Abstract: Early B cell Factor 2 (Ebf2) is a transcription factor involved in cellular differentiation and migration during the mouse embryo development. In our lab, we previously found that the transcription factor Ebf2 may play a significant role in nociceptive perception. Thus, in this work we present our results regarding Ebf2 expression in the trigeminal system, in particular the trigeminal ganglia (TG) and Principal sensory nucleus of trigeminal (PrV) from embryonic and postnatal mice (E14, P10 - P90). We used genetically-modified animals from the $129Sv^{Ebf2\text{-}TauGFP}$ strain where TauGFP is expressed under the control of the Ebf2 promoter, allowing us to map the expression of Ebf2 during development until adulthood. We immunostained heterozygous (HET, Ebf2 +/-) and knockout (KO, Ebf2 -/-) cryosections belonging to embryo and postnatal mice brain samples and trigeminal ganglia (n=12). Our data shows that Ebf2 expression starts from the embryo stage E14 and is maintained through adulthood in the trigeminal system. In the TG of postnatal mice, we found that Ebf2 expressing cells might be supporting mesenchymal cells or ensheathing glial cells, but not Schwann cells, as they do not coexpress associated markers (S100b, GFAP, Sox10). Additionally, in Ebf2 KO animals we found that TauGFP expressing cells increases at the expense of diminishing neuronal density in the TG (Decrease in NeuN+ and TUBB3+ neurons when comparing HET to KO mice). Also, we found that Ebf2-expressing cells in the PrV are neurons innervated by CGRP and Substance P. Finally, our preliminary results show that topical exposure to capsaicin (CAP) and allyl isothiocyanate (AITC) in shaved cheeks of wildtype (WT) and HET animals induce wiping and bunting behaviors (related to pain perception). On the other hand, Ebf2 KO animals only show bunting behavior when exposed to AITC but not when exposed to capsaicin. Because AITC and CAP are two different algogens that activate two different receptors (TRPA1 and TRPV1, respectively), our results suggest that the contrasting responses of KO animals to these algogens could be related to differential effects on expression or circuit development of the receptors and pathways engaged by these two molecules. Taken together, our findings suggest that Ebf2 plays a role during the development of the nociceptive circuits of the trigeminal system. Further experiments are needed in order to elucidate the molecular and cellular mechanisms involved in nociceptive perception in Ebf2 KO mice.

Disclosures: M. Cepeda Varela: None. **L.E. Téllez-Cázarez:** None. **A.N. Salas-Amador:** None. **S. Cárdenas-Lozano:** None. **A.M. Ortiz-Marroquín:** None. **M. Robles-Ramírez:** None. **K. Vazquez-Jasso:** None. **G.R. Luévano-Aranda:** None. **C. Tavera:** None. **I. Meester:** None. **A.C. Martínez-Torres:** None. **V. Zomosa-Signoret:** None. **R. Vidaltamayo:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.05/BB2

Topic: D.02. Somatosensation – Pain

Support: EY034687

Title: Adeno-associated virus (AAV) serotype dependency for efficient transduction of trigeminal ganglia neurons

Authors: X. WANG[1], S. PARIKH[2], *A. MATYNIA[1];
[1]Jules Stein Eye Inst., [2]UCLA, Los Angeles, CA

Abstract: There are significant gaps in the fundamental understanding of neuronal circuits that provides sensory innervation, and particularly of the trigeminal ganglia (TG). The ability to precisely label or manipulate function TG neurons with minimal complications from secondary site expression, damage at the site of viral delivery, and immune activation is crucial to bridging this gap in knowledge. Viruses like HSV are highly transduced but are immunogenic and transported trans-synaptically. By contrast, AAV has low immunogenicity and remains in the transduced neurons. The cornea is the most densely innervated tissue in the body and is easily accessible, making it ideal for understanding AAV tropism. Adult mice of both sexes were transduced with different AAV serotypes expressing eGFP using topical application on naïve or scratched corneas, or by intrastromal injections. Experimenters were masked until analyses were completed. Immunofluorescence microscopy was used to identify virally expressed eGFP and evaluate collateral damage including corneal nerve fibers, corneal scars and immune cell activation in the trigeminal ganglia. Preliminary results indicate that intrastromal injections are the most efficient at transducing corneal nerves, with limited collateral damage. AAV6 and AAV8 serotypes were more effective at labeling cpTGs than AAV2 or 9. No immune activation was observed in the trigeminal ganglia twelve weeks after transduction. Our results indicate that AAV6 viral transduction using intrastromal injections is an effective method to either label or express exogenous genes in cpTGs, with minimal collateral damage. With these new tools, cpTGs can be targeted sparsely or densely, which may be essential for mapping or neural circuits or manipulating neuronal function, respectively.

Disclosures: X. Wang: None. **S. Parikh:** None. **A. Matynia:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.06/Web Only

Topic: D.02. Somatosensation – Pain

Support: PAPIIT-UNAM Mexico Grant IN218122
PAPIIT-UNAM Mexico Grant IN202222
the Fondo Sectorial de Investigación para la Educación (CONACyT-Mexico Grant No. A1-S-23631)

Title: The role of α_2-adrenoceptor subtypes on the neuronal firing of second order wide dynamic range cell at the trigeminocervical complex

Authors: *G. LÓPEZ CÓRDOBA[1], G. MARTINEZ-LORENZANA[2], M. CONDÉS LARA[3], A. GONZALEZ-HERNANDEZ[4];
[1]Neurophysiol., Univ. Nacional Autonoma De Mexico, Queretaro, Mexico; [2]Dept Neurophysiol, UNAM UAQ, Queretaro 76001, Mexico; [3]Univ. Nacional Autónoma De México, Queretaro, Mexico; [4]Inst. De Neurobiología, UNAM, Queretaro, Mexico

Abstract: The trigeminocervical complex (TCC) modulates the nociceptive input from craniofacial structures. At this level, the current consensus is that α_2-adrenoceptor activation induces antinociception. Indeed, this receptor belongs to the seven transmembrane receptors coupled to $G_{i/o}$ proteins and functionally can be subdivided into three subtypes, namely, α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors. Nevertheless, although the antinociceptive action of the noradrenergic system at the trigeminal level is well known, the functional contribution of specific $\alpha_{2A/2B/2C}$-adrenoceptor subtype(s) remains obscure. Using an electrophysiological and pharmacological approach, the present study aimed to determine the effect of clonidine (a non-selective $\alpha_{2A}/\alpha_{2B}/\alpha_{2C}$-adrenoceptor agonist) on the nociceptive signaling in the TCC and the receptor subtype involved. Using anesthetized male and female Wistar rats, electrophysiological unitary recordings of second-order neurons at the TCC region responding to peripheral nociceptive-evoked responses of the periorbital dermatome (V1, V2 trigeminal branch region) of the trigeminal nerve were tested. The effect of clonidine on TCC nociceptive neuronal firing was analyzed by constructing dose-response curves (3.1 - 31 nmol). Furthermore, the role of the $\alpha_{2A/2B/2C}$-adrenoceptor subtype involved in the clonidine's effects was pharmacologically dissected. The results obtained so far show that: (i) clonidine inhibited the nociceptive activity of 45-55% of the total recorded WDR cells in both sexes; (ii) males were more sensitive to clonidine´s antinociceptive effects than females rats; and (iii) the clonidine induced-antinociception was reversed by BRL 44408 (α_{2A}-adrenoceptor antagonist). This electrophysiological study demonstrates that clonidine inhibits the peripheral-evoked neuronal activity at TCC by α_{2A}-adrenoceptor activation. Furthermore, sexual dimorphism in clonidine's antinociceptive effects at the TCC level seems relevant.

Disclosures: G. López Córdoba: None. **G. Martinez-Lorenzana:** None. **M. Condés Lara:** None. **A. Gonzalez-Hernandez:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.07/Web Only

Topic: D.02. Somatosensation – Pain

Title: Neurons of the trigeminal ganglion that project to trigemino cervical complex study

Authors: *X. GARCIA[1], M. CONDES-LARA[2], A. GONZALEZ-HERNANDEZ[3], G. MARTINEZ-LORENZANA[4];
[1]Inst. de Neurobiología, UNAM, Queretaro, Mexico; [2]Inst. de Neurobiologia, UNAM, Queretaro, Mexico; [3]Inst. de Neurobiología, Inst. De Neurobiología, UNAM, Queretaro, Mexico; [4]Dept Neurophysiol, UNAM UAQ, Queretaro 76001, Mexico

Abstract: The trigeminal nerve has a sensitive and motor part. The ophthalmic, maxillary, and mandibular nerves form the largest area of the sensitive part. There's proof that the neurons of the trigeminal ganglion (TG) can send their axons to subnuclei: sensitive (Vp), and also to the caudal nuclei by collateral projections, less frequently the oralis (Vo) and interpolaris. But the projection to the trigemino cervical complex (TCC) hasn't been studied. The most caudal part of the trigeminal spinal nucleus caudalis (SpVC) and the superior cervical segments (C1-C2) are the components of this complex. Consequently, the main goal of this work was to show if the projections to this complex are collateral or go directly from the TG. This work was made with Wistar male rats injected in the SpVC with the retrograde labelled True Blue (TB) and C1-C2 with Diamidino Yellow (DY); after seven days, the brain stem with part of the cervical segments, and both of the ganglion were dissected. Coronal slices were made, so that we could be sure about the place we injected. And horizontal slices for the TG with the labelled neurons. The results show neurons labelled only with DY; neurons labelled only with TB, and double-labelled TB/DY ipsilateral to the place of the injection. This work shows the direct projections and the existence of collaterals from the TG to the TCC.

Disclosures: X. Garcia: None. **M. Condes-lara:** None. **A. Gonzalez-Hernandez:** None. **G. Martinez-Lorenzana:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.08/Web Only

Topic: D.02. Somatosensation – Pain

Title: Interconnection of trigeminal ganglion neurons to the meningeal transverse sinus and infraorbital nerve in the rat.

Authors: *A. CÓRDOVA QUIROGA;
Dolor y epilepsia, Inst. de Neurobiología, UNAM, Queretaro, Mexico

Abstract: INTERCONNECTION OF TRIGEMINAL GANGLION NEURONS TO THEMENINGEAL TRANSVERSE SINUS AND INFRAORBITAL NERVE IN THE RAT.Córdova-Quiroga Aketzalli[1,2]; Condés-Lara Miguel[2]; González-Hernández

Abimael[2];Martínez-Lorenzana Guadalupe[2]. [1]Licenciatura en Biología, FES Zaragoza, [2]Instituto deNeurobiología, Campus Juriquilla UNAM.**Key words:** Trigeminal Ganglion , Meningeal Transverse Sinus, Infraorbital NerveSeveral studies on neuronal tracers have been analyzed separately and differently on theinfraorbital nerve and the meninges. This work aimed to recognize bifurcated ways betweenthe trigeminal ganglion, the infraorbital nerve, and the transverse sinus. In this work, Wistarrats were used, which underwent stereotaxic surgery to inject the neural tracers, Fluoro Gold(FG) into the infraorbital nerve and pellet True Blue (TB) in the transverse sinus area, whichis located in the meninges. Once the surgery is finished, waiting five days approximately is leftbefore sacrificing the rat by perfusion process to obtain the infraorbital nerve, the meninges,and the trigeminal ganglion. When analyzing the slides at 50 microns that were made of theganglion, Three groups of labelled cells were found in the dorso-ventral zone of the ganglion:1) the cells that only had labelled TB, 2) those that only had labelled FG and 3) group that hasbeen both tracers TB/FG. This confirms the existence of the bifurcated connections betweenthe infraorbital nerve and the transverse sinus. This work supports electrophysiological studiesof our working group.

Disclosures: A. Córdova Quiroga: None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.09/BB3

Topic: D.02. Somatosensation – Pain

Support: NIH Grants R01DE031255
NIH Grants R01DE032061

Title: Ferroptosis is involved in comorbid migraine and temporomandibular joint disorder in female mice

Authors: *R. TAO[1], S. LIU[2], F. TAO[2];
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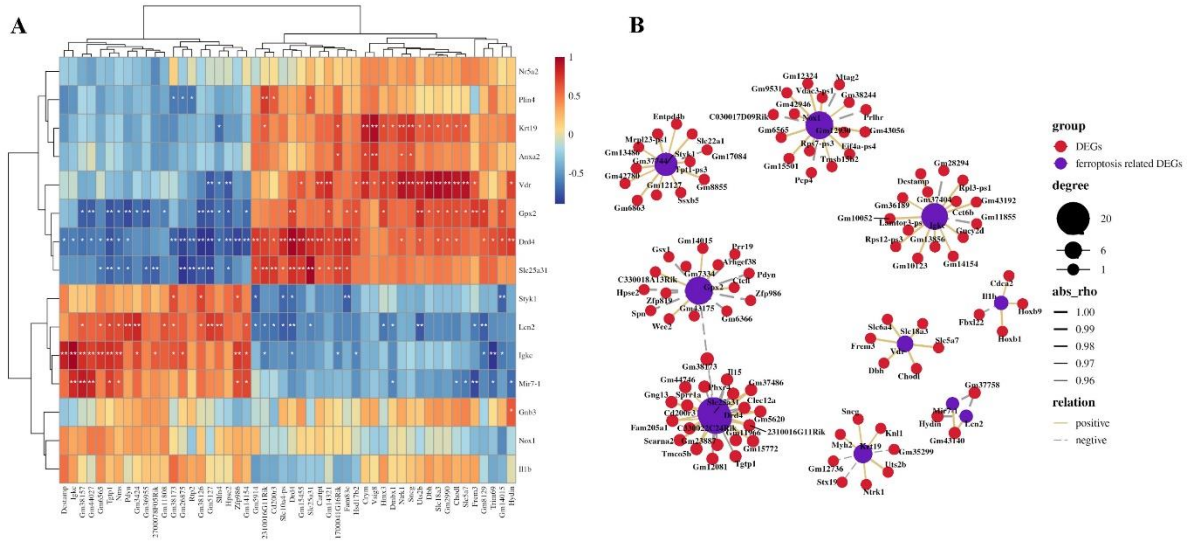
Abstract: Previous studies have shown a higher prevalence of temporomandibular joint disorder (TMD) symptoms among individuals with migraine compared to the general population. However, the underlying mechanisms for this comorbidity remain unclear. In this study, we aim to explore novel mechanisms that underlie the association between migraine and TMD through further analysis of RNA sequencing (RNA-seq) data. We compared ferroptosis-related genes with the differentially expressed genes (DEGs) identified in the RNA-seq. There were 204 upregulated genes and 274 downregulated genes in sham control vs. comorbid migraine and TMD group. More importantly, 15 ferroptosis-related DEGs were identified by mapping with those ferroptosis-related genes. The KEGG analysis showed that the ferroptosis-related DEGs

were largely enriched in the pathways of neurodegeneration, cellular homeostasis, and interleukins signaling. A gene co-expression network was established using Pearson's correlation analysis based on the correlation between ferroptosis-related DEGs and DEGs. Our results suggest that ferroptosis may contribute to the association of the two orofacial pain conditions and the bioinformatic analysis of RNA-seq data can provide valuable insights into the biological processes of the comorbid migraine and TMD. These findings will advance our understanding of the underlying mechanisms for this comorbidity and the identified ferroptosis-related genes could be potentially targeted to develop a novel intervention approach for pain management in patients with such orofacial pain conditions.



Disclosures: **R. Tao:** None. **S. Liu:** None. **F. Tao:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.10/BB4

Topic: D.02. Somatosensation – Pain

Support: NIH Grant UC2AR082195

Title: Characterization of Trigeminal Sensory Neurons Innervating the Temporomandibular Joint

Authors: ***J. ALFARO**, K. A. LINDQUIST, A. HOVHANNISYAN, J. M. MECKLENBURG, A. N. AKOPIAN;
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Abstract: Temporomandibular joint (TMJ) disorders (TMJD) are functionally heterogeneous conditions of the mastication system affecting the jaw joint, masticatory muscles, and ligaments. Despite a large percentage of the population suffering from some type of TMJ pain, treatment remains ineffective. Pathophysiology of TMJD is still unknown. However, there is an agreement that TMJD increases the responsiveness of sensory neurons innervating TMJ ligament and connected lateral pterygoid muscle. In order to gain improved and effective treatment, the subtypes of sensory neurons that innervate the TMJ must be functionally phenotypes and thoroughly characterized in naïve and TMJD subjects. Such a study includes the role of specific receptors and mediators as well as sex and age-dependent plasticity of sensory neuronal groups that innervate TMJ and determines TMJD disorder pathology.As a first step, I used immunohistochemistry with sensory neuronal markers on mouse tissue to identify the specific trigeminal neuronal groups that innervate the TMJ. This initial stage will follow by electrophysiological characterization of TMJ innervating sensory neuronal groups.

Disclosures: J. Alfaro: None. **K.A. Lindquist:** None. **A. Hovhannisyan:** None. **J.M. Mecklenburg:** None. **A.N. Akopian:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.11/BB5

Topic: D.02. Somatosensation – Pain

Support: NIH/NiGMS R35GM138168

Title: Sleep disruption is a predisposing factor for the development of pain associated with temporomandibular joint disorders in rats

Authors: *R. HORNUNG, A. PHERO, L. FERRARI, N. TAYLOR;
Anesthesiol., Univ. of Utah, Salt Lake City, UT

Abstract: Background: Temporomandibular (TM) disorders (TMD) are a diverse group of neuromuscular and musculoskeletal orofacial conditions characterized by pain in the TM joint and masticatory muscles affecting 5% to12% of the population. Clinical studies indicate a link between sleep disruption and TMD pain, with patients reporting a decrease in sleep quality prior to onset of pain. This suggests that sleep disruption may be a predisposing factor for the development of TMD symptoms. Thus, the presence of predisposing factors would increase the risk of patients to develop TMD pain when combined with a triggering insult such as exaggerated mouth opening. Here, we investigated whether sleep disruption increases the predisposition for the development of TMD pain. **Methods**: Chewing function was evaluated in male Sprague Dawley rats (250g) submitted to sleep disruption (SD, a predisposing factor) and jaw hyperextension (JE, a triggering factor). The jaws were anchored to a 3.5N weight to simulate exaggerated mouth opening. The time taken by the rats to chew through a set of

obstacles was determined by the ratgnawmeter (RTG) and used as a surrogate measure to determine the presence of TMD pain. Orofacial Pain Assessment Device (OPAD) accounted for SD-induced anxiety observed as a lack in motivation, since rats could choose between receiving a reinforcing reward, such as sugar water, or escaping an aversive stimulus. Post-JE mechanical allodynia around the TM tissues was evaluated using von Frey filaments. Sleep disruption procedures were performed in a water tank filled with 1-2 inches of water, where the rats were placed on poles during 6 h/day (9am-3pm), 5 days on / 2 days off between the two cycles. JE (1 h/day) was performed right after the SD procedures. Tests in the RTG pain evaluation were performed 15h after JE. **Results**: JE alone did not increase chew time in the RTG. Sleep disruption combined with JE increased chewing time, and decreased the mechanical nociceptive thresholds, as determined by von Frey filaments. The number of contacts during licking activity in the OPAD decreased following SD and JE treatment. **Conclusion**: Sleep disruption can act as a predisposing factor that, when combined with a triggering insult such as exaggerated mouth opening, produces painful TM dysfunction.

Disclosures: R. Hornung: None. **A. Phero:** None. **L. Ferrari:** None. **N. Taylor:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.12/BB6

Topic: D.02. Somatosensation – Pain

Support: R35 DE030045

Title: Silencing of nociceptive afferents attenuate hyperalgesia but not condylar degeneration of TMJ after injury

Authors: *I. ALSHANQITI[1], S. KUMARI[2], J. HU[2], M.-K. CHUNG[2];
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Abstract: A common cause of non-dental pain in the orofacial region is temporomandibular disorder (TMD). One of the painful TMD conditions is temporomandibular joint osteoarthritis (TMJOA). It causes slow degeneration of subchondral bone and cartilage, often accompanied by pain. However, the mechanistic association of nociceptive afferents and TMJ degeneration is not clearly established. Sixty-five percent of trigeminal ganglia afferents projected to TMJ contain calcitonin gene-related peptides. Approximately half of which co-expresses transient receptor potential vanilloid 1 (TRPV1), which likely mediates TMJ pain. To determine the contribution of nociceptors to TMJ degeneration and hyperalgesia, we functionally manipulated the TRPV1-lineage afferents noninvasively. We used an inhibitory designer receptor exclusively activated by designer drugs (DREADD), an engineered receptor coupled with inhibitory G protein which silences targeted neurons upon binding to clozapine-N-oxide (CNO), a specific activator. For

targeting hM4Di expression, we utilized *Trpv*1^{Cre} mice. We used forced mouth opening (FMO) as a model for TMJ injury, leading to TMJ degeneration and hyperalgesia. To activate hM4Di, a CNO-loaded Alzet osmotic pump was implanted in the back of the animal before starting the FMO procedure. This allows for chronic release for 7 days. We performed micro-computed tomography (μCT) to assess subchondral bone phenotypes in mandibular condyles. Silencing of TRPV1-lineage afferents attenuated spontaneous pain assessed by mouse grimace scale, whereas TMJ degeneration was only modestly affected. Our results suggest that TRPV1 afferent fibers may not be a primary contributor to condylar degeneration following TMJ injury.

Disclosures: I. Alshanqiti: None. **S. Kumari:** None. **J. Hu:** None. **M. Chung:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

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Program #/Poster #: PSTR543.13/BB7

Topic: D.02. Somatosensation – Pain

Support: NRF-2017R1A5A2015391
NRF-2021R1A2C1007061

Title: Structural changes in the peptidergic and non-peptidergic C afferent terminals, their pre- and post-synaptic elements in the brain stem in a rat model of craniofacial inflammation

Authors: Y. CHO[1], J. BAE[1], Y. KIM[1], D. K. AHN[2], *Y. BAE[1];
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Abstract: The nociceptive C afferents and their target neurons in the spinal dorsal horn exhibit extensive functional changes in the pathologic pain state. However, little is known about the structural remodeling of synapses of C afferents that may underlie long-term maintenance of these functional changes. To address this issue, we used a model of inflammatory pain following injection of complete Freund's adjuvant (CFA) into the rat vibrissa pad. After 4 days (CFA 4-day group) and 21 days (CFA 21-day group) when the thermal hyperalgesia is severe and has recovered, respectively, we examined calcitonin gene-related peptide (CGRP)-immunopositive (+) and isolectin-B4+ (IB4+) axon terminals (boutons) and their postsynaptic dendrites and presynaptic endings in the medullary dorsal horn, using quantitative immuno-electron microscopy of serial sections. In both CGRP+ and IB4+ boutons, 1) frequency of boutons forming synapse with dendritic spine, 2) the ultrastructural parameters correlated with synaptic strength including bouton volume and mitochondrial volume, and spine head volume and postsynaptic density area increased significantly in the CFA 4-day group, compared to control. The fraction of IB4+boutons receiving axoaxonic synapses and the number density of the GAD65/67+ boutons, which are implicated in pre- and post-synaptic inhibition, decreased

significantly in the CFA 4-day group, compared to control. All the structural changes in the synapse of CGRP+ and IB4+ boutons in the CFA 4-day group were no longer present in the CFA 21-day group. The structural changes following inflammation may represent the morphological basis for the development and long-term maintenance of craniofacial inflammatory pain.

Disclosures: **Y. Cho:** None. **J. Bae:** None. **Y. Kim:** None. **D.K. Ahn:** None. **Y. Bae:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.14/BB8

Topic: D.02. Somatosensation – Pain

Support: University of Toronto Centre for the Study of Pain - Pain Scientist Scholarship
Institute of Medical Science PhD Stimulus Grant
Krembil Foundation Research Grant

Title: Age and sex dependence of hippocampal renormalization following microvascular decompression for trigeminal neuralgia

Authors: *J. LI[1], K. SOHNG[2], T. LATYPOV[1], A. NOORANI[2], P. SRISAIKAEW[4], P. S.-P. HUNG[4], D. JORGENS[4], M. HODAIE[3];
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Abstract: Chronic pain is a silent epidemic. Individuals with trigeminal neuralgia (TN) show evidence of abnormalities in the hippocampus. Previously, we found that reduced hippocampal volume in TN was reversible upon pain relief following successful surgery. However, the roles of age and sex in hippocampal renormalization remain unclear. Microvascular decompression (MVD) is a highly effective method to treat TN and patients who undergo the procedure are generally young; thus, it serves as a valuable platform to investigate the effects of age and sex on the dynamics of grey matter alterations within the hippocampus. We hypothesized that hippocampal recovery would be biased towards females of younger age. We analyzed magnetic resonance imaging (MRI) scans of 50 MVD patients (14 males, 36 females), who rated their pain pre- and post-surgery on a numeric rating scale from 0 (no pain) to 10 (worst pain imaginable). 41 (82%) responded to the surgery, with a post-surgical decrease in pain rating of at least 75%. MRI scans were processed to segment the hippocampus into 10 bilateral regions using FreeSurfer 7.0. After accounting for differences in head size between subjects, females under the age of 50 had a significant increase in total left-hemispheric hippocampal grey matter volume (dependent *t*-test; $n = 12$, $p = 0.02$), including 6/9 of its subregions (dentate gyrus, CA3 and CA4, molecular layer, hippocampal head and body) in addition to 1/9 on the right (hippocampal

head). Correspondingly, an analysis of covariance demonstrated that several of these regions were significantly smaller pre-surgically compared to age- and sex-matched controls but increased to comparable volumes following MVD. No significant changes were observed in male responders nor in female responders over 50 years of age. These results are another instance of sex-related effects in neuropathic pain, where hippocampal normalization is no longer detected beyond an inflection point at age 50. Traditionally known for its role in memory, cognition, and emotions, we provide further evidence that the hippocampus may be a key structure of interest in chronic pain conditions. These findings support a pressing need to expedite clinical timelines for females with TN, as they experience significantly greater delays in receiving surgical treatment than males. Additionally, non-invasive measures like age, sex, and hippocampal volume may have predictive value for grey matter recovery following MVD. Ultimately, knowing that brain abnormalities in chronic pain can be reversed with pain relief presents promising new avenues for future pain research, with a focus on equitable patient treatment.

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Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

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Program #/Poster #: PSTR543.15/BB9

Topic: D.02. Somatosensation – Pain

Support: 5R01DE027929-05

Title: Modulation of Apical Periodontitis by Trigeminal Peptidergic Nociceptors

Authors: ***S. F. HERNANDEZ**, K. LILLIS, R. SIMANAVICIUS, A. DIOGENES; Endodontics, UT Hlth. San Antonio, San Antonio, TX

Abstract: Apical periodontitis (AP) is a prevalent, debilitating disease resulting from odontogenic infection of dental pulp leading to inflammation and bone loss surrounding the apex of the tooth. Evidence suggests that sensory neurons, more specifically peptidergic nociceptors, sense and respond to AP, indicating an active role in modulating the disease. However, their exact role in the pathogenesis of AP remains unknown. We hypothesized that peptidergic nociceptors regulate bone metabolism by inhibiting bone resorption in AP. To test this, we used peptidergic nociceptor-ablated (calcitonin gene-related peptide [CGRP]$^{CreERT+/-}$DTA$^{lox+/-}$) mice and a pulp exposure technique to model AP and isolate the role of peptidergic nociceptors in periapical bone loss. We found that CGRP^{+} nociceptor ablation increased periapical bone loss and expression of inflammatory mediated processes in a sex-specific manner. *In vivo* administration of a CGRP receptor antagonist rigorously validated the increased periapical bone loss seen in peptidergic nociceptor-ablated mice, suggesting a crucial role of the neuropeptide

CGRP itself in modulating AP bone loss. *In vitro* co-culture mechanistic studies demonstrated that peptidergic nociceptors inhibit osteoclast activity while also suppressing osteoblast mineralization. Overall, our studies suggest that peptidergic nociceptors, possibly through the release of CGRP, play a protective role in AP bone loss by directly inhibiting the function of bone metabolism cells, thus reducing the periapical bone remodeling driven by the inflammatory niche. By understanding the neuronal role in AP, we can understand the mechanism behind the protective role of peptidergic nociceptors and develop better therapeutics for the millions suffering from oral diseases such as AP.

Disclosures: S.F. Hernandez: None. **K. Lillis:** None. **R. Simanavicius:** None. **A. Diogenes:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

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Program #/Poster #: PSTR543.16/BB10

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 5R01DE026806-05

Title: Elucidating the role of Schwann cell $PAR_2$and TRPV4 in oral cancer pain

Authors: *S. J. NICHOLSON[1], K. INOUE[1], V. GONZALEZ-BARROS RUBIO[1], Y. MULPURI[1], L. YANG[1], N. W. BUNNETT[2], D. D. JENSEN[2], B. L. SCHMIDT[1]; [1]Translational Res. Center, New York University, Col. of Dent., New York, NY; [2]Pain Res. Ctr., New York Univ., New York, NY

Abstract: Background: Oral cancer patients report high-levels of mechanically-induced pain during oral function. Oral cancer pain (OCP) derives from the release of algogenic substances in the cancer microenvironment that can activate or sensitise neurons and their supporting cells. Schwann cells (SC), which support the neurons innervating the cancer microenvironment, can modulate nociception both indirectly and directly to cause hyperalgesia. The protease-activated receptor 2 (PAR_2) is a G-protein coupled receptor implicated in a range of painful maladies. Activation of PAR_2 can generate mediators that enhance the activity of TRP channels involved in mechanical pain, including TRPV4. The TRPV4 receptor senses mechanical stress and sensitization of this channel by GPCRS can drive mechanical hyperalgesia. However, to our knowledge, PAR_2 and TRPV4 on Schwann cells have not been studied in the context of cancer pain. Since proteases secreted by oral cancer cells can drive pain via PAR_2, we hypothesize that such a mechanism involving PAR_2 and TRPV4 on SCs may contribute to OCP. To test this, we aim to investigate (1) whether oral cancer alters expression of SC PAR_2 and TRPV4 to sensitise SCs, and (2) whether PAR_2 activation is sufficient to sensitise TRPV4. **Methods/Results:** First, we show that human Schwann cells (hSC, Neuromics, HMP303) isolated from human spinal nerve and mouse Schwann cells (mSC, Applied Biological Materials, T0769) express *TRPV4*

mRNA transcripts. Human, but not mouse - Schwann cells express *F2RL1* (PAR_2). Calcium mobilization assays showed a dose-dependent calcium response in hSC and mSC following application of the TRPV4 agonist GSK1016790A (GSK). Human, but not mouse - Schwann cells showed an increase in cytosolic calcium following treatment with the PAR_2 agonist 2-furoyl-LIGRL-NH_2. To examine whether oral cancer increases expression of *F2RL1* and *TRPV4* (Aim 1), human oral cancer cells (HSC-3, JCRB0623, Japan) or murine oral cancer cells (MOC2, Kerafast, EWL002) were co-cultured with hSC or mSC for 24 hours, after which mRNA was collected. qPCR showed a significant increase in *TRPV4* mRNA (*p* < 0.01) and *F2RL1* (*p* < 0.05) in both hSC and mSC cocultured with MOC2 (2-way ANOVA). Co-culture of hSC or mSC with HSC-3 had no effect on *TRPV4* and *F2RL1* levels in either Schwann cell line. **Further Studies** will focus on the more translationally relevant human oral cancer / Schwann cell model. In line with Aim 2, we will elucidate whether the proteases in the cancer microenvironment induce TRPV4 sensitisation via PAR_2, and whether the PAR_2-TRPV4 axis is sufficient to drive oral cancer pain *in vivo.*

Disclosures: **S.J. Nicholson:** None. **K. Inoue:** None. **V. Gonzalez-Barros Rubio:** None. **Y. Mulpuri:** None. **L. Yang:** None. **N.W. Bunnett:** None. **D.D. Jensen:** None. **B.L. Schmidt:** None.

Poster

PSTR543. Trigeminal Circuits and Orofacial Pain

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR543.17/BB11

Topic: D.02. Somatosensation – Pain

Support: DE029187-01S2
DE032599

Title: Diversity in mechanically activated current responses for trigeminal ganglion neurons innervating masseter muscle

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Abstract: Temporomandibular disorders affecting the muscles of mastication are the most prevalent group of chronic orofacial pain conditions, with pain arising from the masseter muscle (MM) most common. We have recently developed a clinically relevant model of chronic masseteric muscle pain (myalgia) by utilizing Collagenase-II (Col-II). Mechanical hypersensitivity is a hallmark of many chronic pain conditions, yet very little is known about the underlying cellular and molecular mechanisms. Using reporter mice to label specific subsets of sensory neurons in combination with back labeling with WGA, I am characterizing properties of mechanically activated (MA) currents of trigeminal ganglion (TG) neurons innervating MM

using whole-cell patch clamp electrophysiology. Using the current signature method, action potentials and outward currents are measured to classify neurons into subclasses I previously characterized. MA currents were studied at several key time-points post-treatment with Col-II to study responses to mechanical stimulation throughout the development of chronic masseteric myalgia. I demonstrate differential responsiveness of unique classes of MM sensory neurons to mechanical stimulation.

Disclosures: K.A. Lindquist: None. **A.N. Akopian:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

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Topic: D.02. Somatosensation – Pain

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UPR RCM Chancellor's Office
UPR Deanship of Medicine

Title: The effects of herbicide consumption on avoidance and pain-related behaviors in rats

Authors: *N. JIMENEZ-RIVERA[1], L. MÉNDEZ-SANTACRUZ[1,2], T. JIMÉNEZ-RIVERA[1], L. VICENTE-RODRÍGUEZ[3], P. VÁZQUEZ-MARTÍNEZ[3], D. NAZARIO-MARTÍNEZ[3], M. CÁCERES-CHACÓN[1], O. MARTÍNEZ-GUZMÁN[1], D. SIERRA-MERCADO[1];
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Abstract: Glyphosate, the activeingredient in glyphosate-based herbicides, was initially considered safe formammals since it exerts its effect by inhibiting a metabolic route that is notpresent in mammals. However, glyphosate has become a significant environmentalthreat due to excessive use. The increased risk of human exposure to glyphosatethrough contaminated water sources and food products suggests a link betweenexposure to glyphosate and the development of emotional disorders such asanxiety and pain. The Environmental Protection Agency regulates glyphosate, andit is unlikely that humans are exposed to levels above those permitted.However, the effect of prolonged exposure to low doses of glyphosate on anxietyand

pain remains poorly studied. Inappropriate interpretation of threat andexcess avoidance are hallmarks of anxiety disorders and are often the mostdebilitating and life altering symptoms of these disorders. This makes studyingthe effect of glyphosate on avoidance and pain-related behaviors extremelynecessary. We administered either glyphosate-contaminated drinking water (n=16)or filtered water (n=16) for controls to female rats and performed behavioralstudies for avoidance and pain-related behaviors. We hypothesized thatglyphosate alters neuronal activity in brain regions important for theregulation of these behaviors. For avoidance, rats were conditioned in anoperant chamber to auditory tones co-terminating with a mild footshock. Anacrylic platform in the opposite corner of the sucrose-delivering bar allowedrats to avoid the shocks. We are evaluating behavioral changes and neuronalactivity in brain regions implicated in avoidance and pain-related behaviors.Our baseline results of the Vonfrey test indicate comparable levels of paintolerance on the left paw (Control: 14.0 g + 1.37 ; Gly: 13.79 g + 1.47) andright paw (Control: 9.0 g + 0.99 ; Gly: 11.21 g + 1.40). Understanding thefundamental mechanism of how herbicide consumption impacts will help in theunderstanding of the biological basis for how environmental exposures impactthe risk for psychiatric disorders.

Disclosures: **N. Jimenez-Rivera:** None. **L. Méndez-Santacruz:** None. **T. Jiménez-Rivera:** None. **L. Vicente-Rodríguez:** None. **P. Vázquez-Martínez:** None. **D. Nazario-Martínez:** None. **M. Cáceres-Chacón:** None. **O. Martínez-Guzmán:** None. **D. Sierra-Mercado:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.02/BB13

Topic: D.02. Somatosensation – Pain

Support: JUST Deanship of research (2021/374)

Title: Effect of enriched environment on long term effects of neonatal noxious stimulation in rats.

Authors: *K. NUSEIR[1], A. ALTARIFI[2], K. H. ALZOUBI[4], S. ABABNEH[3];
[1]Clin. Pharm., [2]Pharmacol., [3]Jordan Univ. of Sci. and Technol., Irbid, Jordan; [4]Jordan Univ. of Sci. & Technol., Irbid, Jordan

Abstract: The concept of an enriched environment (EE) refers to anenvironment that provides enhanced sensory, motor, and cognitive stimulationcompared to a standard environment (SE). It has been observed that an EE canhave positive effects on various aspects of brain function, includingincreasing neuronal plasticity in all brain areas.When it comes to infants, experiencing pain during earlydevelopment can have dire consequences both in the short and long term. This isparticularly relevant for premature babies and mature babies admitted to theNeonatal Intensive Care Unit (NICU), where tests and treatments often induceprocedural pain. Unfortunately, pain management in these settings is ofteninadequate or unavailable.Research has shown that an

enriched environment canalleviate chronic and neuropathic pain. Additionally, studies conducted on ratshave demonstrated that an EE can decrease pain sensitivity to heat in bothmales and females. Based on these findings, the proposed work aims to explorethe effects of an enriched environment on induced repetitive nociceptivestimulation and its long-term consequences.The experimental design involves inserting and quicklyremoving a small needle in the rat pups' paws during their first two weeks oflife. The rats were assigned to either a standard environment (SE) or anenriched environment (EE). When the rats reached adulthood, a series ofbehavioral tests were conducted to assess any deficiencies and inadequaciesresulting from the early pain experiences. Both male and female rats were usedin the study to examine potential sex differences. Preliminary results showed no significant difference betweenmale and female rats in terms of pain sensitivity or spatial learning andmemory. While pooled data showed that noxiously stimulated rats during infancyand reared in standard environment (SE-N) had the most deficiencies later inlife. (SE-N) rats of both sexes had the highest pain sensitivity to thermalstimulation, also, had the highest number of errors on the water maze thus themost deficiency in spatial learning and memory. In addition to behavioral assessments, biochemical markerswill also be tested to explore the effects of the enriched environment on thebrain. These markers can provide insights into the underlying mechanismsthrough which the EE exerts its effects on pain processing and other relatedprocesses. Overall, this research aimed to investigate how an enrichedenvironment influences the response to induced repetitive nociceptive stimulationand the long-term consequences of early pain experiences.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.03/BB14

Topic: D.02. Somatosensation – Pain

Title: Integrated behavioral tracking and calcium imaging in mouse pain models

Authors: *S. KISSINGER, J. KRAJEWSKI, K. W. JOHNSON, A. S. KATO;
Neurosci., Eli Lilly and Co., Indianapolis, IN

Abstract: There is a critical need for robust and quantifiable pain measures in preclinical models that are not solely dependent on observational assessments. Modern technologies allowing for large scale neurophysiological recordings in awake mice and an increased accessibility to machine learning techniques for detailed behavioral tracking represent an opportunity to improve upon long standing pain measures. Additionally, our understanding of pain processing in the brain remains limited compared to the peripheral and spinal mechanisms of nociception and signaling. Though pain is thought to be distributed across many brain areas, the anterior cingulate cortex (ACC) is of particular interest given its suggested role in the affective (’unpleasant’) component of pain. To this end, we utilized head-mounted miniaturized

microscopes (Inscopix) to record calcium transients in the ACC of head-fixed mice expressing GCaMP6f under the Thy1 promoter and developed an integrated system to simultaneously record pain evoked single paw dynamics and pupil dynamics to inform the neurophysiological time series. We find that continuous locomotion (running) occurs with a corresponding increase in the magnitude and duration of ACC activity evoked by Hargreaves stimulation compared to transient paw withdraws. By disrupting the surface of the behavioral platform, we facilitated single paw lifts compared to full body runs without paw restraint, thereby better isolating the transient ACC signal from persistent activity driven by running. We then assessed spontaneous pain with hind paw formalin injections, demonstrating increases in ACC activity, paw withdraws, and pupil increases shortly after the injection (5 mins) that subsequently attenuated (5-10 mins) then ramped up until the end of the experiment (biphasic response). Local maxima of both paw withdraws and pupil increases were informative of ACC activity, with subpopulations of neurons showing significant increases in activity during these periods. Finally, we show that the analgesic gabapentin attenuated formalin driven responses, decreasing the number of pupil and paw events occurring over the same period compared to formalin alone. Taken together, we have validated a new assay that may improve our ability to quantify pain signals in preclinical models without the need for observational assessments by individual researchers.

Disclosures: **S. Kissinger:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **J. Krajewski:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **K.W. Johnson:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **A.S. Kato:** A. Employment/Salary (full or part-time):; Eli Lilly and Company.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.04/BB16

Topic: D.02. Somatosensation – Pain

Support: NS109059
DE018661
DE023090

Title: Assessment of orofacial pain in mice by an artificial intelligence-based method

Authors: *S. GUPTA;
Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Assessment of orofacial pain in mice by an artificial intelligence-based method*Saurav Gupta**, Akihiro Yamada, Jennifer Ling, Jianguo G. Gu Department of Anesthesiology and Perioperative Medicine, School of Medicine, University ofAlabama at Birmingham, Birmingham, AL, 35294Orofacial pain is difficult to be reliably assessed in

experimental animals, which hamper thepreclinical studies of orofacial pain. In the present study, we aimed to develop an artificialintelligence-based (AI-based) method to quantitatively assess orofacial pain in mice. Since oneof the prominent behaviors of orofacial pain is orbital tightening, in our experiments we video-recorded orofacial regions of animals and then quantified orbital tightening of animals using anAI-based method. To measure the orbital dimension, DeepLabCut was utilized to track themidpoints of the upper and lower eyelids of the animals for a duration of 10 minutes usinggraphic machine learning. Subsequently, the distances between these two points were analyzedusing the offline CSV data file generated by DeepLabCut. In one set of experiments, animalsreceived subcutaneous injections of 10 µl saline (control group) or 10 µl capsaicin (10 µMconcentration) (inflammatory pain group) in the cheek regions. The mean orbital dimension inthe capsaicin-induced inflammatory pain group was significantly smaller in comparison with thecontrol group. Orbital dimension histogram also showed an overall reduction of orbitaldimension, i.e., orbital tightening, following orofacial inflammation induced by capsaicin. Inanother set of experiments, $Nav1.8^{ChR2}$ mice were tested. These optogenetic mice displayednocifensive responses (avoidence behaviors) to blue laser light applied to their skin. We appliedblue laser light to their orofacial regions, we found that $Nav1.8^{ChR2}$ mice also showed reductionof orbital dimension over an extended time following the light stimulation. Collectively, thepresent study has established an AI-based method to quantify orofacial pain in mice, which willhelp us in future to better understand orofacial pain and identify therapeutic targets for the reliefof orofacial pain.Keywords: Orofacial pain, orbital tightening, artificial intelligence, machine learning,inflammation, optogenetics.

Disclosures: S. Gupta: None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.05/BB17

Topic: D.02. Somatosensation – Pain

Title: Optimised and scalable reprogramming of human iPSCs to generate nociceptor sensory neurons for the study of pain mechanisms and neuropathies

Authors: *T. OOSTERVEEN, D. PACITTI, L. FOULSER, M. BAHRI, M. ORTIZ, K. LONG, M. BYRNE, V. YIANNI, M. DERMIT-SALACAR, A. WILCZYNSKA, S. MILDE, B. NEWMAN, B. TALSANIA, S. SUR, M. RAMAN SRIVASTAVA, F. PATELL-SOCHA, K. DHALIWAL, O. DOVEY, T. MOREAU, W. BERNARD, M. METZAKOPIAN, M. KOTTER; bit.bio, Cambridge, United Kingdom

Abstract: Nociceptive sensory neurons are a specialised subtype of somatosensory cells residing in the dorsal root ganglia. Nociceptors are able to respond to diverse noxious and pruritic stimuli, and hence are critical for the study of pain mechanisms and neuropathies. It is estimated that 20% of adults suffer from chronic pain, but the current analgesics are limited by short duration,

inadequate efficacy, and/or poorly tolerated adverse events. The discovery of novel drugs have been hampered as the efficacy in animal models of pain cannot be reproduced in the clinic. Consequently, drug classes used to treat chronic pain have essentially not evolved over the past 40 years. Thus, there is an unmet need for reliable and scalable human *in vitro* models to study the molecular mechanisms underlying nociception and develop new, efficacious, and safe pain therapeutics. However, conventional differentiation methods to generate nociceptors from pluripotent cells are complex, inconsistent, and characterised by protracted maturation times. Through our proprietary precision cellular reprogramming technology (opti-ox^{TM}), which enables a robust and controlled expression of transcription factors (TFs), we aimed to generate a rapid and scalable cell culture system for the consistent production of physiologically relevant and functional nociceptor sensory neurons from human iPSCs.
opti-ox^{TM} engineered iPSC lines expressing a combination of key TFs, consistently and efficiently reprogram within a week into a homogeneous population of sensory neurons that display critical features of mature nociceptors.
Morphological, transcriptomic and phenotypic characterisation demonstrated that reprogrammed iPSCs acquired a sensory nociceptor identity. Within 7 days, the sensory neurons expressed the key pan-sensory sensory neuron markers ISL1, POU4F1 and PRPH, as well as key nociceptor markers such as NTRK1, TRPV1, TRPM8, and SCN9A. Neurotrophic factors play a critical role in the subtype specification of sensory neurons and by optimising the culture conditions we were able to further enrich for cells expressing key sensory genes including peptidergic nociceptor markers TAC1, and ADCYAP1. Multi-Electrode Array and calcium assays demonstrated that reprogrammed sensory neurons are functional as displaying asynchronous spontaneous activity and responsiveness to diverse noxious stimuli.
In conclusion, with opti-ox^{TM} precision reprogramming, iPSCs are rapidly converted into functional sensory neurons offering a robust and scalable source of human nociceptors that can be used as an *in vitro* model to study the biology of pain and to develop novel therapies for neuropathies.

Disclosures: **T. Oosterveen:** A. Employment/Salary (full or part-time):; bit.bio. **D. Pacitti:** A. Employment/Salary (full or part-time):; bit.bio. **L. Foulser:** A. Employment/Salary (full or part-time):; bit.bio. **M. Bahri:** A. Employment/Salary (full or part-time):; bit.bio. **M. Ortiz:** A. Employment/Salary (full or part-time):; bit.bio. **K. Long:** A. Employment/Salary (full or part-time):; bit.bio. **M. Byrne:** A. Employment/Salary (full or part-time):; bit.bio. **V. Yianni:** A. Employment/Salary (full or part-time):; bit.bio. **M. Dermit-Salacar:** A. Employment/Salary (full or part-time):; bit.bio. **A. Wilczynska:** A. Employment/Salary (full or part-time):; bit.bio. **S. Milde:** A. Employment/Salary (full or part-time):; bit.bio. **B. Newman:** A. Employment/Salary (full or part-time):; bit.bio. **B. Talsania:** A. Employment/Salary (full or part-time):; bit.bio. **S. Sur:** A. Employment/Salary (full or part-time):; bit.bio. **M. Raman Srivastava:** A. Employment/Salary (full or part-time):; bit.bio. **F. Patell-Socha:** A. Employment/Salary (full or part-time):; bit.bio. **K. Dhaliwal:** A. Employment/Salary (full or part-time):; bit.bio. **O. Dovey:** A. Employment/Salary (full or part-time):; bit.bio. **T. Moreau:** A. Employment/Salary (full or part-time):; bit.bio. **W. Bernard:** A. Employment/Salary (full or part-time):; bit.bio. **M. Metzakopian:** A. Employment/Salary (full or part-time):; bit.bio. **M. Kotter:** A. Employment/Salary (full or part-time):; bit.bio.

Poster

PSTR544. Behavioral and Physiological Pain Models

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.06/BB18

Topic: D.02. Somatosensation – Pain

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Title: Functional Expression of TRPV4 in Human Schwann Cells and its Association with Oral Cancer Pain

Authors: *Y. MULPURI, S. J. NICHOLSON, K. INOUE, V. GONZALEZ-BARROS RUBIO, B. L. SCHMIDT;
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Abstract: Oral cancer (squamous cell carcinoma, SCC) patients suffer from severe, debilitating pain stemming from mechanical stimulation at the site of the cancer. Despite the substantial pain burden in many oral cancer patients, the underlying mechanisms responsible for mechanical hypersensitivity remain poorly understood. TRPV4 is a non-selective cation channel activated by various stimuli including mechanical pressure, osmotic stimuli, warm temperature, and phorbol esters. In the peripheral nervous system, TRPV4 is expressed in sensory neurons and Schwann cells; sensitization and activation of TRPV4 underlie mechanical hyperalgesia in chronic pain. The expression and function of TRPV4 in Schwann cells in the context of oral cancer pain has not been studied. Using patch clamp electrophysiology we studied the functional activity of TRPV4 in the lingual nerve of a patient diagnosed with tongue cancer and in Schwann cells obtained from a commercial vendor (Neuromics, MN). For the oral cancer patient enrolled in the study, the data on reported functional pain was collected using the validated UCSF Oral Cancer Pain Questionnaire (UCSFOCPQ), mechanosensitivity was assessed with von Frey monofilaments (VFM), and chemosensitivity was assessed with taste strips impregnated with varying concentrations of capsaicin (0-10 mM). On the UCSFOCPQ, the patient reported a mean mechanical sensitivity score of 74±15 (0-100 scale). In VFM testing, the mechanical detection threshold for cancer vs. the matched contralateral normal site was 1.7 and 4 grams respectively. The patient reported pain in response to VFM testing on the cancer side; no pain was reported on the contralateral side. In chemosensitivity testing with capsaicin, the area under curve for numerical rating scales was 25 and 18.5 for the cancer and normal sites respectively. In patch clamp electrophysiology, the recording of whole-cell currents with ramp voltage (-100 to 100 mV, 500 ms) in the presence of TRPV4 agonist, GSK1016790A (200 nM) revealed peak inward and outward currents at 5 minutes after drug application. A reduction in the concentration of

GSK1016790A to 100 nM shortened the duration of the inward current. In Schwann cells (n=3) collected from the lingual nerve of the oral cancer patient, the bath application of GSK1016790A, 100 nM, resulted in an average current density of 13.4 (pA/pF) at -100 mV holding potential. We infer from these data that human Schwann cells, including those cultured from oral cancer patients with pain, express functional TRPV4. Our future studies will explore the sensitization and activation of TRPV4 in Schwann cells by oral cancer mediators and related effects on oral cancer pain.

Disclosures: Y. Mulpuri: None. **S.J. Nicholson:** None. **K. Inoue:** None. **V. Gonzalez-Barros Rubio:** None. **B.L. Schmidt:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant K23DA048972
NIH Grant R61NS118651-01

Title: A physical and psychological profile for high impact chronic pain

Authors: *O. ALTIRKAWI[1], D. S. YOU[3], T. C. DILDINE[4], T. MARONESY[1], K. A. WEBER[3], S. MACKEY[2];
[1]Stanford Univ., Palo Alto, CA; [2]Anesthesia & Pain Mgmnt, Stanford Univ., PALO ALTO, CA; [3]Stanford, Palo Alto, CA; [4]Natl. Ctr. for Complementary and Integrative Hlth., NIH, Bethesda, MD

Abstract: Background: Chronic pain is associated with many negative health outcomes including impacts on the structure, organization, chemistry, and circuitry of the nervous system. These changes likely lead to alterations in cognitive and behavioral processes. Indeed, chronic pain has been associated with increased incidence of comorbidities (e.g., anxiety, depression) and worse subjective health (e.g., increased pain interference). Most studies of chronic pain assess how patients compare to healthy volunteers; however, great variability likely exists within these patients. Previous work indicates high impact chronic pain (HICP) is associated with worse health status, increased rates of opioid use, higher healthcare costs and is more common in older age, females, and racial/ethnic minorities. **Objective**: To identify a PROMIS physical and psychological profile for HICP and to assess for behavioral and cognitive differences within a chronic pain sample. This study specifically compares patients with and without HICP.**Methods:** Participants were recruited from a tertiary pain clinic, enrolled in a pain registry (choir.stanford.edu). A total of 452 adults (M age= 58.8 years, 72.3% female, 81% White) completed PROMIS-measures (www.promishealth.org) for average pain, pain interference, physical function, depression, anxiety, and anger as well as the Graded Chronic Pain Scale-

Revised (GCPS-R). The GCPS-R was used to classify people with (67%) and without HICP (33%). A MANOVA was conducted to compare subjective health status between the two groups. **Results:** We observed a significant group difference (Λ= 0.54, p< .001, η^2= 0.46). The HICP group reported worse health status in all measures (M average pain= 6.6, SD= 1.7 ; M pain interference = 66.3T, SD= 7.0; M physical function= 34.8T, SD= 5.9; M depression= 57.1T, SD= 8.6; M anxiety = 57.5T, SD= 9.0; M anger= 51.5T, SD= 9.8) than the non-HICP group (M average pain= 4.8, SD= 1.8; M pain interference= 56.8T, SD= 5.3; M physical function= 44.3T, SD= 7.2; M depression= 51.6T, SD= 8.1; M anxiety= 53.3T, SD= 9.0; M anger= 48.7T, SD= 8.4, all p's< 0.003) with a large difference in pain interference (η^2=0.42) and physical function (η^2=0.33), a moderate difference in average pain (η^2=0.20), and a small difference in depression (η^2=0.09), anxiety (η^2=0.05) and anger (η^2=0.02). An additional MANOVA was conducted with age, female sex, and minority status as covariates and all results remained. **Conclusion**: Greater pain interference and poorer physical function, followed by worse pain ratings and cognitive symptoms, compose a PROMIS symptoms profile associated with HICP, indicating health outcomes vary with chronic pain impact.

Disclosures: O. Altirkawi: None. **D.S. You:** None. **T.C. Dildine:** None. **T. Maronesy:** None. **K.A. Weber:** None. **S. Mackey:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.08/BB20

Topic: D.02. Somatosensation – Pain

Support: NIH Grant K23DA048972
NIH Grant R61NS118651-01

Title: Somatization and sensory hypersensitivity: which is a better predictor for pain ratings and pain interference in patients with chronic pain?

Authors: *T. MARONESY[1], D. S. YOU[1], T. C. DILDINE[3], O. ALTIRKAWI[1], K. A. WEBER[1], S. MACKEY[2];
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Abstract: Somatization and sensory hypersensitivity are associated with worse pain and greater pain interference in chronic pain patients. DSM-5 defines somatization as having somatic symptoms accompanied with excessive thoughts, emotional distress, or high health seeking behaviors. Sensory hypersensitivity refers to an ability to detect low levels of sensory input and is associated with lower tolerance to sensory stimuli (e.g., heat). To date, studies have investigated somatization and sensory hypersensitivity separately for their relationship with pain. The current cross-sectional study examined these two predictors simultaneously to identify

which better predicts pain interference and intensity in chronic pain patients. The Symptom Checklist 90-somatization subscale (SCL90-somatization) and Sensory Hypersensitivity Scale (SHS) were administered to people seeking treatments at a tertiary pain clinic who enrolled in a pain registry (https://choir.stanford.edu). In addition, PROMIS measures were administered to assess average pain and pain interference in the past 7 days. A total of 452 adults (mean age = 58.8 years, 72.3% female) completed questionnaires. SCL90-somatization and SHS scores were moderately correlated (r = 0.41, $p < .001$). When examining the predictors separately, SCL90-somatization scores was a significant predictor of pain intensity (β= .44, $p < .001$, r^2=0.20) and pain interference T scores (β= .52, $p < .001$, r^2=0.27). SHS was also a significant predictor of pain rating (β= .17, $p < .001$, r^2=0.03) and PROMIS-pain interference (β= .21, $p < .001$, r^2=0.04). These two separate regression analyses revealed somatization was moderately associated with pain ratings and largely associated with pain interference and sensory hypersensitivity was minimally associated with pain and pain interference. When examining the two predictors simultaneously in a multiple regression, only SCL90-somatization scores significantly predicted pain ratings (β= .45, $p < .001$, r^2=0.20) and PROMIS-Pain Interference (β= .52, $p < .001$, r^2=0.27). An exploratory analysis was conducted to examine just the SHS pain subscale. Exploratory analyses revealed SCL90-somatization and SHS pain subscale scores were significant predictors of pain ratings (r^2=0.22), whereas results remained the same for pain interference. In conclusion, somatization is more strongly associated to average pain and pain interference than sensory hypersensitivity. Future research should consider how somatization and sensory hypersensitivity impact future pain interference and intensity and develop treatments for those with greater somatization and/or sensory hypersensitivity.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.09/BB21

Topic: D.02. Somatosensation – Pain

Support: KAKENHI
AlphaNavi Pharma Inc

Title: Analgesic effects of a novel sodium channel blocker on a mouse model of episodic pain syndrome

Authors: *H. OKUDA[1], S. INOUE[2], Y. OYAMADA[3], A. KOIZUMI[4], S. YOUSSEFIAN[2], A. KOIZUMI[4];
[1]Kyoto Prefectural Univ. of Med., Kyoto, Japan; [2]Kyoto University, Kyoto, Japan., Kyoto, Japan; [3]AlphaNavi Pharma Inc., Osaka, Japan; [4]Kyoto University, Inst. of Publ. Hlth. and Welfare Res., Kyoto, Japan

Abstract: The Nav1.7, 1.8, and 1.9 sodium channels that are expressed in DRG neurons are known to contribute to the action potentials of the pain signaling pathway. Mutations in these channels can result in hyperexcitability of the DRG neurons and lead to both painful and painless disorders. We recently identified several Nav1.9 mutations, in numerous pedigrees of familial episodic pain syndrome (FEPS), which lead to pain symptoms that appear during infancy, are predominantly confined to the extremities, and are often induced by fatigue and bad weather. We subsequently developed knock-in mice lines harboring these mutations and demonstrated their association with pain symptoms by both behavioral and electrophysiological studies. A novel non-opioid drug, ANP-230, which has strong inhibitory effects on Nav1.7, 1.8, and 1.9, has already completed Phase I trials in Japan, the US, and Europe and is currently undergoing Phase I/II trials for FEPS patients in Japan. We performed behavioral experiments to determine the analgesic effects of ANP-230 on the thermosensitivity and mechanosensitivity of model mice (male, 6-8 weeks) carrying p.R222S, one of the Nav1.9 mutations discovered in our FEPS patients. We also performed *in vitro* pharmacological studies to characterize the nonclinical effects of ANP-230 on the mice. Our results clearly demonstrate that ANP-230 not only significantly reduced the hyper-thermal sensitivity in a concentration-dependent manner but also the hypo-thermal sensitivity even at low concentrations. ANP-230 also attenuated the hypersensitivity to mechanical stimuli in a concentration-dependent manner, and this effect was sustained for 3 hours even at low doses. From the electrophysiological experiments, we determined that administration of ANP-230 (10 μM or 30 μM) resulted in a reduction in the current density and a change in the properties of Nav1.7, 1.8, and 1.9, as well as a reduction in the shape of the action potentials so as to reduce the number of repetitive action potentials derived from the p.R222S mutation. Overall, our results clearly suggest that ANP-230 has an analgesic effect on FEPS and that this results from reduced DRG neuron hyperexcitability. It is highly likely that the effects of this non-opioid drug will be of clinical significance not only for FEPS but also for other peripheral nerve pain symptoms.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.10/BB22

Topic: D.02. Somatosensation – Pain

Support: 1F31NS129281-01A1
GR5271591

Title: Closed-loop optogenetic system for deep behavioral phenotyping

Authors: *R. MEIR, A. NAGARAJ, S. SAMADOV, J. JANG, T. SERRE, D. SHEINBERG, J. T. RITT, D. LIPSCOMBE;
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Abstract: The field of pain behavior is developing behavioral paradigms for deeper phenotyping to reduce experimenter bias and facilitate replication across experimenters and labs. The goal is to reduce labor, reduce experimenter-based differences associated with stimulus positioning and improve response interpretation. To reduce subjectivity and increase reproducibility, we have developed a new optogenetic closed-loop system to monitor and record light-evoked responses in sensory neuron subpopulations in the mouse hindpaw (CLOSER: Closed-Loop Optogenetic System for Evoked Responses).
Mice are placed in a clear box situated on an acrylic floor and allowed to move freely while their hindpaws are tracked continuously with DeepLabCut-Live (DLC-Live). We use a laser and galvanometer mirror system for directed targeting of the mouse hindpaw based on DLC-Live determined coordinates. Laser stimulation of the hindpaw only occurs if specified criteria, such as time from last stimulation, degree of movement, and paw detection confidence level, are met. The laser can be tuned to various intensities for generating stimulus input-output relationships. Cameras below and beside the testing box record the behavior session for offline analysis. Mice expressing ChannelRhodopsin2 (ChR2) in Trpv1 or Cacna1h sensory neurons were assessed and compared with CLOSER.
Using CLOSER, we are able to dissect features of laser-evoked responses, such as response latency, response probability, and paw displacement. Moreover, through machine learning for behavior classification, we are beginning to classify response subtypes. Ethograms of behavioral responses allow for deep phenotyping to distinguish behaviors based on stimulus intensity and stimulus targets (e.g. Trpv1 nociceptors compared to Cacna1h Aδ-LTMRs). Beyond the immediate applications, CLOSER, in combination with newly developed analysis pipelines, could be used to document more nuanced differences in behaviors associated with spontaneous compared to stimulus-evoked responses. Behavior stratification could inform the assessment and development of novel therapeutics optimized to mitigate spontaneous and stimuli-evoked pain. To our knowledge, CLOSER is the first fully-automated system for non-surgical, targeted, optogenetic-induced nocifensive behavior in freely-moving rodents. The code and design blueprints will be available on the Lipscombe Lab Github.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.11/BB23

Topic: D.02. Somatosensation – Pain

Support: NSF 1932707

Title: Development and evaluation of a behavioral assay for nociception using zebrafish larvae

Authors: *A. BUNNELL, E. MARSHALL, M. DEADMOND, S. ESTES, S. LOESGEN, J. STROTHER;
Whitney Lab. for Marine Bioscience, Univ. of Florida, St. Augustine, FL

Abstract: Chronic pain is a debilitating disease that affects more than 20% of U.S. adults, and current therapeutic approaches often have severe side effects, including dependence and addiction. Zebrafish larvae are a potentially excellent model for studying the neural circuits associated with the sensing of noxious stimuli and other pathways associated with pain. Many genes associated with human health are conserved in zebrafish, zebrafish larvae are amenable to low-cost and high-throughput experimental approaches, and the small size and optical transparency of zebrafish larvae enables optical approaches that are infeasible in other models. However, although there are many well-established behavioral assays for nociception in rodents, there are no widely accepted assays in zebrafish larvae. In this study, we develop and evaluate a novel zebrafish larvae-based assay for nociception. Hatching-stage zebrafish larvae are loaded into a well plate and dosed with a test compound, their movement is continuously monitored, and the response to an introduced noxious chemical is recorded. Extensive preliminary experiments were performed to optimize each experimental parameter. To evaluate how different types of pharmacological activity present in this assay, we next examined a panel of established pharmaceuticals with a range of targets. We found that most of the tested human TRPA1 antagonists were active in this assay, although the behavioral phenotypes varied, and the EC50 was not always well correlated with previously reported values from *in vitro* assays using mammalian cells. Similarly, local anesthetics abolished the response to noxious stimuli. In contrast, non-steroidal anti-inflammatory drugs (NSAIDS), anxiolytics, and sedatives showed no activity in this assay. Interestingly, mu-opioid agonists (e.g., morphine) showed no activity in our assay, which may suggest that the opioid system is not yet fully functional at this developmental stage. These results indicate that this behavioral assay provides a robust measure of nociceptive activity and has substantial promise for drug discovery efforts, with the caveat that some leads may exhibit species-specific differences in potency.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Topic: D.02. Somatosensation – Pain

Support: The majority of this research was supported by departmental funds. A subset of the single-dose data was supported in part by funds from MyMD pharmaceuticals, Inc. and MIRA Pharmaceuticals, Inc.

Title: Ratio combinations influence the anti-nociceptive effects of cannabinoids delta-9-tetrahydrocannabinol and cannabidiol in a rat model of inflammatory pain.

Authors: *B. W. JENKINS, C. F. MOORE, E. M. WEERTS;
Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Chronic inflammatory pain affects 100 million Americans. Inflammatory pain is produced by pro-inflammatory signaling that persists beyond the initial injury or disease. Symptoms include hyperalgesia (increased pain sensitivity) and allodynia (pain sensitivity to non-painful stimuli). Cannabinoids, such as delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), are of therapeutic interest for inflammatory pain treatment as data suggests possible anti-nociceptive and anti-inflammatory properties. While select THC:CBD formulations are in clinical trials for chronic pain treatment, moderate efficacy and adverse effects related to THC are reported. Co-administration of CBD with THC may attenuate the adverse effects of THC; thus, continued investigation of different dose ratios of THC:CBD are needed to identify optimal combinations that improve treatment outcomes. In our ongoing study, we tested in a rat model of inflammatory pain the anti-nociceptive effects of THC and CBD, alone or combined in different ratio combinations. Using a mixed design, treatment groups of adult Sprague Dawley rats (n = 5-10 per condition) were administered sesame oil vehicle (negative control), THC (1-10 mg/kg, p.o.), CBD (10-100 mg/kg, p.o.), or THC+CBD combinations under blinded conditions. Inflammatory pain was induced via intraplantar injection of carrageenan in the rat hind paw. Thermal hyperalgesia and mechanical allodynia were assessed using the Hargreaves and Von Frey tests, respectively, prior to carrageenan (baseline) and at 1-, 3-, and 5-hrs post-treatment. Paw edema was measured using an electronic digital caliper at the same timepoints. Preliminary data were analyzed using repeated-measures ANOVAs with treatment as the between-subject factor and timepoint as the within-subject factor. Compared to vehicle control, THC was anti-hyperalgesic ($p < 0.05$) and anti-allodynic ($p < 0.05$) at multiple doses tested and across timepoints. CBD alone did not significantly reduce pain-related outcomes at any dose tested ($p > 0.05$). Administration of THC in combination with CBD either augmented or attenuated the effects of THC alone, depending on the dose of CBD. Neither THC, CBD, nor the combinations reduced paw edema. Taken together, these data can inform further therapeutic investigation of THC:CBD dose combinations for inflammatory pain.

Disclosures: B.W. Jenkins: None. **C.F. 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.; C.M. has received funds from MyMD pharmaceuticals, Inc. and MIRA Pharmaceuticals, Inc for contract preclinical research. **E.M. Weerts:** 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.; E.W. received funds from MyMD pharmaceuticals, Inc. and MIRA Pharmaceuticals, Inc for contract preclinical research and Cultivate Biologics LLC, and Canopy Growth Corp. for clinical research projects.

Poster

PSTR544. Behavioral and Physiological Pain Models

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Topic: D.02. Somatosensation – Pain

Support: CIHR Foundation Grant 154281 to J.S.M.
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Title: Postoperative pain shows circadian influence in a mouse model of incisional wounds.

Authors: *E. MCEACHERN[1], M. ZILIC[3], N. GHASEMLOU[4], J. S. MOGIL[2];
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Abstract: Postoperative pain is experienced by over 80% of people undergoing surgeries each day. Usually managed with NSAIDs, opioids, and acetaminophen, which offer their own set of negative side effects, an alternate method to manage postoperative pain alongside the current options is needed. An emerging approach is the use of chronotherapy (i.e. timing of interventions). Circadian rhythms, controlled by molecular clocks, are involved in nearly every physiological function, including immune responses and pain mechanisms. While time-of-day effects on pain levels of various pain conditions have been well studied, it remains poorly understood whether time of injury can influence subsequent pain behaviours. This study aims to assess postoperative pain in a mouse model of hind paw incision in a circadian-dependent manner. Incisions were made at four time points (ZT2, ZT8, ZT14, ZT20), indicated with *Zeitgeber* time (ZT) which is used to standardize time across laboratory light:dark cycles, with ZT0 indicating lights-on and ZT12 lights-off. Evoked pain behaviours were measured in mice using the von Frey mechanical sensitivity and Hargreaves' radiant heat paw-withdrawal assays. Testing at ZT8 was performed 1, 3, 5, 7, and 10 days following incision, then once weekly until pain resolution. Interestingly, mice receiving surgery in the resting phase at ZT2/8 appeared to show faster pain resolution compared to the active phase surgeries at ZT14/20 for mechanical hypersensitivity, while no significant differences were observed for heat sensitivity. These data suggest that pain from resting phase injuries may resolve more rapidly than pain from active phase injuries, as well as having a modal-specific pain resolution. A sex difference was not observed for either pain assay. Oscillations in clock-controlled gene expression show very tightly orchestrated rhythms in mice, with *Per1/2* and *Reverb-a* peaking in the dorsal root ganglion around mid-resting phase, and oppositely expressing *Bmal1*, *Clock*, and *Npas2* mid-active phase. Other immune and hormonal functions also show circadian rhythmicity, which could account for pain resolution differences between groups. We aim to establish whether recovery from surgeries would benefit from specific time windows, holding therapeutic potential for more biologically informed postoperative care as well as greater experimental control in the lab.

Disclosures: E. McEachern: None. **M. Zilic:** None. **N. Ghasemlou:** None. **J.S. Mogil:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.14/CC1

Topic: D.02. Somatosensation – Pain

Support: DA046537

Title: Using a novel pain scale to assess morphine-derived antinociception in young and aged rats

Authors: C. DRESSLER[1], B. DUNHAM[3], M. JIWANJI[4], V. V. PARIKH[5], N. T. FRIED[6], I. ABDUS-SABOOR[7], *M. WIMMER[2];
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Abstract: Chronic pain is a socio-economic burden affecting more than 30% of people worldwide. This multi-dimensional condition can lead to severe disabilities and has a significantly negative impact on quality of life. Pain management is particularly challenging in elderly populations, who are disproportionally impacted by the deleterious effects of pain. Measuring pain reliably and systematically is inherently difficult both in patients as well as in pre-clinical models of pain. We recently established a novel high speed videography-based pain scale by combining scoring of nocifensive behaviors (i.e. face grimace) with paw kinematics into a single pain score in rats. By mapping sub-second mechanically-evoked behaviors to innocuous and/or noxious stimuli in both males and females, we transformed the data into a single dimension using statistical and machine learning approaches to generate an easily interpretable rat pain scale. This approach consistently distinguished innocuous stimuli from painful pinpricks, reliably predicting which stimuli would be perceived as innocuous or painful in adult male and female rats. Here, we deployed this novel tool to examine sensory reflexes and pain in young and aged Wistar rats. In humans, normal aging is accompanied by changes in pain perception, mu opioid receptor signaling and in the doses of opioids required to achieve pain relief. We examined baseline sensory reflexes to innocuous and noxious stimuli in young (2-4 months) and aged (22-24 months) animals. Aging did not affect scores in response to innocuous or noxious stimuli but there was a significant difference between scores elicited by innocuous stimuli compared to pin pricks. This scale had been previously only tested in Sprague Dawley and Long Evans rats. These results indicate that the novel pain scale is a reliable assay in both young and aged Wistar rats. We next examined morphine-derived antinociception using pin pricks at baseline, fifteen and sixty minutes following a subcutaneous morphine injection (3mg/kg). All animals showed a reduction in pain scores following a morphine injection and aging had no impact on this effect. Taken together, these results indicate that our novel high-speed videography-based pain scale reliably discriminates sensory reflexes elicited by innocuous stimuli to the paw from those produced by painful pin pricks in young and aged rats. These studies lay the foundation to further probe the mechanisms underlying age-related changes in

pain perception, opioid signaling and antinociception in preclinical models of acute and chronic pain.

Disclosures: C. Dressler: None. **B. Dunham:** None. **M. Jiwanji:** None. **V.V. Parikh:** None. **N.T. Fried:** None. **I. Abdus-Saboor:** None. **M. Wimmer:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.15/CC2

Topic: D.02. Somatosensation – Pain

Support: CONACYT A1-S-27869

Title: Effect of experimental gestational diabetes mellitus on the postoperative recovery of the adult offspring

Authors: *C. A. CANTU-CEPEDA, M. A. SALINAS-CASTILLO, A. MARTINEZ-MARTINEZ, R. I. ACOSTA-GONZALEZ, V. M. VARGAS-MUÑOZ, J. M. JIMENEZ-ANDRADE, E. MUÑOZ-ISLAS;
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Abstract: Gestational diabetes mellitus (GDM) is an obstetric metabolic disease that causes short- and long-term maternal and offspring complications. We determined whether GDM had an influence on the hind paw postoperative recovery in the female adult offspring.GDM was induced by i.p. administration of streptozotocin (STZ) in mouse dams. At 13 weeks of age, a plantar surgery on the right paw of the female offspring was made. The mechanical sensitivity (von Frey filaments) was recorded under the "up-down" method under 1, 2, 4, 6, 8, 10, 12, 14, and 21 days post-surgery. Additionally, other groups with plantar surgery received or did not the administration of i.p. baclofen (10 mg/kg) before every evaluation. Offspring mice born from STZ-treated dams had similar values of blood glucose compared to offspring born from vehicle-treated dams. The offspring from STZ dams showed mechanical hypersensitivity as compared to the control group. The offspring born from control dams reach 50% of recovery post-surgery at 3-4 days, meanwhile, offspring born from STZ dams reach the same percentage of recovery at 8 days post-surgery. The administration of baclofen led to a recovery of 100% on day 2 post-surgery in the offspring from the control group and on day 3 for offspring from STZ dams. These results taken together showed that GDM decreases post-surgery recovery capacity in the offspring, which is improved after the i.p. administration of baclofen.

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Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR544.16/CC3

Topic: D.02. Somatosensation – Pain

Support: CIHR Foundation Grant 167276
SickKids Commercial Proof-of-Principle Grant

Title: Inferring painfulness of a stimulus through its modulation of ongoing behaviour: Beyond latency and threshold measurements

Authors: *C. DEDEK[1,2], S. A. PRESCOTT[1,2];
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Abstract: Preclinical pain research typically measures the latency and/or threshold of stimulus-evoked behaviors. Chronic pain is often associated with hypersensitivity reflected in latency and/or threshold changes but the bigger clinical complaint is ongoing pain. Accordingly, measuring ongoing behaviors (e.g. activity level, gait and facial grimace as well as guarding, licking or flinching of an affected paw) in models of persistent pain has grown in popularity. Evoked and ongoing behaviors are treated separately but this need not be the case. We developed a device capable of reproducible optogenetic and radiant heat stimulation and precise latency measurement. Video of the mouse from below, which is used for aiming, records behavior before, during and after stimulation. We found that increasingly strong optogenetic stimuli triggered faster reflexive paw withdrawal as well as more guarding and licking, and even facial grimacing. Mice also exhibited more sniffing and rearing after strong stimulation. In contrast, weak (perithreshold) stimuli typically evoked (slower) paw withdrawal without affecting other behaviors. After intraplantar injection of capsaicin, mice withdrew from radiant heat faster than during pre-capsaicin testing, but also exhibited more guarding, licking and flinching. These behaviors occurred infrequently if at all prior to stimulation, suggesting that a latent pain state was uncovered by stimulation. Such differences point to differential affective consequences. Artificial intelligence offers an unprecedented opportunity to thoroughly quantify and dissect these non-reflexive but nonetheless stimulus-modulated behaviors. To facilitate collection and analysis of such data, we developed a user-friendly GUI written in Python that automatically stores video and metadata for all trials conducted on our device. Users can then import video into software of their choice for subsequent analysis. Our device and software bridge a crucial gap between pain researchers and modern advancements in behaviour analysis, enabling reproducible stimulation and thorough yet efficient analysis of multifaceted behaviors. This research was supported by the Canadian Institutes of Health Research and the Industry Partnerships and Commercialization Office at SickKids.

Disclosures: **C. Dedek:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent. **S.A.**

Prescott: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR544.17/CC4

Topic: D.02. Somatosensation – Pain

Support: Transpharmation Ltd., London, United Kingdom
Hertfordshire Local Enterprise Partnership's Single Local Growth Fund
European Union's European Regional Development Fund

Title: Oxaliplatin-induced neuropathic pain is associated with poor sleep quality and reduced EEG alpha power in the rat

Authors: M. T. LANIGAN[1,2], L. GIGGINS[2], L. A. LIONE[1], M. DUXON[2], *S. KANTOR[2];
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[2]Transpharmation Ltd., London, United Kingdom

Abstract: Platinum-based chemotherapeutic treatments of cancer such as oxaliplatin are important tools in an oncologist's arsenal. However, their use is limited because of serious side effects such as neurotoxicity that can lead to neuropathic pain, sleep disturbances, attention or memory deficits. A better understanding of the pathophysiological changes induced by chemotherapeutic treatments can help choosing treatments with less side effects, leading to better patient compliance. In this study, we monitored the changes induced by oxaliplatin (10mg/kg i.p) treatment on neuropathic pain, sleep-wake behaviour and electroencephalogram (EEG) power spectra in adult male Sprague Dawley rats after implanting the rats with intraperitoneal wireless transmitters (HD-S02, DSI USA) and with epidural EEG and electromyogram (EMG) electrodes. To assess the chemotherapy-induced changes in pain processing, we measured 15°C cold plate induced thermal hyperalgesia (paw withdrawal latency) in the rats before and 4 days after treatment. Starting from light onset, we also recorded EEG, EMG, locomotor activity and body temperature in the rats for 24 hours before oxaliplatin treatment (baseline) as well as 3- and 6-days post treatment. The recordings were semi-automatically scored as wake, rapid eye movement (REM) sleep or non-REM (NREM) sleep using SleepSign (Kissei Comtec, Matsumoto, Japan), and then visually inspected and corrected when appropriate by a trained analyst who was blind to the treatment conditions. Our results show that oxaliplatin decreased paw withdrawal latency to noxious cold stimuli, indicating neuropathic pain in the rats. We also found decreased EEG delta (1-4 Hz) power during NREM sleep that indicates reduced NREM sleep intensity after oxaliplatin treatment. The total number of wake bouts was increased, whilst their average length decreased, suggesting a fragmented sleep-wake behaviour in the oxaliplatin treated animals that resembles the reduced sleep quality seen in chemotherapy patients. Interestingly, oxaliplatin also reduced alpha (8-12 Hz) power in the rats during wakefulness.

Since early neuropathic pain development is often associated with a decrease in EEG alpha power, our data further support the notion of reduced alpha power being a marker of neuropathic pain. Thus, our results demonstrate that the sleep and EEG changes seen after oxaliplatin treatment are possible markers of neuropathic pain that can help to monitor the pathophysiological changes induced by chemotherapeutic treatments and guide therapeutic dose selections.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.18/CC5

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS107356-05

Title: Chronic unpredictable stress induces somatosensory hypersensitivity and prelimbic hypoactivity during acute pain

Authors: *L. MAILE[1], L. BAIG[1], S. DAVIDSON[2];
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Abstract: The experience of both acute and chronic pain can be intensified by comorbid anxiodepressive disorders, which are often mediated by chronic stress. The prelimbic medial prefrontal cortex (PL) shows structural and physiological plasticity in both chronic pain and chronic stress models. Additionally, modulation of PL in rodents affects both pain and anxiety-like behavior. PL is therefore a likely hub for the overlap of stress and pain. A subset of cells in the PL projects to the ventrolateral periaqueductal gray (vlPAG). This circuit has been identified as part of the descending pain modulatory system and has the capacity to modulate pain behavior. The purpose of this study was to determine how chronic unpredictable stress (CUS) affects pain processing, and to determine a potential role of the PL and its projections to vlPAG in stress-induced pain hypersensitivity. We implemented a two-week CUS model using pseudo-randomized stressors presented twice a day. Following CUS or control conditions, C57BL/6 mice underwent one of several pain models, followed by a battery of pain and affective behavior testing. We show that CUS reliably reduced weight gain, reduced time in the center of the open field test (OFT), and increased both mechanical and thermal sensitivity. In a model of chemotherapy-induced neuropathic pain, stressed mice showed a maintenance of thermal hypersensitivity and anxiety-like behavior across a two-week time period. To determine a candidate brain region that could be mediating this stress-induced enhancement of pain, we stained for cFos following CUS and subsequent exposure to intraperitoneally injected acetic acid, which induces acute visceral pain. Mice with a history of stress showed fewer cFos-positive

cells in the PL after an acute painful stimulus, indicating hypoactivity. To identify a specific population of PL neurons that may underly both the observed hypoactivity and stress-induced hypersensitivity, mice were injected with a retrograde AAV tagged with mCherry in the vlPAG. After two weeks of CUS or control conditions, we examined the PL-vlPAG cells using patch clamp electrophysiology. We found no differences in membrane properties in these cells between stress and control mice, indicating that PL neurons projecting to vlPAG are not intrinsically more or less excitable. Our findings indicate that chronic stress induces hypersensitivity to noxious stimuli in mice. PL likely plays a role in the overlap of pain and stress, but it is not yet determined whether PL-vlPAG is necessary or sufficient for the hypersensitivity observed in chronically stressed mice.

Disclosures: L. Maile: None. **L. Baig:** None. **S. Davidson:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.19/CC6

Topic: D.02. Somatosensation – Pain

Support: P30DA048742

Title: Impact of chronic alcohol consumption on tactile hypersensitivity in models of persistent pain

Authors: *R. E. SCHORN[1,2], M. S. RIEDL[2], L. S. STONE[3], A. M. LEE[2,4], L. VULCHANOVA[2];
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Abstract: Chronic pain and chronic alcohol consumption are two prevalent healthcare issues that can significantly impact the quality of life. The relationship between these conditions is important as alcohol is often used as a means of self-medication by individuals with chronic pain, and persistent pain is commonly reported by people with alcohol use disorder. Despite the high prevalence of chronic pain and alcohol use disorder, little is known about the neural mechanisms underlying this comorbidity. The interplay between chronic ethanol consumption and persistent pain was investigated using two different mouse models of persistent pain. We tested the hypothesis that chronic alcohol exposure increases the duration of recovery from mechanical hypersensitivity. Using the two-bottle free-choice chronic alcohol consumption paradigm, baseline mechanical withdrawal thresholds were reduced in alcohol-consuming mice around 4-5 weeks of drinking compared to age and sex-matched controls who consumed water (males n=7 per group). We used unilateral intradermal hind paw injection of 3 mg/ml capsaicin to determine whether chronic alcohol consumption affects the time course of mechanical hypersensitivity in a model of acute inflammatory pain. At 2 hours post-injection, withdrawal

thresholds significantly decreased in all capsaicin-treated mice compared to baselines. Alcohol-consuming mice who received capsaicin failed to fully recover to their baseline mechanical threshold over a 24-hour period, whereas the capsaicin-treated mice who consumed water significantly recovered to their pre-treatment thresholds at that 24-hour time point. In a separate cohort, we examined the effects of chronic alcohol consumption on the time course of nerve injury-induced hypersensitivity using the sciatic nerve crush injury. Similarly, alcohol-consuming mice exhibited slower recovery of mechanical withdrawal thresholds relative to their pre-injury levels compared to water-consuming mice. Mechanical hypersensitivity in water-consuming mice began to decrease 2 weeks post-injury and returned to their pre-surgical thresholds by the end of the experiment 5 weeks post-injury, while alcohol-consuming animals' recovery was both delayed and partial (males n=4/females n=3 per group). These results suggest chronic voluntary alcohol consumption prolongs the duration of recovery of tactile hypersensitivity in both acute inflammatory pain and nerve injury-induced hypersensitivity models. Ongoing neuroanatomical analysis is investigating differentially activated brain regions in our experimental groups.

Disclosures: R.E. Schorn: None. **M.S. Riedl:** None. **L.S. Stone:** None. **A.M. Lee:** None. **L. Vulchanova:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.20/CC7

Topic: D.02. Somatosensation – Pain

Support: NIH R01

Title: Investigating the role of dorsal column nuclei and nucleus tractus solitarius in colon nociception

Authors: *S. LEE[1], B. WILSON[2], E. A. LOEZA[1], M. S. GOLD[1], R. P. SEAL[1];
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Abstract: Visceral pain is one of the most prevalent types of pain and negatively impacts patients' quality of life. The underlying neural circuits of visceral pain differ in many ways from those for somatic pain and remain poorly understood. It is widely accepted that spinal projection neurons that ascend through the anterolateral tract carry somatic nociceptive information to brain areas such as the parabrachial and thalamic nuclei. Intriguingly, an increasing body of evidence suggests that visceral nociceptive information is sent to two distinct areas of the caudal brainstem - nucleus tractus solitarius (NTS) and dorsal column nuclei (DCN). However, the molecular identity of NTS and DCN brainstem neurons that respond to nociceptive visceral information is not well characterized. Furthermore, the functional importance of NTS and DCN for visceral pain processing has yet to be established. Utilizing knock-in mice for activity-dependent genetic

labeling (TRAP2), we identified neurons that were responsive to noxious (70mmHg) colorectal distension CRD. More gracile and cuneate nuclei neurons were active in mice that received noxious CRD compared to mice that received no colon stimulation (n=5, $p<0.001$). Surprisingly noxious CRD did not increase the number of active NTS neurons compared to no CRD condition (n=5). Using *in situ* hybridization, we next determined whether the labeled neurons were excitatory or inhibitory. Among gracile nuclei neurons that responded to noxious CRD, the majority were *VGLUT2* positive (73.80%) and only 13.54% of labeled neurons expressed *VGAT*. More cuneate nuclei neurons that responded to noxious CRD expressed *VGAT* (39.5%) while 45% of neurons expressed *VGLUT2*. Our preliminary data show that following dextran sulfate sodium induced colitis, there was no change in the number of active gracile and cuneate nuclei neurons in response to noxious CRD (n=3) but there was an increase in number of active cells in the no distension condition ($p<0.05$). Based on these findings, we hypothesize that inhibition of excitatory brainstem neurons will lead to a decrease in the VMR in response to noxious CRD. To test this we plan to inhibit the activity of *VGLUT2* positive brainstem neurons using the designer receptor exclusively activated by designer drug (DREADD) approach and measure changes in CRD induced visceromotor response, a reliable method to assess visceral sensitivity to mechanical stimuli in both naïve and DSS conditions. The data will shed light on the functional importance of these brainstem nuclei in colon nociceptive transmission.

Disclosures: **S. Lee:** None. **B. Wilson:** None. **E.A. Loeza:** None. **M.S. Gold:** None. **R.P. Seal:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR544.21/Web Only

Topic: D.02. Somatosensation – Pain

Support: Research partially funded by the project SIP 20230938 IPN.
Thanks to CONAHCYT for the postdoctoral fellowship, CVU 487025.

Title: Copulatory analgesia in male rats and assessment of spectral changes in blood plasma

Authors: *C. E. AGUILAR PÉREZ[1], R. DELGADO-MACUIL[1], J. L. ENCARNACIÓN SANCHEZ[2], A. GALVAN-ROSAS[3], O. GONZÁLEZ-FLORES[3];
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Abstract: Pain perception is a complex process involving the brain's interpretation and experience of pain signals. This response can vary among individuals and is influenced by various physical, emotional, and cognitive factors. Using the Vocalization Threshold to Tail Shock (VTTS) method has shown that copulatory activity increases the pain threshold, leading to

copulatory analgesia. However, the correlation between serum levels of certain hormones and neurotransmitters, such as β-endorphin and oxytocin, and the intensity of copulatory analgesia remains unknown. In this study, we evaluated the VTTS in rats during the first ejaculatory series (ES1; n=4) and second ejaculatory series (ES2; n=5), as well as their respective post-ejaculatory intervals (PEI1; n=3, PEI2; n=3), compared to a control group not exposed to receptive females (n=5). Blood plasma samples were obtained through decapitation, and the plasmas were evaluated using infrared vibrational spectroscopy in the mid-region. The results were processed using Principal Component Analysis (PCA) within specific bands (1500-1600 cm^{-1}, 1725-1745 cm^{-1}, and 2800-3000 cm^{-1}) associated to proteins, lipids, and CH bonds, respectively, which are present in the serum sample. The analysis revealed an increase in %VTTS (indicating analgesia) in ES2 (46.3%) compared to ES1 (26.9%) (p=0.016) and in PEI2 (57.9%) compared to PEI1 (3.9%) (p=0.20). The bands between 1500-1600 cm^{-1} and 2800-3000 cm^{-1} exhibited significant changes in absorbance compared to the control group during ES1, while the other groups showed lower absorbance. Conversely, the 1725-1745 cm^{-1} band demonstrated the opposite effect. PCA analysis demonstrated a clustering trend among the experimental groups in all analyzed regions, indicating modifications in endogenous pharmacological concentrations related to the behavioral context. Considering the unknown levels of endogenous pharmacological agents that influence copulation and analgesia, optical biosensors are proposed. Biosensors offer high detection sensitivity, making them ideal for detecting molecular changes in blood plasma and tissues. As a next step, changes concentration in oxytocin and β-endorphin will be measured in rats' blood plasma and central nervous system (CNS) tissues during and after two ejaculatory series, providing further insights into the relationship between copulation and analgesia.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.22/CC8

Topic: D.02. Somatosensation – Pain

Support: DNRF121

Title: Increased cortical responses to painful punishment during operant conditioning

Authors: *C. CERUTI[1], C. GRAVERSEN[2], E. NEDERGAARD SØHOLM[2], L. PETRINI[2], C. D. MORCH[3];
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Abstract: Background and aims: The development and maintenance of chronic pain is associated with operant learning theory. Operant learning uses reinforcement and punishment to voluntary modify behavior by associating a behavioral response to a following consequence.

Negative reinforcement increases the probability of a repeated correct behavior by decreased unpleasant consequences (such as pain), while positive punishment decreases the probability of an incorrect behavior by increased unpleasant consequences. Insights into the cortical responses associated with the consequences may provide new insights into why some patients develop chronic pain. Therefore, the aim of this study was to explore differences in the evoked brain potentials (ERPs) following the consequence feedback stimuli.
Methods:In this pilot study, five healthy volunteers experienced tonic pain using a cuff algometer. Participants scored perceived stimulation using a visual analogue scale (VAS) (0 = no sensation, 5 = pain threshold (PT), 10 = worst imaginable pain). The pressure was kept constant at PT during the cognitive task (listening and repeating hearing-in-noise-test sentences of 5 words). The speech repetition threshold was individually adjusted to approximately 50% to have an equal amount of correct and in-correct answers. The experiment consisted of two conditions: 1) negative reinforcement (NR) with pain relief VAS = 3 as reward (NRc) and unaltered pain VAS = 5 (NRic) for correct/in-correct responses, respectively; and 2) positive punishment (PP) with increased pain at VAS = 7 as punishment (PPic) and unaltered pain VAS = 5 (PPc) for in-correct/correct responses, respectively. The feedback indicating either a correct or in-correct response was delivered by two different auditory tones. Test participants were exposed to 120 trials with conditions randomized in blocks of 10 trials. EEG data was recorded from 64 surface electrodes referenced to ear lobes. Data from the Cz electrode was epoched from -500ms to +1000ms after feedback stimuli onset. ERP peaks were manually identified, and amplitudes and latencies for the main peaks were analyzed by RM-ANOVA with two factors, condition (PP and NR) and consequence (correct and in-correct).Results: The P1 (135±17 ms), N1(194±18 ms), P2(276±25 ms), N2(370±14 ms) and P3(426±27 ms) waves were identified. The amplitude of the P2-N2 for NRc (11.7±1.0μV) was statistically lower than for PPic (17.2±0.9μV; $F_{1,4}$ = 22.334, $p < 0.01$).Conclusions: The P2-N2 amplitude was lower when expecting a following reward as compared to punishment expectancy. This might indicate altered cognitive or affectional processing during the operant learning.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Program #/Poster #: PSTR544.23/CC9

Topic: D.02. Somatosensation – Pain

Support: R01NS112266
DE022912

Title: Spontaneous activation of nociceptors fragments sleeps by brief arousals after nerve injury in mice.

Authors: *V. A. DUARTE HOLANDA[1], A. SHARMA[1], W. HU[1], K. KOURBANOVA[1], T. SCAMMELL[2], C. WOOLF[3], C. ALEXANDRE[1], A. LATREMOLIERE[1];
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Abstract: The major complaints expressed by patients with neuropathic pain are spontaneous pain and poor sleep quality. Because painful stimuli trigger an awakening, we investigated if neuropathic pain causes an increase in arousal from sleep in mice. Adult male and female C57BL/6J mice (aged 2-6 months; 5-8 mice/group) and Na(v)1.8::Tet-tox mice (6 mice/group) were used in this study. All animals were instrumented with a 2-EEG/1-EMG configuration After 2 weeks of recovery, the EEG and EMG signals were recorded for baseline, then mice were subjected to peripheral nerve injury [Spared Nerve Injury - (SNI) injuring tibial and peroneal nerves, sparing sural nerve., or Chronic Constriction Injury - (CCI) ligatures around sciatic nerve induce gradual constriction injury], and recorded their sleep two to three weeks after nerve injury. EEG/EMG signals were semi-automatically scored in 4-s epochs, this preliminary scoring was visually inspected by blinded, trained experimenters (blinded to animal's treatment) and corrected when appropriate. For each group/condition, we calculated the percentage of time spent in wakefulness, rapid-eye-movement sleep (REMS), and non-REMS (NREMS), as well as the mean duration and number of behavioral state episodes. Both nerve injury models caused a significant non-rapid eye movement sleep (NREMS) fragmentation by increasing the number of brief arousals (wake episodes <16s) without affecting the total sleep or wake amount. Both male and female SNI mice developed NREMS fragmentation to a similar degree. To determine where the neural signal responsible for the brief arousals was generated, we crossed mice that express the Cre-recombinase under the Na(v)1.8 promoter (a sodium channel only expressed by small and medium diameter sensory neurons) with mice that express the light chain of the tetanus toxin in a Cre-dependent manner. The resulting animals (Na(v)1.8::Tet-tox mice) were protected against NREMS fragmentation after SNI. Blocking the activation of sensory fibers in the skin of SNI mice by local anesthetics (lidocaine 2%/QX314 0.5% and flagellin 0.9 µg/ QX314 2%) did not change the nerve injury-induced NREMS fragmentation. Together, these results indicate that the neural activity responsible for NREMS fragmentation after nerve injury is generated in sensory neurons (that include nociceptors) but do not require external stimuli. We propose that measuring the number of brief arousals after nerve injury can represent a surrogate measure for spontaneous pain in mice.

Disclosures: V.A. Duarte Holanda: None. **A. Sharma:** None. **W. Hu:** None. **K. Kourbanova:** None. **T. Scammell:** None. **C. Woolf:** None. **C. Alexandre:** None. **A. Latremoliere:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.24/CC10

Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS R01 NS109541 to A Nackley
R61/R33 NS123753 to A Nackley

Title: A novel mouse model of chronic primary pain conditions that integrates COMT genotype and environmental stress/injury

Authors: *Y. WANG[1], S. KIM[1,2], M. E. KLEIN[1], J. CHEN[1], E. GU[1], S. SMITH[1], A. BORTSOV[1], G. D. SLADE[3], X. ZHANG[1,4], A. G. NACKLEY[1,5];
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Abstract: A novel mouse model of chronic primary pain conditions that integrates COMT genotype and environmental stress/injury

Authors*Y. WANG[1], S. KIM[1,2], M.E.KLEIN[1], J. CHEN[1], E. GU[1], S. SMITH[1], A. BORTSOV[1], G. D. SLADE[3], X. ZHANG[1, 4], A. G. NACKLEY[1,5]; [1]Anesthesiol., Duke Univ., Durham, NC; [2]Anesthesiol. and Pain Med., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [3]Ctr. for Pain Res. and Innovation, Univ. of North Carolina, Chapel Hill, NC; [4]Anesthesiol. and Pain Med., The Affiliated Wuxi People's Hosp. of Nanjing Med. Univ., Wuxi, China; [5]Pharmacol. and Cancer Biol., Duke Univ. Sch. of Med., Durham, NC

Abstract

Chronic primary pain conditions (CPPCs) affect over 100 million people, predominantly women. Yet, they remain ineffectively treated due, in large part, to lack of valid animal models with translational relevance. Here, we characterized a novel mouse model of CPPCs that integrated clinically-relevant genetic (catechol-o-methyltransferase; COMT knockdown) and environmental (stress and minor injury) factors. Compared to wildtype mice, *COMT*+/- mice undergoing the repeated swim stress and molar extraction surgery intervention exhibited pronounced multi-site body pain and depressive-like behavior lasting more than 3 months. The *COMT*+/- mice undergoing the intervention also exhibited enhanced activity of primary afferent DRG nociceptors innervating hindpaw and back sites and increased plasma levels of norepinephrine and the pro-inflammatory cytokines IL-6 and IL-17A. Notably, the pain and depressive-like behavior was of greater magnitude and longer duration (lasting at least 12 months) in females compared to males. Further, increases in anxiety-like behavior and IL-6 levels were female-specific. The effect of COMT genotype x stress interactions on pain and IL-6 and IL-17A levels was evaluated in our CPPC study, demonstrating clinical relevance. Finally, we assessed the predictive validity of the CPPC model for analgesic screening and found that it successfully predicted the lack of efficacy of minocycline and the CB2 agonist GW842166X, which were shown to be effective in the spared nerve injury (SNI) and complete Freund's adjuvant (CFA) models, respectively, but failed clinical trials.

Meanwhile, pain in the CPPC model was alleviated by the beta-3 adrenergic antagonist SR59230A. Thus, our novel mouse model reliably recapitulates clinically- and biologically-relevant features of CPPCs and can be further implemented to test underlying mechanisms and discover new therapeutics.**Funding:** NIH/NINDS R01 NS109541 and R61/R33 NS123753 to A Nackley.

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Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.25/CC11

Topic: D.02. Somatosensation – Pain

Support: R01CA249939

Title: Metformin and other metabolic inhibitors attenuate neuropathic pain and tumor growth in mice with paraneoplastic syndrome and CIPN

Authors: *P. KUPPUSAMY[1,2], M. HAQUE[1,2], O. K. MELEMEDJIAN[2];
[1]Univ. of Maryalnd, Baltimore, MD; [2]Univ. of Maryland Dent. School, Dept. of Neural and Pain Sci., Univ. of Maryland Dent. Sch., Baltimore, MD

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) and paraneoplastic neurological syndrome are two conditions that can cause significant pain and discomfort in cancer patients. CIPN is a common side effect of certain chemotherapeutics and can result in numbness, tingling, and pain. Paraneoplastic neurological syndrome, on the other hand, is a rare disorder that occurs when cancer-fighting antibodies attack parts of the nervous system. Both neuropathies can persist which can adversely affect the quality of life and the rehabilitation of cancer patients. Unfortunately, therapies that can alleviate tumor or chemotherapy-induced neuropathic pain that do not interfere with tumor growth do not currently exist. The main goal of this study was to identify a therapeutic strategy that can achieve both anti-tumor and analgesic effects. The chemotherapeutic, bortezomib, has been shown to induce aerobic glycolysis in sensory neurons which lead to bortezomib-induce neuropathic pain. Aerobic glycolysis is also a hallmark of cancer cells, suggesting a common metabolic vulnerability. Paraneoplastic neuropathies are commonly associated with lung cancers. Hence, we used Lewis Lung Carcinoma cells (LLC1) to develop a mouse model of paraneoplastic neuropathy. We hypothesized that blocking metabolic pathways could alleviate CIPN and paraneoplastic neuropathic pain without compromising on tumor control. To test our hypothesis, we demonstrated that mice implanted with LLC1 developed significant allodynia. Treatment with bortezomib attenuated tumor growth but exacerbated the neuropathic pain. However, co-treatment with metformin, which blocks bortezomib-induced aerobic glycolysis in sensory neurons and prevents CIPN, attenuated both tumor growth and neuropathic pain. Similarly, inhibition of lactate dehydrogenase and pyruvate dehydrogenase kinase with oxamate and dichloroacetate respectively, also reduced tumor growth and pain. These results suggest that targeting metabolic pathways is a promising strategy to improve oncologic outcomes and alleviate neuropathic pain in cancer patients.

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Poster

PSTR544. Behavioral and Physiological Pain Models

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.26/CC12

Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS 1R61NS123758-01A1

Title: Development of a fibromyalgia analog model index to comparatively evaluate the face and construct validities of various rat models of fibromyalgia

Authors: *L. F. FERRARI, G. DONALDSON, A. WILKINSON, N. E. TAYLOR; Anesthesiol., Univ. of Utah, Salt Lake City, UT

Abstract: Millions of Americans suffer from fibromyalgia syndrome (FMS) and experience severe disability and diminished quality of life, making it a significant public health problem. The goal of this project was to develop and validate a multivariate regression index in rats that will mimic current clinical fibromyalgia indices. We hypothesize that this index will improve the evaluation of face and predictive validities of animal FMS models, and provide a defined method to compare them. We used the index to compare the established reserpine-injection model of FMS and an innovative model, the Dahl salt-sensitive (SS) rat. Male (n=30) and female (n=30) rats were used; FMS was induced in Sprague Dawley (SD) rats by injection of reserpine. Six behavioral endpoints were tested, in the same individual, for FMS traits: behavioral aspects of fatigue (24-h home cage distance traveled), muscle tenderness (bilateral evaluation of mechanical nociceptive threshold in the gastrocnemius muscle), widespread pain (hind paw and facial withdrawal thresholds), anxiety (zero maze test), and depression (forced swim test). The results were analyzed using regression modelling within a rigorous multivariate framework to define relationships in observable clinical phenotypes to develop the Fibromyalgia Analog Model (FAM) index. The data was used to maximize the internal validity of the measurements. Five statistical milestones were considered in evaluation of the FAM index including measures of model fit (Root Mean Squared Error of Approximation = 0.039), variance inclusion (R^2 = 0.564), reliability ($r_{jj} \geq 0.7$ for all 6 tests along with a factor score determinacy coefficient = 0.98), and robustness (Cohen's d = 2.4). We certified the external validity of the FAM index using two additional strains, SS (both sexes, n=30/group) and saline-treated SD rats (both sexes, n=30/group). The analogous model for the SS rats also converged (RMSEA = 0.109) while the one-factor FAM model did not converge for the saline-treated SD rats. This indicates that one factor does not adequately explain the covariation in saline-treated SD rats. This is according to our twofold prediction that the model would fit well in reserpine-treated but not in saline-treated rats. The hypothesis that reserpine treatment induces a central organizing mechanism for objectively different behavioral tasks (analogous to fibromyalgia) is therefore supported. Additionally, the FAM model also identified the SS rat as a promising FMS model. There is a

provisional suggestion that future work with this population could yield an even better fit and index with the FAM model.

Disclosures: L.F. Ferrari: None. **G. Donaldson:** None. **A. Wilkinson:** None. **N.E. Taylor:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR544.27/Web Only

Topic: D.02. Somatosensation – Pain

Support: DE026677
DE031477

Title: Elucidation of neuronal activity in a mouse model of TMJ injury by in vivo GCaMP imaging of trigeminal ganglion neurons

Authors: *H. SON[1], J. SHANNONHOUSE[1], R. GOMEZ[1], Y. ZHANG[1], M.-K. CHUNG[2], Y. KIM[1];
[1]Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [2]Neural & Pain Sci., Univ. of Maryland Dent. Sch., Baltimore, MD

Abstract: Temporomandibular disorders (TMD) affect 4% of Americans each year. TMD patients typically experience facial pain and discomfort or tenderness in the temporomandibular joint (TMJ). Unfortunately, existing treatments for TMD are not always efficacious in all patients, necessitating more advanced, mechanism-based therapies. The pain experienced in TMJ arthralgia results mainly from peripheral sensitization of joint afferents originating in the trigeminal ganglion (TG) neurons, suggesting that monitoring the activities of TG neurons could provide direct mechanistic information to identify novel targets for treating TMD. In this study, we have demonstrated the properties of TG neurons under living conditions, specifically in a TMD animal model, using in vivo intact TG GCaMP3 imaging. This imaging allows us to observe neuronal activity in its natural milieu and discover its function, connectivity, and response to stimuli and enables the simultaneous monitoring of up to 2,800 neurons, which can be classified into three groups - small, medium, and large - based on their cell body diameter. We observed a significant increase in spontaneously activated neurons and transiently activated small-diameter neurons in response to stimuli, including mechanical and chemical stimuli, in the TG of forced mouth open (FMO) mice. In addition, a specific neuropeptide level increased in FMO mice, and injection of this neuropeptide directly into the TMJ caused hypersensitivity in the V3 region. An inhibitor of the specific neuropeptide attenuated the FMO-induced sensitization of TG neurons and facial hypersensitivity. Our findings highlight the role of increased neuronal activity in the TG of TMD animal model, with particular emphasis on the function of a specific neuropeptide in the induction of hypersensitivity. This study exemplifies

the utility of in vivo GCaMP3 imaging to unravel the function of TG neurons in the TMD animal model, bringing us closer to understanding the pathophysiological processes underlying TMD and developing more targeted and effective treatments.

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Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.28/CC13

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 2R01DK099201-05-NIDDK

Title: Visceral analgesic effect of eluxadoline (viberzi®); a central action, but not peripheral

Authors: *J. SENGUPTA[1], B. MEDDA[2], M. TERASHVILI[2];
[2]Med., [1]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The current treatment options for irritable bowel syndrome (IBS) and bladder pain syndrome are ineffective and the pain management strategies for these sets of symptoms remain controversial. The risk of prolonged use of opioids is high including constipation, respiratory depression, tolerance, and importantly addiction. The bifunctional opioid ligands with mixed agonist/antagonist profiles at multiple types of opioid receptors possess therapeutic advantages over conventional opioids. In gastrointestinal (GI) tract, peripheral δ-opioid receptor (DOR) antagonism attenuates the development of constipation by μ-OR (MOR) agonists. Bifunctional opioid ligand Eluxadoline (ELX) normalizes the condition of diarrhea-predominant (d-IBS) patients by slowing the peristalsis (a MOR agonism effect) without causing constipation (a DOR antagonism effect). It is thought that peripherally acting ELX also alleviates pain associated with d-IBS. However, the exact underlying mechanism is not known. The objective of this study was to investigate the site of action of ELX in visceral pain signaling neuraxis of rats. In behavioral experiments, pain was measured by recording viscero-motor responses (VMRs), a '*pseudaffective response*', to painful distension of colon or bladder before and after systemic injection of ELX (10mg/Kg, i.v.). ELX produced analgesia to noxious distension of bladder or colon. The peripherally restricted MOR antagonist naloxone-methiodide (NLX-meth) failed to reverse the ELX-induced analgesia, while naloxone (NLX) which crosses blood-brain barrier reversed the analgesia. Electrophysiology recordings from the mechanosensitive afferent fibers in L6 and S1 dorsal roots innervating bladder and colon, respectively, ELX failed to inhibit the excitation of these afferent fibers to colon or bladder distension. In contrast, ELX inhibited responses of colon- and bladder-responsive lumbo-sacral (L6-S1) spinal dorsal horn neurons (SDHNs) in spinal (C1-C2) transected rats suggesting a direct effect of ELX on viscero-responsive SDHNs. The inhibition of SDHNs by ELX was reversed by NLX, but not by NLX-

meth. Overall, our results of visceral pain measurement and electrophysiology recordings from SDHNs indicate that ELX produces analgesia by inhibiting the functions of spinal neurons, not by attenuating mechanotransduction of sensory afferents fibers. In conclusion, ELX inhibits excitation of spinal neurons to produce analgesia.

Disclosures: **J. Sengupta:** None. **B. Medda:** None. **M. Terashvili:** None.

Poster

PSTR544. Behavioral and Physiological Pain Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR544.29/CC14

Topic: D.02. Somatosensation – Pain

Support: CIHR Project Grant

Title: Consistent features of healthy human nociceptive processing in the brainstem and spinal cord identified by means of fMRI

Authors: *M. UMRAW[1], H. ALGITAMI[2], S. HASSANPOUR[3], P. W. STROMAN[4];
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Abstract: Chronic pain affects approximately 20% of the population worldwide and has substantial socioeconomic impacts. Previous studies describe connections between regions of the brainstem (BS) and spinal cord (SC) that are involved in nociceptive processing and thus may be important in chronic pain conditions. However, much of this understanding relies heavily on behavioural studies or animal models. To understand the neural mechanisms underlying chronic pain, it is first necessary to characterize nociceptive processing in healthy humans. Therefore, this study aims to identify common patterns of neural signalling involved in nociceptive processing by means of fMRI in the BS and SC.
Previous fMRI studies in our lab provided fMRI data from the BS and SC of 55 healthy adult participants. During each fMRI run, participants experienced brief repeated heat stimuli to their right hand at a calibrated temperature, followed by periods without pain. The participants were told 1 minute in advance when to expect the noxious stimulus and were asked to rate their pain at the end of each run. Subsequent connectivity analyses were carried out for each participant, using 2-source structural equation modelling (SEM). The results demonstrated functional connectivity between all possible combinations of anatomical regions. Connectivity values were then analyzed to identify those with consistent strengths (significantly different than zero) or correlated with pain ratings across the group.
The results showed consistent connections in the BS and SC during both pain anticipation and pain experience ($p < 0.05$). While some of these connections are in line with behavioural studies and animal models, their relevance in human nociceptive processing is unclear. For example,

SEM results demonstrated a consistent connection between the nucleus raphe magnus (NRM) and the nucleus gigantocellularis (NGc), which have been commonly grouped together as the rostral ventromedial medulla (RVM). The RVM is known to consist of distinct regions with potentially different roles. Our results provide evidence that the NRM and NGc have distinct signalling pathways to other regions. Exploring these poorly understood regions provides insight into their functional organization within the nociceptive processing network. This contributes to the developing model of network connections involved in nociceptive processing in humans. Understanding the consistency and variability of these connections is important to provide a reference point for future studies investigating nociceptive processing in chronic pain conditions.

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Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.01/CC15

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01DC020097

Title: Predicting auditory midbrain responses to natural sounds with interpretable Gabor integrate and fire receptive field models

Authors: *J. BLAIN[1], M. A. ESCABI[1,2,3], I. H. STEVENSON[3,1,4];
[1]Biomed. Engin., [2]Electrical and Computer Engin., [3]Psychological Sci., [4]Statistics, Univ. of Connecticut, Storrs, CT

Abstract: Spectrotemporal receptive fields (STRFs) are widely used in auditory neuroscience to model the time-frequency sensitivity of auditory neurons. In many instances, STRFs are derived using unbiased synthetic stimulus ensembles, such as dynamic ripples or random chords, which can easily be estimated using spike-triggered averaging. When natural sounds are used, however, decorrelation and regularization techniques are needed to remove residual stimulus correlations that can distort the estimated STRFs. Furthermore, nonlinearities and non-stationarities (such as adaptation) make it difficult to predict neural responses to natural sounds.Here, we obtained neural recordings from the inferior colliculus of unanesthetized rabbits in response to both a sequence of natural sounds and dynamic moving ripple sounds (DMR). We developed a model-based approach for deriving auditory STRFs and for predicting single trial spike trains to either the DMR or the natural sounds. The model consists of a nine parameter Gabor STRF (gSTRF; Qiu et al. 2003), which accounts for the neuron's spectro-temporal integration of the stimulus and a four parameter nonlinear integrate-and-fire compartment which incorporates intrinsic noise, cell membrane integration, and nonlinear thresholding in order to generate simulated output spikes. We used Bayesian optimization to fit neural data and derive optimal model

parameters by maximizing the model's log-likelihood.To validate our spiking gSTRF model, we first compared the optimal gSTRFs to more common approaches such as regularized regression and a generalized linear model (GLM). We compared STRFs derived using both DMR and natural sounds for each of these estimators and subsequently compared the spike train predictions obtained from each model. We also carried out these comparisons with simulated data where the "ground truth" STRF and spiking activity was known a priori. For these simulations, we demonstrate that the gSTRF converges to the original simulation parameters and replicates the spiking activity from the original simulations down to millisecond precision. Furthermore, for real neural data the gSTRF allows us to quickly estimate physiologically interpretable parameters, such as the neuron's best frequency, delay, and best temporal and spectral modulation frequency directly from the optimized gSTRF parameters. Collectively, this new approach allows one to derive auditory STRFs and predict neural spiking activity to natural sounds using functionally interpretable basis functions. The small number of parameters make exploration of nonlinear and nonstationary effects due to natural sound statistics more feasible.

Disclosures: J. Blain: None. **M.A. Escabi:** None. **I.H. Stevenson:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.02/CC16

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant RO1DC020097

Title: The influence of spectrum and modulation cues on the neural representation of vocalizations in natural background sounds

Authors: *J. DION[1], M. A. ESCABI[2,3,1], I. H. STEVENSON[1,3,4];
[1]Psychological Sci., [2]Electrical and Computer Engin., [3]Biomed. Engin., [4]Statistics, Univ. of Connecticut, Storrs, CT

Abstract: Real-world listening poses significant challenges for humans and other animals when communicative sounds occur in competing background noise. These same acoustic scenarios are often the most challenging for the hearing impaired and artificial speech recognition systems. Although perceptual studies have shown that both the spectrum and modulation statistics of a background sound can influence the perception of a foreground target, how the brain separates sound mixtures and solves this computational problem is poorly understood. Here, we recorded neural activity from populations in the auditory midbrain in order to assess how the statistics of natural background sounds alter the neural representation of a foreground vocalization.Multi-unit population activity was obtained from the inferior colliculus of head-fixed unanesthetized rabbits listening to natural sound mixtures using linear 64-channel recording arrays (Neuronexus). Speech sentences or zebra finch song motifs were presented as foregrounds in the presence of

seven competing natural background sounds and perturbed variants at multiple signal-to-noise ratios. These backgrounds encompassed a wide range of modulation statistics and included speech babble, bird babble, running water, and construction noise. The backgrounds were delivered in the original unmodified (ORIG) or the perturbed phase randomized (PR) or spectrum equalized (SE) configurations. The PR perturbation preserves the original sound spectrum but distorts (whitens) the original sound modulations; whereas the SE perturbation distorts the spectrum (whitens) and preserves the original sound modulations.Using shuffled correlation methods, we separated the foreground- and background-driven neural response components for each of the sound mixtures and conditions (ORIG, PR and SE). Preliminary results show that the distance between the foreground-driven population activity with noise and without noise strongly depends on the background sound statistics. For some background sounds, the spectrum dominates and distorts the foreground sound encoding. While for other backgrounds, the modulation statistics more strongly interfere with the encoding of the foreground. Collectively, the findings demonstrate how both the spectrum and modulation statistics of natural backgrounds influence and interfere with the representation of vocalization foreground sounds suggesting that these are both critical features underlying masking of real-world natural sounds.

Disclosures: **J. Dion:** None. **M.A. Escabi:** None. **I.H. Stevenson:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.03/CC17

Topic: D.05. Auditory & Vestibular Systems

Support: DFG

Title: The bat cerebellum and its role in vocalisation and hearing

Authors: *S. HARIHARAN, L. L. JURY, J. C. HECHAVARRIA;
Inst. of Cell Biol. and Neurosci., Goethe-University Frankfurt, Frankfurt am Main, Germany

Abstract: Echolocating bats have been used as a relevant example in neuroscience to understand auditory processing and adaptive behaviour mechanisms. Though studies dealing with bat auditory networks are abundant, research outside of the auditory system of these animals is scarce. Here we present our findings from neurophysiological investigations of the bat cerebellum. Previous studies had shown that the cerebellum of a bat is a brain region implicated in sensorimotor integration, orientation, and auditory processing. We tested this idea in our experiments by investigating cerebellar responses to auditory stimuli across the cerebellar hemispheres through single-unit and field potential recordings in anaesthetized fruit-eating bats (species *Carollia perspicillata*). In addition, we measured neural activity in awake, head-fixed, vocalizing bats that emitted sounds at their own volition. Since characteristics of the mammalian

brain are preserved across species, we believe our study would help understand the role of the mammalian cerebellum for vocalization and hearing. Moreover, the results of this study could bring us a step closer to understanding cerebellar function and the neurodegenerative diseases that affect it.

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Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.04/CC18

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01DC018621
NSF IOS-1942480

Title: The impact of early acoustic environment on noise invariance in the avian auditory cortex

Authors: *S. M. MOSELEY, C. FEHRMAN, C. MELIZA;
Univ. of Virginia, Charlottesville, VA

Abstract: Vocal communication requires the ability to perceive a signal of interest in a background of similar acoustics (the “cocktail-party problem”). Many neurons in the auditory cortex exhibit noise invariance, responding selectively and consistently to vocalizations in the presence of background noise, but it is not known how this computation is implemented or how it develops. We investigated this question in zebra finches (*Taeniopygia guttata*), a colonial songbird with individually distinctive, learned vocalizations. Zebra finches need to be able to identify other individuals from their vocalizations amidst a noisy, complex acoustic background; furthermore, fledgling males must successfully isolate the song of their tutor from this background in order to learn how to copy it. We therefore hypothesize that early exposure to a complex acoustic environment is required for cortical circuits to learn how to solve the cocktail-party problem. We predict that birds reared in a complex acoustic environment will have more noise-invariant neurons and will be better at discriminating conspecific songs in noisy conditions, compared to birds reared in an acoustically simple environment. Behavioral noise invariance was measured using a two-alternative forced choice task in which birds were trained to discriminate between four conspecific songs and then tested for how well they could identify the songs in the presence of synthetic colony noise at varying signal-to-noise ratios. Neural noise invariance was measured using single-unit recordings from the major subdivisions of the avian auditory cortex. Neurometric curves were calculated using two complementary approaches based on a population decoder and single-unit spike-train distances. Our results show that neural responses in colony-reared birds are more consistent in the presence of loud (0 dB SNR) background noise, suggesting that early experience of a noisy environment shapes auditory

circuits to filter out cocktail-party noise. This points to a non-Hebbian form of neural plasticity that may be critical to vocal communication.

Disclosures: S.M. Moseley: None. **C. Fehrman:** None. **C. Meliza:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.05. Auditory & Vestibular Systems

Support: NSERC CGSM
CIHR FRN 148365

Title: Vocalization-related responses in marmoset area 32

Authors: *R. E. GILLILAND, K. D. JOHNSTON, S. EVERLING;
Physiol. and Pharmacol., Univ. of Western Ontario, London, ON, Canada

Abstract: The anterior cingulate cortex is widely known for its involvement in higher-order functions such as affect regulation and conflict processing. Brodmann area 32, a region of the anterior cingulate cortex, remains poorly understood. Recent functional connectivity studies have suggested the involvement of area 32 in an auditory-related circuit. Additionally, we have observed functional activation of area 32 for conspecific vocalizations in a recent fMRI study conducted on marmosets (*Callithrix jacchus*).
In this study, we sought to characterize the responses of marmoset area 32 neurons to a suite of auditory stimuli. The common marmoset has a well-characterized repertoire of calls, making it a valuable NHP model for systems-level investigations of vocal communication. We performed single-neuron recordings in area 32 of two adult marmosets (2 females, aged 27 and 38 months) using ultra-high density 384-channel Neuropixels probes. During the recordings, marmosets passively listened to a range of auditory stimuli including marmoset, human, cat, and bird vocalizations, a water drip sound, as well as control stimuli consisting of scrambled versions of each sound. We carried out a total of eight recording sessions, each with a duration of 45 minutes.
The recordings were first sorted automatically using Kilosort 2.0, then manually curated using Phy. We identified a total of 1380 well-isolated single units across all sessions. Out of these units, 753 (54.6%) exhibited significant auditory-related activity. The response profiles of these units varied, with some units responding selectively to one stimulus, while others responded to a range of stimuli. There were 536 (38.8%) units that responded to at least one type of marmoset call, with 22 (1.6%) units responding to all marmoset calls. Furthermore, 542 (39.3%) units responded to at least one non-marmoset stimulus. Across the population of recorded units, all tested unscrambled auditory stimuli were represented.
In summary, we found that neurons in marmoset area 32 are responsive to a wide range of

auditory stimuli, particularly conspecific vocalizations. These results support the presence of vocalization-related responses in marmoset area 32 and suggest that this area may play a significant role in vocal communication.Top of FormBottom of Form

Disclosures: R.E. Gilliland: None. **K.D. Johnston:** None. **S. Everling:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.06/CC20

Topic: D.05. Auditory & Vestibular Systems

Support: DFG 428645493

Title: Activity in supra-granular layers of the auditory cortex precedes social vocalization but not echolocation in bats

Authors: *J. HECHAVARRIA;
Neurobio. & Biosensors, Auditory Computations Lab., Goethe-University Frankfurt, Frankfurt am Main, Germany

Abstract: Acoustic communication is of paramount importance for many animal species. Yet, at present, we know surprisingly little about the neural networks that participate in vocalization in species that rely heavily on sounds for everyday natural behaviors. Here, we characterize field potentials, current source density and neural spiking patterns across layers of the auditory cortex in spontaneously vocalizing bats (species: *Carollia perspicillata*). The data shows previously un-reported differences between neural activity patterns associated to two distinct vocalization modes: social vocalization and echolocation. Before social vocalization, strong synaptic currents occur in supra-granular layers of the auditory cortex (layers I & II). Pre-vocal supra-granular currents are strongest when the bats are producing sequences of social calls, and weakest when they are producing single echolocation pulses. The strength of pre-vocal supra-granular currents correlates negatively with neural spiking, and can be used to predict the degree of isochronicity in social vocalization sequences. Taken together, the data suggest that the supragranular layers of the auditory cortex represent an important hub for social vocalization, but not echolocation, in bats. These findings may have broader implications for other species known for their extensive social vocalization abilities.

Disclosures: J. Hechavarria: None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.07/DD1

Topic: D.05. Auditory & Vestibular Systems

Support: 2021ZD0204100
E215102013

Title: A hub for call processing in auditory cortex of awake marmoset revealed by wide-field calcium imaging

Authors: *H. ZENG;
Lingang Lab., Shanghai, China

Abstract: Marmoset is becoming a powerful investigation tool for the studies in neuroscience. It will potentially be a bridge between rodents' field which owns a variety of analyzing tools and macaques' field which is capable of interrogating complicated cognitive functions unique to primates. Marmosets are highly vocal animals, and their calls convey rich social meanings. Their auditory cortex, in principle, shall co-evolve with their vocal system so that they could receive/perceive con-species vocalizations with high sensitivity and robustness, given the fact their habitat is in rain forest, where is full of all kinds of natural sounds, and a single call is very likely to be masked by or mixed with other sounds. A partial call chunk shall activate a unique circuitry for that specific call. With the aid of widefield calcium imaging technique, we have analyzed the global activity patterns of the auditory cortex of marmoset. We found there is a unique "call hub" area, which synergistically co-activated with all the other areas to encode the marmoset calls. Besides, the neural trajectories have demonstrated "a piece of" the call intrigues the activation pattern/trace of its original, instead of that from those phonemically similar sounds. These results provided us new evidence to further investigate how meaningful sequences, instead of random sequences of events, are stereotypically encoded in the animals'brain via the use of marmosets.

Disclosures: H. Zeng: None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: PSTR545.08/DD2

Topic: D.05. Auditory & Vestibular Systems

Support: Office of Naval Research Grant No. ONR N00014-17-1-2736

Title: Calcium imaging of auditory cortical subfields in an echolocating bat reveals hierarchical stages of echo processing

Authors: R. PAL[1], *M. SMOTHERMAN[2];
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Abstract: The auditory cortex of echolocating bats is organized like other mammals, except that it is biased towards the spectral and temporal parameters of their self-generated echoes. The primary auditory cortex (A1) extracts echo acoustic information to reconstruct the auditory scene and guide behavior. However, many questions remain about the cellular and circuit mechanisms used to encode complex sounds, including the location and potential roles of auditory subfields dedicated to echolocation or communication, the path of information flow from A1 to decision-making areas, and the precise nature of the information relayed to higher brain regions such as prefrontal cortex (PFC). While many electrophysiological details are known for bat A1, little is known about how the surrounding cortical areas contribute to the processing of complex sound features. To address this, we transfected the brains of echolocating free-tailed bats (*Tadarida brasiliensis*) with an adeno-associated viral vector (AAV) carrying the gene for a neuronal calcium indicator (GCaMP7s), and developed a protocol for optically imaging acoustically-evoked cortical neural activity in stationary bats. Bats first underwent surgery for injection of the AAV-GCaMP into cortical regions of interest (ROI), during which the tissue above the ROI was cleared down to the skull to create a 3-4 mm diameter window. The skull was sufficiently thin and transparent to allow for visualizing GCaMP fluorescence without a craniotomy. After 6 weeks, bats were lightly anesthetized, restrained and placed under a widefield mesoscope with a loudspeaker centered 10 cm in front of the animal. Stimuli included tone-pips, downward frequency-modulated (FM) sweeps, and communication calls. Results revealed rapid and selective activation of A1 and neighboring regions, with subsequent progressive activation of a restricted region of mPFC. The spatial and temporal patterns of activation varied with stimulus features and temporal patterns, revealing new details about ensemble coding of sounds in bat and mammalian auditory cortex.

Disclosures: R. Pal: None. **M. Smotherman:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.05. Auditory & Vestibular Systems

Support: R01DC018353 (NIDCD)
Nancy Lurie Marks Family Foundation
5R01DC000937-30

Title: Cellular and widefield responses to salient vocalizations across primary and higher order fields of auditory cortex

Authors: *Z. GHASEMAHMAD[1,2], M. E. THOMAS[3,2], C. G. SWEENEY[3,2], J. J. WENSTRUP[4], A. E. TAKESIAN[3,2];
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Abstract: Mice emit a repertoire of complex vocalizations during different behavioral contexts, including courtship and aggressive social interactions. Playback of these vocalizations can elicit specific, stereotyped behavioral responses in the listening mice. Auditory cortex is thought to provide information about the vocalization identity to the motor and emotion centers of the brain involved in shaping these behavioral reactions. However, the representation of these specific vocalizations within neuronal subpopulations across auditory cortical regions is not well understood. Using a transgenic mouse line that expresses the calcium indicator, GCaMP6s, in a subset of cortical pyramidal neurons (Thy1-GCaMP6s mice), we performed widefield imaging from the auditory cortex in awake head-fixed mice during playback of mouse vocalizations emitted in distinct affective contexts. Playback of a range of mouse vocalizations induced robust activity across the primary auditory cortex (A1), anterior auditory field (AAF), and multiple higher-order auditory cortical fields, identified by mapping best frequency spatial gradients. Our ongoing studies are using two-photon calcium imaging to examine the responses of L2/3 pyramidal neurons in A1 to vocal stimuli. We observe that these pyramidal neurons show diverse responses to vocalizations, with subsets of neurons that show a fast, transient activation, neurons that show a prolonged increase in activity and neurons that are suppressed. Moreover, subsets of these neurons show robust responses to a range of vocalizations, whereas others show reliable and selective responses to specific vocalizations. Our future experiments will further examine the responses to these vocal stimuli within various auditory cortical subregions and across several distinct excitatory and inhibitory cell types. Together, these studies will provide insight into the processing of salient vocalizations within specific circuits across auditory cortical fields and the possible role of these circuits in shaping sensory-driven behaviors.

Disclosures: Z. ghasemahmad: None. **M.E. Thomas:** None. **C.G. Sweeney:** None. **J.J. Wenstrup:** None. **A.E. Takesian:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

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Program #/Poster #: PSTR545.10/DD4

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC003180
NIH Grant DC005808

Title: Voice patches in the marmoset auditory cortex revealed by wide-field calcium imaging

Authors: *Y. ZHANG[1], X. SONG[1], Y. GUO[1], C. CHEN[1], X. WANG[2];
[1]Johns Hopkins Univ., BALTIMORE, MD; [2]Johns Hopkins Univ., baltimore, MD

Abstract: Species-specific vocalizations are behaviorally critical sounds. Recognizing vocalizations is important for the survival and social interactions of vocal animals. In humans, a voice patch system has been identified on the lateral superior temporal gurus (STG) that is selective to human voices. In non-human primates, both in macaques and marmosets, vocalization-selective regions are found on the rostral portion of the temporal lobe, which are outside of the auditory cortex, using functional magnetic resonance imaging (fMRI). It is yet unclear whether vocalizations are uniquely processed in the auditory cortex. Using wide-field calcium imaging, a technique with both high temporal and high spatial resolution, we discovered two voice patches in marmoset auditory cortex that prefer species-specific vocalizations over other vocalizations and sounds. One patch is located on the posterior A1 (primary auditory cortex), and the other one is located on the anterior non-core regions. These voice patches are hierarchically organized based on latency and selectivity analyses. In addition, call types and identity information are carried by population responses from the voice patches. Furthermore, we found that the voice patches are functionally connected. Overall, these results reveal the existence of voice patches in the auditory cortex of marmosets and support the notion that, for different primate species, similar cortical architectures are adapted for recognizing communication signals for both vocalizations and faces.

Disclosures: Y. Zhang: None. **X. Song:** None. **Y. Guo:** None. **C. Chen:** None. **X. Wang:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: PSTR545.11/DD5

Topic: D.05. Auditory & Vestibular Systems

Support: JSPS KAKENHI 23H02593

Title: Neural mechanisms underlying complemental individual and species song discrimination in the zebra finch higher auditory cortex

Authors: *Z. CHENG[1], Y. YAZAKI-SUGIYAMA[2];
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Abstract: Male zebra finches sing individually different songs. They identify individual birds by hearing their songs, while also recognize all of them as their own species songs. The zebra finch higher auditory cortex, caudomedial nidopallium (NCM) has been reported to be involved in song discriminating behavior (Canopoli et al., 2014; Tomaszycki and Blaine, 2014; Yu et al., 2023), and our previous work showed highly selective auditory responses to specific individual

songs in NCM neurons as well (Yanagihara and Yazaki-Sugiyama, 2016). However, neural mechanisms for discriminating individual songs while accommodating species identity within NCM have remained largely unknown. Here, we examined how NCM neurons identify individual songs by recording their auditory responsiveness extracellularly from freely behaving adult male zebra finches. We found two types, narrower and broader spike (NS and BS), neurons which were distinct in spike shapes and firing rates as previously reported. NS neurons responded to all the zebra finch song stimuli we presented (18 songs). In contrast, most BS neurons responded to only a small subset of song stimuli (5.64 ± 4.70), while NCM neurons as an ensemble recorded from one bird (51.33 ± 24.66 neurons/bird) responded to all song stimuli. BS neurons were further tested how they responded to specific songs. We found most BS neurons showed responses to specific syllables in a song and responded to those syllables presented individually. But in most neurons (37/45) the response strength to a specific syllable increased/decreased when that syllable was presented with preceding syllable(s). Taken all together, our results suggest each NCM neuron identifies a subset of individual songs by detecting specific syllable sequences, while recognizing species specific songs as a whole NCM neuronal network, balancing auditory information about individual differences and species identities.

Disclosures: Z. Cheng: None. **Y. Yazaki-Sugiyama:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

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Program #/Poster #: PSTR545.12/DD6

Topic: D.05. Auditory & Vestibular Systems

Support: NIH F32 MH123016
NIH R01 HD088411
NIH U19 NS107616
NIH R01 DC012557

Title: Neural circuitry underlying cortical control of a vocalization-guided maternal behavior.

Authors: *A. M. LEMESSURIER, A. A. AGHA, R. C. FROEMKE;
Neurosci. Inst., NYU Grossman Sch. of Med., New York, NY

Abstract: Perception of vocalizations is crucial for social behavior. A conserved example of this is mothers responding to distress calls from infants. In mice, experienced mothers (dams) find and retrieve isolated pups into the nest when pups emit ultrasonic vocalizations (USVs). Virgin females generally don’t retrieve pups until they gain experience, for example by co-housing with a dam and litter. The onset of retrieval behavior is correlated with heightened sensitivity to USVs in left auditory cortex (AC) (Marlin et al.2015, Schiavo et al. 2020). This plasticity may support learning via projections from cortex to early structures in the auditory pathway. The central

auditory pathway is highly interconnected, with dense “corticofugal” projections from auditory cortex to earlier structures that may support vocal perception by filtering incoming auditory input. To test whether projections from left AC are required for retrieval, we chemogenetically silenced activity in layer 5 during retrieval. In expert retrievers the fraction of pups retrieved was substantially reduced in the CNO condition relative to vehicle ($N=6$, $p<0.05$). Silencing only neurons projecting to inferior colliculus (corticocollicular) led to a similar decrease ($N=5$, $p<0.05$). However, silencing neurons projecting to striatum (corticostriatal) had no effect ($N=5$, $p>0.05$), suggesting that corticocollicular projections are particularly critical for linking perception to behavior. To test this we used 2-photon Ca^{2+} imaging in awake mice to compare encoding of USVs in corticostriatal and corticollicular neurons. Corticocollicular neurons in expert retrievers ($N=4$ mice) exhibited sustained increases in activity during USV playback compared to presentation of pure tones, while activity was equivalent during USV and pure tone presentation in corticostriatal neurons ($N=3$ mice). This was corroborated by in vivo patch recordings in optotagged projection neurons ($N=8$ corticocollicular and 5 corticostriatal neurons). The sustained activity we observed in corticollicular neurons may reflect increased excitability in a dedicated network of recurrently-linked cortical and subcortical areas. To examine whether this develops with experience, we tracked activity in corticocollicular neurons in an additional cohort of virgins ($N=4$) over several days before and during co-housing as performance improved. This revealed robust population responses to USVs on each day; however, response durations in individual neurons changed over days. Overall these results suggest that corticofugal projections are crucial for pup retrieval, and plasticity in these projections could drive learning.

Disclosures: **A.M. Lemessurier:** None. **A.A. Agha:** None. **R.C. Froemke:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

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Program #/Poster #: PSTR545.13/DD7

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD R01DC017141
Univ. of Pittsburgh

Title: How active listening modulates vocalization processing in the primary auditory cortex

Authors: *M. KAR[1,2,3], K. WILLIAMS[1], S. SADAGOPAN[1,2,3,4,5];
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Abstract: The modulatory effects of active listening on the activity of single neurons in the primary auditory cortex (A1) is a subject of ongoing debate. Reasons for this lack of consensus

include: 1) the lack of theoretical frameworks at single-neuron levels in the auditory system that can explain how activity modulation can lead to improved task performance, 2) the use of simple or non-relevant stimuli that might not be optimal to drive A1 activity, and 3) the treatment of all A1 layers as a single processing stage. Here, we leveraged our previously developed auditory categorization model and a unique vocalization (call) categorization task to investigate the role of active listening in reshaping individual neural responses and population codes in different cortical laminae while processing complex sounds in different task contexts. First, to verify the necessity of A1 activity for call processing, we trained guinea pigs (GPs) on a calls vs. noise discrimination task, which GPs learned to perform with high accuracy. Unilateral inactivation of A1 did not affect task performance (consistent with a previous study in humans), but bilateral A1 inactivation resulted in performance dropping to near-chance levels, suggesting that A1 activity is critical for call processing. Next, we extended an auditory categorization model developed in our lab to include modulation by active listening. The model generalizes over the variability in calls and categorizes them by detecting informative features in calls. Here, we explored power-law scaling of neural outputs as a possible neural mechanism that could underlie enhanced separation of auditory categories during task performance. Scaling A1 L4 outputs did not lead to consistent performance changes, but scaling A1 L2/3 feature-detector outputs led to systematic performance increases. To test these predictions, we recorded from A1 of GPs performing a call categorization task using chronically implanted Neuropixels probes, and compared neural activity in different A1 laminae across the passive and active conditions. Preliminary results reveal a lamina-specific effect, with the superficial and deepest layers of A1 showing the highest modulation during task performance. Consistent with the model, A1 L2/3 neurons retained their high feature selectivity across passive and active conditions, but showed increase output nonlinearities in active conditions. Together, our results demonstrate the necessity of A1 for call perception, and indicate that active listening modulates the activity of feature selective neurons in a manner that leads to better separation of call categories at a population level during active behaviors.

Disclosures: **M. Kar:** None. **K. Williams:** None. **S. Sadagopan:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH R00EY028964
T32 NINDS Award T32NS115656
DC-IDDRC Award P50HD105328

Title: High frequency ultrasonic vocalizations are disrupted in a mouse model of Angelman syndrome

Authors: *C. D. GUOYNES[1], G. PAVALKO[2], M. S. SIDOROV[3];
[1]Ctr. for Neurosci. Res., Children's Natl. Hosp., Washington, DC; [2]Univ. of Maryland Col. Park, College Park, MD; [3]Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC

Abstract: Angelman syndrome (AS) is a disorder that leads to a severe language deficit, seizures, sleep disturbances, and motor and cognitive impairments. Recent work has led to promising therapies that unsilence the paternal copy of UBE3A and restore its function in neurons. To test effectiveness of these therapies, it is important to have a mouse model that characterizes meaningful clinical features. Vocalizations are used in many social contexts in mice. Previous work in AS mice found increased vocalizations in pups and decreased vocalizations in adults. Here, we sought to assess vocalizations and characterize their spectral properties in two different behavioral paradigms—courtship and distress. To elicit vocalizations, we used a modified 3-chamber social preference test to compare courtship USVs in male WT and AS mice. In our second test, we examined undirected USVs using a brief distress test where mice were suspended by their tail for 60 seconds. After USVs were recorded, we used the program deepSqueak analyze the USVs. In both behavioral paradigms, male AS mice made fewer USVs compared to WT mice, and this effect was driven by a decrease in vocalizations in the higher frequency (55-120 kHz) We tested a separate cohort of both female and male WT and AS on the distress USV paradigm and found that differences in USV replicated across both females and males. To determine if we could predict genotypes based on results from our distress USV analysis, we used principal component analysis and *k*-means clustering. We could accurately detect genotypes based on phenotype with up to 80% accuracy based on three features of USVs. This work supports previous findings of differences in USV production between WT and AS mice and adds new insights into the vocal features that may be most affected. Adding USV recording and analysis to behavior tests that screen the effectiveness of treatments may improve sensitivity to detect group differences and/or changes in communication.

Disclosures: C.D. Guoynes: None. **G. Pavalko:** None. **M.S. Sidorov:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: PSTR545.15/DD9

Topic: D.05. Auditory & Vestibular Systems

Support: ARL W911NF-19-1-0503-P0000

Title: Multisensory activity in the auditory cortex of behaving macaques

Authors: H. CAI, *Y. COHEN;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Because our environment is inherently multisensory, it is reasonable to speculate that our brain has evolved to preferentially process such multisensory information. Indeed,

multisensory activity has been found throughout the entire auditory hierarchy: from the middle ear to the prefrontal cortex. However, despite the large literature on multisensory processing, we do not have a full picture of the relationship between multisensory behavior and neural activity, especially in primate models. Here, we recorded auditory-cortex neural activity at different spatiotemporal scales in order to evaluate its contributions to multisensory behavior. Specifically, we recorded EEG, LFP, and single-unit activity while a monkey performed an audiovisual detection task, in which an ecologically relevant 'coo' call embedded in a chorus was delivered with or without a corresponding 'coo' video. The signal to noise ratio was varied from -15 - 10dB with a step size of 5dB. We report how auditory neurons encode multisensory signals and how well we can decode stimulus and task parameters from individual neural signals (e.g., LFP or single units).

Disclosures: H. Cai: None. **Y. Cohen:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

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Fondation Art-Thérapie

Title: Newborns neural representation of instrumental and vocal music

Authors: *M. FILIPPA[1], S. LOUKAS[1], J. SA DE ALMEIDA[1], C. BORRADORI-TOLSA[2], F. BARCOS-MUNOZ[3], D. GRANDJEAN[4], D. VAN DE VILLE[5], P. S. HUPPI[2];
[1]Univ. of Geneva, Geneva, Switzerland; [2]Geneva Univ. Hosp., Geneva, Switzerland; [3]Univ. Hosp. of Geneva, Geneva, Switzerland; [4]Neurosci. of Emotion and Affective Dynamics Lab., Geneva, Switzerland; [5]École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: Music is ubiquitous, both in its instrumental or vocal forms. The ontogeny of instrument and voice neural representation has yet to be defined. To assess the origins of the ability to discriminate instrumental or vocal melodies, 45 newborns were scanned using functional magnetic resonance imaging while listening to a melody played by a musical instrument, a flute or sang by a female voice. To investigate the dynamic task-based effective connectivity, we employed a psychophysiological interaction of co-activation patterns (PPI-CAPs) analysis, using the auditory cortices as seed region, to investigate moment-to-moment changes in task-driven modulation of cortical activity during an fMRI task. Our findings revealed unique, condition-specific, dynamically occurring patterns of co-activation (PPI-CAPs). During

vocal condition, auditory cortex co-activated with sensorimotor and salience network, while during instrumental condition, it co-activated with visual and superior frontal cortex. In line with adult studies, the vocal condition was recognized as relevant stimulus (salience activation) in CAP1, it induced somatomotor network activations, evoking motor responses. In CAP2 a cognitively oriented network with temporal pole activation rTPG, while the instrumental condition activated (CAP3/4) visual imaginary and mind wandering networks. Common neural signatures for vocal and instrumental melodies were found in the precuneus and posterior cingulate gyrus, indicating a perceived musicality of the sound stimuli. Finally, this study adds knowledge on the dynamic brain connectivity underlying the newborns capability of early and specialized auditory processing, highlighting the relevance of dynamic approaches to study brain function in newborn populations.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

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Title: Neural representation of emotions conveyed by brief vocal bursts

Authors: *S. YAMAGISHI[1], T. MATSUDA[3], T. HORIKAWA[2];
[1]NTT Communication Sci. Labs., Atsugi-shi / Kanagawa, Japan; [2]NTT Communication Sci. Labs., Kanagawa, Japan; [3]Brain Sci. Inst., Tamagawa Univ., Tokyo, Japan

Abstract: Brief emotional vocalizations provide profound insights into one's mental state. A recent study by Cowen et al. (2018) collected behavioral ratings of emotional judgments for a wide range of vocal sounds, such as cries, sighs, and growls, demonstrating that people can reliably identify and distinguish up to two dozen distinct emotions from these vocal bursts. The study highlights the importance of diverse emotion categories (e.g., awe and relief) over affective dimensions (e.g., valence and arousal) in understanding how people recognize the meaning of brief vocalizations. However, despite the theoretical advancement supported by behavioral experiments, the neural mechanisms underlying vocal emotion recognition remains largely unexplored. Here, we used the rich experimental resources of the previous study and functional magnetic resonance imaging (fMRI) to explore the relationship between brain responses to various types of vocal busts and ratings of diverse emotions (30 emotion categories and 13

affective dimensions). First, we examined whether activity patterns within multiple brain areas covering the entire brain could be used to predict ratings of individual emotions. On average, ratings of most emotions were accurately predicted from fMRI activity in areas around the auditory cortex. In particular, the higher-order auditory cortex showed higher accuracy than the primary auditory cortex, suggesting that higher-order auditory information processing is involved in the recognition of emotion conveyed by vocal bursts. Next, we examined the effectiveness of emotion categories in explaining brain responses to vocal bursts by comparing the performance of encoding models based on emotion category ratings with those based on the affective dimension ratings or acoustic features (cochleagrams) of vocal sounds. This analysis confirmed that the model based on the emotion categories outperformed the model based on the affective dimensions, providing the neural basis for the significance of diverse emotion categories previously supported by behavioral experiments. In addition, the category model outperformed the acoustic-feature model in the higher-order auditory cortex, which may reflect the processing of high-level emotion-related feature extraction from sensory inputs. These results demonstrate the feasibility to capture neural representations associated with the processing of rich and nuanced emotions conveyed through brief vocal sounds. Furthermore, the present study provides a neuroscientific foundation for the theory that underscores the importance of diverse emotion categories to understand human vocal emotion recognition.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

Title: Using deep neural networks and intracerebral recordings to reveal encoding properties of human auditory cortex

Authors: *K. RUPP, T. ABEL;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The human auditory system can seamlessly parse auditory scenes into individual components and sort them into sound categories. Many studies have attempted to explain the underlying neural representations of sound categorization, focusing largely on human-defined stimulus features or matrix decomposition techniques, which both fall short in capturing the rich, complex stimulus transformations involved. Meanwhile, recent deep neural network (DNN) models have solved this problem with few constraints on the specific stimulus transformations that the models can use. Assuming the models have identified an optimal set of stimulus features that grow increasingly complex and abstract with increasing layer depth, we can view it as a data-driven feature extractor with representations ranging from low-level acoustics to abstract category-level descriptions. Guided by this framework, we built encoding models to predict

neural responses in auditory cortex using layer activations within a sound categorization DNN as input features, which we refer to as DNN-derived encoding models. Neural data was recorded via stereoelectroencephalography (sEEG) in 16 patient-participants while they listened to a set of 165 two-second clips of natural sounds from categories including speech, non-speech vocalizations, music, and environmental sounds. We were able to predict neural responses with state-of-the-art accuracy, with supratemporal plane (STP) channels modeled best by shallow DNN layers, and channels in superior temporal gyrus and superior temporal sulcus (STG/S) modeled best by deeper layers. DNN-derived encoding models consistently outperformed spectrotemporal receptive field models, suggesting more complex representations than simple spectrotemporal tuning throughout auditory cortex. Furthermore, the category encoding strength for human vocalizations (as determined by a separate analysis) correlated strongly with the best DNN layer across channels: voice category-selective channels were most closely associated with deep DNN layers. We then used the DNN-derived encoding models to estimate integration windows by identifying the shortest stimulus inputs that did not appreciably change the predicted neural responses; integration windows segregated anatomically, with windows of ~85-185 ms in STP and ~245-500 ms in STG/S. These results elucidate the functional properties in auditory cortex: STP encodes acoustic properties (albeit with higher complexity than spectrotemporal tuning) at short timescales, while STG/S integrates over longer timescales to encode higher order stimulus transformations more akin to voice category selectivity.

Disclosures: **K. Rupp:** None. **T. Abel:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.19/DD13

Topic: D.05. Auditory & Vestibular Systems

Support: NRF Grant 2021R1F1A1054810

Title: Audio-visual speech perception of Korean bisyllabic words: An event-related potential study

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Abstract: Adding visual information, such as “lip-reading”, enhances speech perception, as shown by psychophysiological studies that have observed the modulation of the N1/P2 complex by visual speech cues. However, previous research has primarily focused on auditory illusions like the McGurk effect or non-meaningful stimuli, overlooking real words.The aim of this study was to investigate how auditory and visual information are processed and integrated during the

perception of two-syllable Korean words using event-related potentials (ERPs). Four experimental conditions were utilized: AV (congruent), MM (incongruent), AO (audio only), and VO (video only), with adult participants.The analysis focused on two distinct time-locked epochs: the sound onset of the first syllable and the start of the video. Time windows (N1, P2, P350/P400, Late Negativity) were defined to examine ERP modulations and cognitive processes occurring after the initial N1-P2 complex, aiming to understand the integration of auditory and visual information at later stages of speech perception.Comparing the AV, MM, and AO conditions based on the sound of the first syllable (excluding VO due to the absence of sound triggers), no significant differences were found in the N1 component. However, both AV and MM conditions exhibited decreased P2 amplitudes compared to AO, with no significant differences between AV and MM. In the P350/P400 component, the MM condition showed decreased amplitudes in the frontal and central regions compared to AV and AO, but no significant differences were observed between AV and AO. No amplitude differences were detected in the parietal region. The Late Negativity component showed no significant differences among the three conditions.Analyzing the video-based epochs revealed similar differences among the AV, MM, and AO conditions as observed in the sound-based analysis. The VO condition displayed a flat component without significant amplitude changes, distinguishing it from the other three conditions.In summary, the findings indicate that visual speech cues influence the N1/P2 complex. However, no significant differences were observed in the N1 component across the experimental conditions. On the other hand, the P2 component was influenced by visual cues, but there were no differences based on congruency. The P350/P400 component demonstrated patterns consistent with previous research on mismatched multisensory integration, indicating no differences between AV and AO conditions. The MM condition exhibited a positive deflection, suggesting that the integration of mismatched information at the word level occurs at a later stage.

Disclosures: **J. Chung:** None. **Y. Lim:** None. **T. Chung:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

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Program #/Poster #: PSTR545.20/DD14

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant U01NS117765
NIH Grant R01DC012379
Shanghai Pujiang Program 22PJ1410500

Title: Dissecting neural computations of the human auditory pathway using deep neural networks for speech

Authors: ***Y. LI**[1], G. K. ANUMANCHIPALLI[2], A. MOHAMED[3], P. CHEN[1], L. H. CARNEY[4], J. LU[5], J. WU[5], E. F. CHANG[6];

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Abstract: The human auditory system extracts rich linguistic abstractions from the speech signal. Traditional approaches to understand this complex process have used classical linear feature encoding models, with limited success. Artificial neural networks have recently achieved remarkable speech recognition performance and offer potential alternative computational models of speech processing. We used the speech representations learned by state-of-the-art deep neural network (DNN) models to investigate neural coding across the ascending auditory pathway from the peripheral auditory nerve to auditory speech cortex. In particular, we trained different DNNs on large natural speech corpora of English and Mandarin. We also recorded neural responses from auditory cortex using electrocorticography (ECoG) in native English and native Mandarin speakers when they listened to the natural speech of the two languages. Using neural encoding models, we evaluated the similarity between the representations in the speech DNNs and in the auditory pathway of the human brain. We found that representations in hierarchical layers of the DNN correlated well to neural activity throughout the ascending auditory system. Unsupervised speech models achieve the optimal neural correlations among all models evaluated. Deeper DNN layers with context-dependent computations were better correlated with neural activity in high order auditory cortex, and the computations were aligned to phonemic and syllabic context structures in speech. Accordingly, DNN models trained on a specific language (English or Mandarin) predicted cortical responses in native speakers of each language. These results reveal convergence between representations learned in DNN models and the biological auditory pathway and provide new approaches to modeling neural coding in the auditory cortex.

Disclosures: Y. Li: None. **G.K. Anumanchipalli:** None. **A. Mohamed:** None. **P. Chen:** None. **L.H. Carney:** None. **J. Lu:** None. **J. Wu:** None. **E.F. Chang:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.21/DD15

Topic: D.05. Auditory & Vestibular Systems

Title: Replicating fast auditory intracranial responses using fMRI and large neural network models

Authors: *A. R. VAIDYA[1,2], L. S. HAMILTON[3,5], A. G. HUTH[2,4];
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Abstract: High spatial and temporal resolution intracranial recordings (iEEG, including sEEG and ECoG) have rapidly advanced our understanding of how human brains process speech at fine

timescales. However, these methods have limited anatomical coverage and opportunities to acquire data are rare. Non-invasive fMRI, in comparison, offers whole-brain coverage and is easier to obtain, but is considered unsuitable for many questions due to its low temporal resolution. Here we overcome this limitation using computational models and large fMRI datasets (LeBel et al. 2023) collected while subjects listened to up to 20 hours of natural speech. Our technique replicates two discoveries made using intracranial recordings: the presence of onset-selective speech areas in STG (Hamilton et al. 2018), and a hierarchy of temporal integration windows (Norman-Haignere et al. 2022). Both results rely on differentiating responses at the scale of ~100 ms, something previously thought impossible with fMRI. We fit voxelwise encoding models using activations from WavLM, an artificial neural network (ANN) trained to model speech sounds that captures many aspects of speech and provides unsurpassed brain prediction performance (Vaidya et al. 2022). WavLM features are computed at 100 Hz, but are then downsampled to 0.5 Hz and (effectively) convolved with a hemodynamic response function (HRF) before being linearly combined in the encoding model. We can thus simulate underlying neural responses by applying the same linear combination—excluding the downsampling and HRF—directly to the 100 Hz WavLM features. To map onset-selective speech areas we fit WavLM-based encoding models that predict "onset" and "sustained" ECoG response components from Hamilton et al. 2018. We then correlated the resulting model weights with those obtained from fMRI. Despite the short timescale of the onset response (~100 ms), we replicated the finding of onset-like responses in a small area in posterior superior temporal gyrus (pSTG). To map temporal integration windows, we directly replicated Norman-Haignere et al. 2022 in silico. In their temporal context invariance (TCI) paradigm, stimuli of various durations are presented in different contexts. A brain area's integration window is then the smallest stimulus duration that produces a context-invariant response. We used our WavLM-based models to simulate 100 Hz responses for each voxel under the same paradigm, and then applied the TCI procedure to estimate integration windows. Consistent with iEEG findings, this revealed a gradient of integration windows in primary auditory cortex ranging from <100 ms to 1 s, far finer than the raw temporal resolution of fMRI.

Disclosures: **A.R. Vaidya:** None. **L.S. Hamilton:** None. **A.G. Huth:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.22/DD16

Topic: D.05. Auditory & Vestibular Systems

Support: CDMRP HRRP W81XWH-20-1-0485

Title: Using chirped speech (Cheech) to uncover the neural, perceptual, and cognitive determinants of speech perception during a dynamic, spatial attention task.

Authors: *D. C. COMSTOCK[1], K. M. MANKEL[1], B. M. BORMANN[4], S. DAS[2], L. M. MILLER[3];
[1]Ctr. for Mind and Brain, [2]Psychology, [3]Univ. of California Davis, Davis, CA; [4]Neurosci. Grad. Group, Univ. Of California Davis Neurosci. Grad. Program, Pleasanton, CA

Abstract: Given the complexity of speech processing and the number of neural systems involved, adequately characterizing speech perception difficulties requires evaluation of neural, cognitive, and perceptual factors, in addition to basic hearing capabilities. To aid in this task, we use Cheech, continuous speech blended with synthetic frequency sweep chirps designed to elicit neural responses from brainstem to cortex simultaneously as measured with EEG (Backer et al, 2019). Cheech is a tool that can provide unique insight into how neural factors relate to auditory processing and cognition, and potentially uncover the source(s) of underlying speech perception difficulties. Here, we employed Cheech in a dynamic, multi-talker, spatial-attention-switching task to assess the neural contributions to speech processing. Cheech-modified short stories were played in silence or in the presence of a spatially separated competing talker to normal hearing, young adult listeners. Listening performance was assessed through several behavioral metrics, including narrative comprehension and embedded target word identification. Individual differences in cognitive factors (e.g., selective attention, inhibitory control, working memory) and perceptual factors (e.g., pitch discrimination, temporal fine structure, speech-in-noise perception) were also evaluated as potential moderators of speech listening performance. Cheech-evoked neural potentials originating from multiple points along the auditory pathway including the Auditory Brainstem Response (ABR), Middle Latency Response (MLR), Long Latency Response (LLR), as well as the linguistic component, N400, were extracted. Listening to a single talker resulted in more robust neural responses in lower levels of the auditory pathway (i.e., ABR, MLR) relative to dual talker conditions. Attending to the target talker also elicited enhanced neural responses at higher-level stages of the auditory pathway (i.e., LLR, N400) compared to distracter talker responses. We additionally found both listening performance and selective attention cognitive test results were reflected in neural encoding in both early and late cortical responses, while working memory test results are reflected only in late cortical responses. These results indicate that among normal hearing individuals, our Cheech paradigm is capable of revealing the neural encoding differences between good and poor listeners. Taken together, this Cheech-based paradigm provides a novel, speech-based neurodiagnostic that is sensitive enough to detect and characterize a broad range of speech perception difficulties within the auditory brain.

Disclosures: D.C. Comstock: None. **K.M. Mankel:** None. **B.M. Bormann:** None. **S. Das:** None. **L.M. Miller:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

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Program #/Poster #: PSTR545.23/DD17

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD F30 FP00027756

Title: Investigation of intracerebral auditory cortex electrophysiology and spectrotemporal feature processing in human auditory cortex using voice-like acoustic stimuli

Authors: *J. L. HECT[1], K. RUPP[3], E. E. HARFORD[2], L. L. HOLT[4], T. J. ABEL[3]; [1]Neurosurg., [2]Children's Hosp. of Pittsburgh, Pittsburgh, PA; [3]Univ. of Pittsburgh, Pittsburgh, PA; [4]Psychology and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Voice perception engages characteristic regions of auditory cortex. However, it remains unclear to what extent these regions rely on shared or unique mechanisms for processing voice and non-voice sounds and for assessing for sound patterns specific to voice. We used direct intracerebral recordings from seven patient-participants with epilepsy undergoing chronic monitoring to test the hypothesis that temporal voice areas (TVAs) rely on shared representations of voice and other natural sounds across human auditory cortex, including supratemporal plane (STP), superior temporal gyrus (STG) and superior temporal sulcus (STS). Participants listened to 1) voice and non-voice stimuli (Voice Localizer) and 2) synthetic sounds generated from modulated noise, called Gaussian Sound Patterns (GSPs). The GSPs stimuli mirror spectrotemporal features of natural sounds, while remaining perceptually distinct, in line with prior fMRI work. We used a convolutional neural network (CNN) to classify GSPs into sound categories and selected stimuli most and least likely to be classified as voice (250 total). We extracted broadband high-gamma activity (HGA; 70-150 Hz) and identified sound-responsive channels (two-sample t-test, FDR-corrected, $q < 0.01$). We tested decoding (80% train, 20% test) of stimulus category from HGA prior to cross-task decoding, used to examine similarities in the neuronal representation between voice and GSPs within sound-responsive channels. Decoding of voice from non-voice was significant for all patients (61-76%, $p < 0.001$). Decoding accuracy of GSPs was significantly above chance for two patients (63% and 68% accuracy, $p < 0.001$), and nonsignificant for two (46% and 55% accuracy). We found cross-task decoding did not perform above chance when GSPs (41-48%) or Voice Localizer (41-52%) were used as the training set. These preliminary data suggest TVAs may employ unique representations of voice, even when spectrotemporal properties are controlled between artificial and natural sound stimuli.

Disclosures: J.L. Hect: None. **K. Rupp:** None. **E.E. Harford:** None. **L.L. Holt:** None. **T.J. Abel:** None.

Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR545.24/DD18

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01-DC04290
NIH Grant UL1-RR024979

Title: Processing of spectrally degraded speech in the human cortex: An intracranial electrophysiology study

Authors: *K. V. NOURSKI[1], M. STEINSCHNEIDER[2], A. E. RHONE[1], J. I. BERGER[1], E. R. DAPPEN[1], H. KAWASAKI[1], M. A. HOWARD, III[1];
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Abstract: Electrical stimulation of the auditory nerve with a cochlear implant (CI) is the method of choice for treatment of severe-to-profound hearing loss. Auditory cortical function and plasticity are major contributing factors to the variability in speech perception outcomes. Spectrally degraded stimuli, presented to normal-hearing individuals, can serve as a model of cortical processing of speech by CI users. This study leveraged the superior spatio-temporal resolution of intracranial electroencephalography (iEEG) to study processing of spectrally degraded speech across multiple levels of the cortical hierarchy, test for hemispheric asymmetries, and determine the relationship of cortical activity to speech perception. Participants were adult neurosurgical epilepsy patients with normal hearing (N=14, 6 women). Stimuli were utterances /aba/ and /ada/, spectrally degraded using a noise vocoder (1-4 bands) and presented in a one-interval discrimination task. Cortical activity was recorded using depth and subdural iEEG electrodes (>1900 contacts). Recording sites were assigned to regions of interest, hierarchically organized into several groups: auditory core in posteromedial Heschl's gyrus (HGPM), superior temporal plane, superior temporal gyrus (STG), ventral and dorsal auditory-related, prefrontal, and sensorimotor cortex. Event-related band power was examined in broadband gamma (30-150 Hz) and alpha (8-14 Hz) bands. Stimuli yielded chance identification performance when degraded to 1-2 spectral bands. Performance was variable in the 3-4 band conditions and near-ceiling in the clear condition. Analysis of iEEG data revealed regional differences in cortical activation with respect to stimulus spectral complexity and intelligibility. HGPM was characterized by strong bihemispheric activation regardless of task performance. A progressive preference for clear speech emerged along both the ventral and the dorsal auditory processing pathways. Better task performance in the 3-4 band conditions was associated with gamma activation on the STG and alpha suppression along the dorsal pathway (supramarginal gyrus) in response to all vocoded stimuli. Within sensorimotor cortex, differences in task performance were paralleled by different patterns of stimulus-induced activity. Direct recordings reveal a hierarchical organization of degraded speech processing. Examination of responses to noise-vocoded speech provides insights into the neural bases of variability in speech perception in CI users. This work will aid in the development of novel objective measures of CI performance and of neuromodulation-based rehabilitation strategies.

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Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

Location: WCC Halls A-C

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Program #/Poster #: PSTR545.25/DD19

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD R01DC018805
NIDCD R00DC018051

Title: Temporal integration throughout human auditory cortex is predominantly yoked to absolute time and not the duration of speech structures

Authors: *S. NORMAN-HAIGNERE[1], M. KESHISHIAN[2], O. DEVINSKY[3], W. DOYLE[3], G. M. MCKHANN, II[4], C. SCHEVON[4], A. FLINKER[3], N. MESGARANI[2];
[1]Univ. of Rochester, Rochester, NY; [2]Columbia Univ., New York, NY; [3]NYU Langone Med. Ctr., New York, NY; [4]Columbia Univ. Med. Ctr., New York, NY

Abstract: The auditory system must integrate across many different temporal scales to derive meaning from complex natural sounds such as speech and music. A key challenge is that sound structures - such as phonemes, syllables, and words in speech - have highly variable durations. As a consequence, there is a fundamental difference between integrating across absolute time (e.g., a 100-millisecond window) vs. integrating across sound structure (e.g., a phoneme or word). Auditory models have typically assumed time-yoked integration, while cognitive models have often assumed structure-yoked integration, which implies that the integration time should scale with structure duration. Little empirical work has directly tested these important and divergent assumptions, in part due to the difficulty of measuring integration windows from nonlinear systems like the brain and the poor spatiotemporal resolution of noninvasive neuroimaging methods. To address this question, we measured neural integration windows for time-stretched and compressed speech (preserving pitch) using a novel method for estimating integration windows from nonlinear systems (the temporal context invariance paradigm) applied to spatiotemporally precise intracranial recordings from human neurosurgical patients. Stretching and compression rescale the duration of all sound structures and should thus scale the integration window if it is yoked to structure but not time. Across the auditory cortex, we observed significantly longer integration windows for stretched vs. compressed speech, demonstrating the existence of structure-yoked integration in the human auditory cortex. However, this effect was small relative to the difference in structure durations, even in non-primary regions of the superior temporal gyrus with long integration windows (>200 milliseconds) that have been implicated in speech-specific processing. These findings suggest that the human auditory cortex encodes sound structure using integration windows that are mainly yoked to absolute time and weakly yoked to structure duration, presenting a challenge for existing models that assume purely time-yoked or structure-yoked integration.

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Poster

PSTR545. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.05. Auditory & Vestibular Systems

Support: Institute of Information & Communications Technology Planning & Evaluation (IITP) grant (2017-0-00432)
KIST Institutional Program (2E31084)
National Research Council of Science & Technology (NST) (No. CAP21052- 200)

Title: Exploring the influence of topic familiarity and volatility of auditory scene on selective auditory attention

Authors: J. PARK[1], S.-C. BAEK[2], M.-W. SUH[3], J. CHOI[4], S. KIM[1], ***Y. LIM**[4,5]; [1]Electrical and Computer Engin., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Max Planck Inst. for Empirical Aesthetics, Frankfurt, Germany; [3]Otorhinolaryngology-Head and Neck Surgery, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [4]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [5]Dept. of HY-KIST Bio-convergence, Hanyang Univ., Seoul, Korea, Republic of

Abstract: This study investigates the impact of topic familiarity and volatile listening environments on selective auditory attention during dichotic listening, using electroencephalography (EEG). Previous research has demonstrated that auditory attention can influence the cortical representation of speech, particularly in challenging acoustic conditions. However, the role of top-down factors, such as topic familiarity, in this process is not well understood, despite evidence that semantic information can enhance speech perception in noisy environments. Additionally, the effects of dynamic and irregular changes in auditory scenes - volatile listening environments - have received less attention in previous studies. To address these gaps, we examined how topic familiarity and volatile listening affect selective auditory attention by analyzing EEG data. Results showed that when participants listened to stories with unfamiliar topics, their comprehension was significantly impaired. However, their cortical activity accurately tracked the speech of the target story, indicating that topic familiarity has minimal influence on the neural index of speech tracking, particularly when sufficient bottom-up information is available. On the other hand, in volatile listening environments where listeners had to constantly adjust to new speech whenever auditory scenes changed, the neural correlates of attended speech were diminished. Specifically, the cortical response to the attended speech and the spatial asymmetry of attention-related responses between the left and right hemispheres were significantly reduced around 100-200 ms after the onset of speech. These findings suggest that volatile listening environments can negatively impact the modulation effect of selective attention, potentially due to increased perceptual load and hindered attentional processes.

Disclosures: J. Park: None. **S. Baek:** None. **M. Suh:** None. **J. Choi:** None. **S. Kim:** None. **Y. Lim:** None.

Poster

PSTR546. Optic Diseases and Neuropathies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.01/DD21

Topic: D.06. Vision

Support: FFB Grant BR-CMM-0619-0767-DUKE

Title: Understanding the function of a novel photoreceptor-specific isoform of the retinal disease geneCrb1

Authors: *E. DEMBLA, J. VALDEZ-LOPEZ, C. KOZLOWSKI, D. SUL, J. KAY;
Duke Univ., Durham, NC

Abstract: Loss of CRB1 causes a variety of different pathologies, including ~10% of all cases of Leber congenital amaurosis. To devise treatments for CRB1 disease it is important to understand the normal function of this gene and how mutations cause pathology. However, the mechanism by which loss of CRB1 causes photoreceptor death remains unknown. Our lab recently discovered a new CRB1 transcript, denoted CRB1-B, which is the most abundant isoform in both mouse and human retina. Unlike the canonical isoform, CRB1-A, the new B isoform is selectively expressed by photoreceptors. We therefore hypothesized that CRB1-B, acting in photoreceptors, has a central role in disease pathology. To test this, we first established an improved mouse model of the human disease, as existing mouse alleles show minimal degeneration. We therefore generated a $Crb1^{null}$ mouse that lacks all CRB1 isoforms including CRB1-B. Unlike other Crb1mutants, this null mutant showed a significant loss of photoreceptor cells by 3.5 months, accompanied by behavioural defects in the Optomotry assay for visual acuity. By 6 and 9 months of age, mutants showed a range of anatomical phenotypes consistent with progressive cell loss. To test the role of CRB1-B in this degenerative process, we generated CRB1-B rescue mice in which a Crb1B-ires-GFP transgene was expressed selectively in rod photoreceptors. Crossing the rescue transgene into the null background substantially ameliorated photoreceptor loss, implicating the CRB1-B isoform in disease pathobiology. Finally, we investigated the pathological mechanism underlying photoreceptor death in $Crb1^{null}$ mutants. The CRB1-A isoform is expressed by Müller glia and transported to the outer limiting membrane (OLM). We found that this protein localizes to the inner segment region of the photoreceptor cell, where the OLM junctions with Müller cells are located. Therefore, we tested the hypothesis that CRB1 isoforms have a role in the formation or maintenance of OLM junctions. Using a new anatomical assay for OLM integrity, in retinal whole-mounts, we found striking holes in the null mutant OLM. These holes appeared prior to photoreceptor loss, became progressively worse over time, and were often occupied by microglia/macrophages, suggesting inflammatory processes. Strikingly, rod-specific expression of the CRB1-B rescue transgene reduced the number and size of OLM holes, suggesting a potential mechanism underlying enhanced photoreceptor survival in rescue mice. Altogether, our results establish key disease-related functions for the newly discovered CRB1-B isoform and suggest possible gene replacement strategies for treating the human disease.

Disclosures: **E. Dembla:** None. **J. Valdez-Lopez:** None. **C. Kozlowski:** None. **D. Sul:** None. **J. Kay:** None.

Poster

PSTR546. Optic Diseases and Neuropathies

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Program #/Poster #: PSTR546.02/DD22

Topic: D.06. Vision

Support: NIH Grant R15EY029866-01.

Title: The effects of tributyltin, an endocrine disrupting compound, on zebrafish retinal physiology

Authors: *J. S. JENSEN, V. P. CONNAUGHTON;
American Univ., Washington, DC

Abstract: Estrogen is a key hormone of retinal development. Disrupting the synthesis of estrogen can lead to the thinning of the cornea and retina, abnormal and delayed eye growth, and cell death. A brief chemical disruption during development has been shown to have long-term consequences. Tributyltin (TBT), an organotin compound and an environmentally relevant endocrine disrupting compound (EDC), is an estrogen synthesis inhibitor that prevents the synthesis of estrogen from testosterone. Unfortunately, not a lot is known about the long-term effects of atypical estrogen signaling due to transient developmental exposure to TBT. The purpose of this work was to examine if transient developmental exposure to TBT can disrupt retinal physiology in adults. Zebrafish (*Danio rerio*) aged 72 hours postfertilization (hpf) or 7 days (d) pf were exposed to either water, vehicle control (0.1% ethanol), low TBT (0.2 µM), or high TBT (2 µM) for 24-hours. After exposure, larvae were returned to control conditions and raised to adulthood (~1 year), at which point electroretinograms (ERG) were recorded. Mean ERG response component amplitudes and peak times were obtained from the different treatment groups (n=2-5). Our results show decreased ON-bipolar b-wave amplitude, OFF-bipolar d-wave amplitude, and photoreceptor a-wave amplitude in fish exposed to TBT at 72 hpf. Differences were not widely noted in adult fish exposed at 7 dpf, but there were differences when comparing them to adults exposed at 72 hpf. Fish exposed at 72 hpf had more delayed responses in comparison to those exposed at 7 dpf, and smaller amplitudes. These results indicate that a short, 24-hour exposure to TBT during development causes long-term effects on retinal physiology. The difference in response between exposure groups suggests that TBT exposure may disrupt the formation of synapses if introduced at 72 hpf. Though all cell layers are present at 72 hpf, the cell synapses may not be. Considering this possibility, the prevention of these connections would cause a weaker signal to be transmitted through the retina. The observed effects of TBT on retinal physiology could be due to TBT interacting with hormone systems. Estrogen plays a key role in visual system development and function and disrupting estrogenic expression can cause physiological deficits.

Disclosures: J.S. Jensen: None. **V.P. Connaughton:** None.

Poster

PSTR546. Optic Diseases and Neuropathies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.03/DD23

Topic: D.06. Vision

Support: MOST 108-2320-B-030-004
MOST 109-2314-B-281-002-MY3
110-CGH-FJU-03

Title: Long-term light expose upregulates expression of transcription factor AP-2 delta by activating IL1B and IL8 in retinal epithelial cells

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Abstract: Environmental factors, such as long-term light exposure, especially to the blue end of the light spectrum, are thought to damage the retina causing degeneration. Recently, a greater understanding of genetics has indicated that some genes may be potential biomarkers for retinal degenerative diseases. In this study, we investigated the expression of transcription factor AP-2 delta (TFAP-2D) in the retina and sought to determine the mechanism of retinal degeneration. We used a long-term light exposure animal model to study TFAP-2D expression in the retina. TFAP-2D expression was observed in the retinal pigmented epithelial (RPE) area in the retina of the light-exposed rats, but no TFAP-2D expression was detected in the retina of control (normal) rats. We also inserted two vectors overexpressing different TFAP-2D genes in retinal pigmented epithelial cells (ARPE-19 cells) and investigated the expression of angiogenesis molecules. We found that interleukin 1 beta (IL1B) and interleukin 8 (IL8) were upregulated in the TFAP-2D-overexpressed cells. This upregulation was attenuated by the proteinase-activated receptor 1 (PAR1) antagonist, vorapaxar. These studies demonstrate that expression of TFAP-2D is upregulated by long-term light exposure in the RPE area of the retina, and that IL1B and IL8 may be involved in this upregulation of TFAP-2D, which is suppressed by vorapaxar.

Disclosures: Y. Lee: None. **C. Chien:** None. **C. Huang:** None. **C. Ke:** None. **L. Tien:** None. **P. Lin:** None. **Y. Cheng:** None.

Poster

PSTR546. Optic Diseases and Neuropathies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.04/DD24

Topic: D.06. Vision

Support: MOST104-2325-B-010-005
CY10809
109J002

Title: The electrophysiology of a wireless subretinal retinal prosthesis

Authors: *P.-K. LIN[1], Y.-C. TSAI[3], C.-Y. WU[2];
[1]Sch. of Med., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; [2]Natl. Yang Ming Chiao Tung Univ., Hsinchu City, Taiwan; [3]A-Neuron Electronic Corp., Zhubei City, Taiwan

Abstract: Introduction: The photovoltaic-powered wireless subretinal prosthesis with a divisional power supply scheme (DPSS) was designed to restore vision for patients with retinitis pigmentosa (RP) or age-related macular degeneration (AMD). It achieves complete wireless functionality by utilizing 850nm infrared light for power supply. This technology offers the advantages of a simplified and fast surgery process. In our study, we characterized the surgical implantation technique and electrophysiology using retinal degenerated mice (*rd1*) and minipigs as experimental models before conducting human implantation trials.
Methods: Our prosthetic chip contains a centrally located 16x16 CMOS sensor array. We systematically investigated the chip to confirm the electrophysiological response of retinal ganglion cells (RGCs) from isolated *rd1* mouse retinas using patch clamp technologies. Subsequently, we implanted the chips subretinally in minipigs for a duration of 4-12 weeks. Fundus imaging, electroretinogram (ERG), and visual evoked potential (VEP) measurements were performed to monitor the positioning of the prosthesis, detect any adverse events in the retina, and assess the efficacy of the photovoltaic-powered subretinal prosthesis through electrophysiological tests.
Results: Through *ex vivo* experiments, we successfully recorded the response of RGCs to localized current biphasic stimulation of rd1 mouse retinas using patch clamp techniques, demonstrating the effectiveness of DPSS stimulation. After successful subretinal implantation, we confirmed through ophthalmoscopy that the retina reattached, adhered tightly to the electrodes, and remained stable without severe adverse events. Additionally, from 4 to 12 weeks after implantation, we successfully recorded the electrophysiological responses, including ERG and VEP, induced by the photovoltaic-powered subretinal prosthesis using 850nm infrared light for wireless power supply.
Conclusion: Successful retinal stimulation was achieved using an 850nm light source in the photovoltaic-powered wireless subretinal prosthesis, and stability was maintained after implantation. This study demonstrates the surgical safety, feasibility, and efficacy of the photovoltaic-powered subretinal prosthesis. It provides a foundation for potential future clinical tests aimed at restoring vision.

Disclosures: P. Lin: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Yang Ming

Chiao Tung University. **Y. Tsai:** A. Employment/Salary (full or part-time):; A-Neuron Electroni Corp. **C. Wu:** F. Consulting Fees (e.g., advisory boards); A-Neuron Electronic Corp..

Poster

PSTR546. Optic Diseases and Neuropathies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.05/DD25

Topic: D.06. Vision

Title: Sodium iodate induces the decrease of Vegfa expression in retinal pigment epithelium and choriocapillaris atrophy in mice

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Abstract: Sodium iodate causes a degeneration of the photoreceptor subsequently to the loss of the retinal pigment epithelium (RPE) and is used for the development of an animal model of age-related macular degeneration. We have shown that the treatment of ICR mice with sodium iodate resulted in an accumulation of photoreceptor debris in the subretinal space likely due to the failure in phagocytosis of RPE. The aim of this study is to investigate the details of pathological changes in the retina, RPE, and choroid of mice treated with sodium iodate. Female ICR mice at 8 weeks old were intravenously injected with 50 mg/kg sodium iodate and analyzed by electron microscopy, immunohistochemistry, and reverse transcription and quantitative PCR (RT-qPCR). Electron microscopy showed the loss of fenestrated choriocapillaris adjacent to the RPE 2 days after sodium iodate administration. We examined the expression of choriocapillaris-specific protein plasmalemma vesicle-associated protein (PLVAP) in cross-sections and choroidal flat-mounts and found the decreased expression of PLVAP in the choroid after sodium iodate administration. In contrast, immunostaining of retinal flat-mounts with CD31, an endothelial cell marker, did not show changes in retinal vessels of mice treated with sodium iodate. Immunostaining of flat-mounts with IBA1, a microglia/macrophage marker, indicated the activation of microglia/macrophage after sodium iodate administration. We examined the mRNA expression of several genes in the retina and RPE/choroid by RT-qPCR and found that the significant decrease of Vegfa expression in the RPE/choroid, but not in the retina, 1 day after sodium iodate administration. Taken together, our results showed that sodium iodate leads to loss of fenestrated choriocapillaris in the early stages of pathology, which may be partly due to a rapid decrease of the Vegfa expression in the RPE.

Disclosures: **T. Iwagawa:** A. Employment/Salary (full or part-time):; CHUGAI PHARMACEUTICAL CO.,LTD.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FUJIFILM Wako Pure Chemical Corporation. **K. Saita:** None. **H. Sagara:** None. **S. Watanabe:** A. Employment/Salary (full or part-time):; CHUGAI

PHARMACEUTICAL CO.,LTD.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FUJIFILM Wako Pure Chemical Corporation.

Poster

PSTR546. Optic Diseases and Neuropathies

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Topic: D.06. Vision

Support: NRF Grant 2017R1D1A1B05028221
Ministry of Health & Welfare and Ministry of Science and ICT (HI20C0206).

Title: Zinc protects against A2E-induced toxicity in ARPE19 cells: Possible involvement of lysosomal acidification

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Abstract: Accumulation of extracellular aggregates called drusen and degeneration of photoreceptor and retinal pigment epithelial (RPE) cells are hallmark features of dry age-related macular degeneration (AMD). It has been proposed that dysfunctional lysosomes in RPE cells contribute to dry AMD pathology by hindering the degradation of shed photoreceptor membranes. We have previously shown that raising intracellular zinc levels can restore lysosomal acidity and its degradative function (*Yoon et al. IOVS, 2010; Seo et al Neurobiol Aging, 2015*). In the present study, we examined the effects of zinc and cAMP on lysosomal alkalization and dysfunction in an *in vitro* and *in vivo* model of AMD. To induce lysosomal dysfunction in a human RPE cell line (ARPE-19), we used A2E. To examine the effect of raising intracellular zinc against A2E-induced changes, we used zinc ionophores (Zn-clioquinol). A2E accumulation in ARPE-19 cells was evaluated by measuring intracellular A2E using LCMSMS. Lysosomal pH was measured by using pHrodo™ Red-AM. In addition, A2E was injected into the subretinal space of adult mice to test the in vitro results. After A2E treatment, ARPE-19 cells exhibited A2E accumulation and decreases in pHrodo™ Red fluorescence, and subsequently underwent cell death. Zinc ionophores reduced A2E accumulation and restored lysosomal pH. In addition, zinc ionophores substantially reduced cell death induced by A2E. All the effects of zinc ionophores on A2E-induced changes were blocked by the addition of zinc chelator. In the A2E-injected eyes, a large amount of A2E was found accumulated in the photoreceptor layer and the RPE layer. Co-injcetion of ZnClioQ with A2E, however, significantly reduced both A2E accumulation. Our results support the possibility that zinc, especially in lysosomes, may help

overcome such lipofuscin-induced cytotoxic changes in the RPE, which may contribute to the pathogenesis of Dry-AMD.

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Poster

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Topic: D.06. Vision

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Title: Vsx2-egfp reporter dynamically monitors human retinal development in hipsc derived retinal organoids and reveals cell fate transition and gene networks

Authors: *X. ZHONG[1,2], D. ZHENG[2], Y. WANG[2], P. XU[2], G. GAO[2], M. XIANG[2];
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Abstract: Purpose: Mechanisms underlying human retinal development and diseases are largely unknown due to the limited source of human tissues. In this study, we aim to dynamically trace the development and transcriptomes of human retinal cells in retinal organoids (RO) using a VSX2 (a key transcriptional factor for retinal development) reporter (VSX2-eGFP) human induced pluripotent stem cell (hiPSC) line. **Methods:** ROs were generated from VSX2-eGFP hiPSCs using methods we reported previously. The lineage trajectory of human VSX2-eGFP+ cells were observed under an inverted fluorescence microscope during RO differentiation and maturation. Immunofluorescence staining was used to confirm the spatiotemporal expression of VSX2 and other retinal cell markers. VSX2-eGFP+ cells at distinct developmental stages were positively sorted by the fluorescence-activated cell sort. The dynamic transcriptomes of human VSX2-eGFP+ retinal cells were analyzed by RNA-seq analysis. **Results:** The VSX2-eGFP reporter labeled retinal progenitor cells (RPC) at early stages and bipolar cells at late stages, recapitulating endogenous VSX2 protein expression patterns. RNA-seq analysis of VSX2-eGFP+ cells at distinct developmental stages highlighted the switch from cell proliferation, specification, differentiation towards maturation with the increase of culture time, and indicated a transitional cell state of human VSX2+ retinal cells from multipotent RPCs to oligopotent

RPCs and finally bipolar cells. **Conclusions:** This study firstly provides a global database of human VSX2+ retinal cells, which will serve as a valuable reference for studying genetic regulation underlying human retinal and neural development, especially for the development of VSX2+ RPCs and bipolar cells.

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Poster

PSTR546. Optic Diseases and Neuropathies

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Program #/Poster #: PSTR546.08/DD28

Topic: D.06. Vision

Support: BRAIN Initiative, NIH/NINDS, NS110575
DoD/CDMRP, VR170089

Title: Structural changes in axon initial segments of ON-sustained alpha retinal ganglion cells in retinal degenerated mice.

Authors: *M. YUNZAB[1], F. NADAL-NICOLAS[2], P. WERGINZ[3], B. R. HUBER[4], S. F. STASHEFF[5], S. FRIED[6];
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Abstract: The axon initial segment (AIS) is a portion of the proximal axon that underlies action potential initiation and back propagation. The AIS structural properties, such as length and distance from the soma, are known to be tailored to optimize the input/output properties of individual neurons and can be altered in response to changes in network excitability. The level of spontaneous spiking in retinal ganglion cells (RGCs) increases at the onset of retinal degeneration and then reduces over time. While it is well established that alterations in synaptic input underlie the spiking increases, the issue of whether such changes result in alterations to the AIS has not been well studied. Here, we examined AIS properties in a single type of RGC, the ON-sustained alpha cells, known to exhibit significant increases in spontaneous spiking levels during degeneration and compared results in the *rd10* mouse model of retinal degeneration to those from age-matched wild type (*wt)* animals. We used specific strains of both mouse models that express GFP in subpopulations of RGCs (GFP-*Thy*1-EGFP-M). The AIS was identified by immunolabeling with a pan-sodium channel (pan-Na_v) antibody, a widely used marker for the AIS, in GFP-expressing RGCs; followed by confocal microscopy and morphometric analysis of fluorescent AIS markers. The ON-sustained alpha RGCs were identified using previously

described morphological features based on soma and dendritic field sizes and the stratification location of the terminal dendrites relative to ON- and OFF- ChAT bands. Our results showed that both the length of the AIS as well as its distance from soma were significantly reduced in *rd10* RGCs (compared to *wt*). While further testing is needed, our results suggest that AIS properties are indeed altered in at least some RGC types. Based on previous studies outside the retina, the changes observed here may help to reduce excitability and thus, may compensate for the network hyperactivity arising following photoreceptor degeneration.

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Poster

PSTR546. Optic Diseases and Neuropathies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.09/EE1

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

Support: NRF-2020R1A2C1102368

Title: Retinal neurodegeneration in intraocular pressure fluctuation rat model

Authors: *J. HAN[1], C. PARK[2], K. JUNG[2];
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Abstract: Glaucoma develops even in normal range of intraocular pressure (IOP) and can progress when IOP is within in normal range with treatment, even though IOP is the most important risk factor for glaucoma. The role of IOP fluctuation is controversial in development and progression of glaucoma in clinical trials. We investigated effects of intraocular pressure fluctuation on retinal neurodegeneration and their mechanisms in rats. Male Sprague-Dawley rats were treated with IOP-lowering eyedrops (brinzolamide and latanoprost) or saline for 8 weeks. IOP-lowering eyedrops were put intermittently on Monday and Thursday for the irregular instillation group or daily for the regular instillation group, and saline was put daily for the control group. IOP was measured daily under general anesthesia using a rebound tonometer. Mean IOP was lower both in the irregular instillation group (9.6±0.4 mmHg) and the regular instillation group (9.5±0.4) than the control group (10.1±0.4 mmHg, $P<0.001$). Standard deviation (SD) of IOP was higher in the irregular instillation group (0.8±0.1 mmHg) than the regular instillation group (0.5±0.1 mmHg) or the control group (0.6±0.3 mmHg, $P<0.001$). Number of retinal ganglion cells (RGCs) stained with anti-Brn3a antibody was lower in the irregular instillation group than other two groups ($P=0.002$). Expression of cleaved caspase-3 was greater in the irregular instillation group than other two groups ($P<0.001$). Expression of glial fibrillary acidic protein in the optic nerve head was increased by irregular instillation of

IOP-lowering eyedrops than the control group, but not in the regular instillation of eyedrops (P=0.001). Expression of anti-ionized calcium binding adaptor molecule (Iba-1), a marker for microglia and P2Y12 known as a chemotactic receptor for microglia were greater in the irregular instillation group than regular instillation group (P=0.031, 0.018). Oxidative stress measured by dihydroethidium, oxidative DNA damage exhibited by 8-hydroxy-2'-deoxyguanosine, and immunostaining for nitrotyrosine were upregulated (All P <0.001). In conclusion, IOP fluctuation induced by treatment of irregular instillation of IOP-lowering eyedrops reduced the number of RGCs. This study suggested that gliosis and oxidative damage could be related to retinal neurodegeneration caused by IOP fluctuation.

Disclosures: **J. Han:** None. **C. Park:** None. **K. Jung:** None.

Poster

PSTR546. Optic Diseases and Neuropathies

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.10/EE2

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

Support: NEI Grant RO1EY032542

Title: Discovering the role of long-noncoding RNAs in retinal ganglion cell loss upon optic nerve injury

Authors: ***N. C. MATHEW**[1], L. KONSTANTIN[2], A. C. AYUPE[2], G. NASCIMENTO-DOS-SANTOS[1], K. PARK[2];
[1]Neurosci., Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Neurolog. Surgery, Univ. of Miami, Miami, FL

Abstract: Long noncoding RNAs (lncRNAs) are an exciting frontier in the realm of gene regulation. Many researchers are identifying the mechanisms through which lncRNAs are capable of regulating gene expression. With the advancements in high-throughput RNA sequencing, lncRNAs are a target of interest in different disease and injury models. Injury to the optic nerve induces different transcriptomic profiles between the subsets of retinal ganglion cells (RGCs), including lncRNA expression. One lncRNA which we named optic nerve injury induced lncRNA-1(Onil1) is highly upregulated in RGCs post insult to the optic nerve both in optic nerve crush (ONC) and retinal ischemia models. Prevention of Onil1 expression provides neuroprotection of RGCs after ONC. We verify Onil1 upregulation in glaucoma (closed angled), and upregulation of Onil1 expression in multiple subtypes of RGCs after injury in the ONC model. We examine neuroprotection of RGCs in these models with silencing of Onil1 expression through intravitreal injection of a small-hairpin RNA (shRNA) and quantify RGC survival through immuno-histochemistry analysis. To examine the mechanisms by which Onil1 operates, we identified the binding partners of Onil1 through chromatin isolation through RNA purification (CHIRP) mass spectrometry and CHRIP-sequencing. To help identify which genes

are regulated by Onil1, we perform RNA sequencing of RGCs whose expression of Onil1 has been manipulated through intravitreal injection of AAV2 shRNA targeting Onil1. The goal of this project is to verify Onil1 expression in multiple injury and disease models to show its prevalence in RGC loss. Through better understanding of the mechanism behind Onil1 and other lncRNA functions, a potential therapeutic target may be uncovered for RGC loss prevention due to optic nerve injury.

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Poster

PSTR546. Optic Diseases and Neuropathies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.11/EE3

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

Title: A Novel Experimental Glaucoma Model for Uncovering Neurodegenerative Pathways in Chronic Ocular Hypertension

Authors: ***Q. WANG**, S. RAO;
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Abstract: Elevated intraocular pressure (IOP) is a major risk factor in the progression of glaucoma, a debilitating condition that leads to irreversible blindness in millions of individuals. To advance our understanding of the pathophysiological mechanisms driving glaucoma progression and facilitate the development of effective treatments, it is crucial to establish reliable in vivo experimental models capable of inducing chronic, controllable IOP elevation. In this study, we have successfully developed an experimental glaucoma model in mice using injectable viscoelastic polymeric microbeads designed to achieve chronic IOP elevation. The functionality and reliability of this in vivo approach depend on the engineering of the polymer and surface chemistry modifications of the microbeads, which effectively obstruct the trabecular meshwork (TM) outflow pathway, ensuring long-term IOP elevation. Our initial findings demonstrate that a single intracameral injection of these "viscobeads" can effectively induce chronic IOP elevation at desired levels and durations. Furthermore, we have observed that viscobeads-mediated ocular hypertension leads to retinal ganglion cell (RGC) apoptosis and alters electrophysiological responses to optical stimulation. These observations have been extended to controlled ocular hypertension conditions, encompassing various levels and durations of IOP elevation, through refined approaches in viscobead synthesis. Unlike disruptive techniques that impair the TM or microbeads composed of rigid materials, this viscobeads-mediated experimental glaucoma model offers several advantages, including: (1) efficient and chronic IOP elevation with a single injection, (2) tunable control over the level and duration of IOP elevation through microbead surface modification, and (3) compatibility with in vivo electrophysiology measurements and imaging systems. The surgical procedure is

straightforward, and the technique can be easily adapted for investigations in other animal species, such as nonhuman primates (NHPs), which closely resemble human glaucoma. By providing an accessible and versatile in vivo platform, this technique facilitates the study of neurodegenerative mechanisms underlying glaucomatous progression and holds promise for future applications in translational research.

Disclosures: **Q. Wang:** None. **S. Rao:** None.

Poster

PSTR546. Optic Diseases and Neuropathies

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.12/EE4

Topic: D.06. Vision

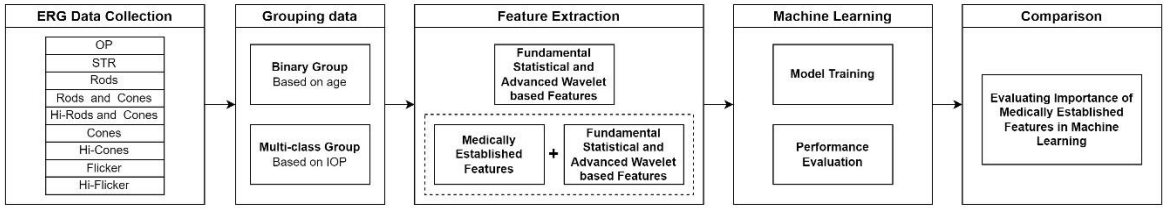
Support: NIH Grant EY031248

Title: Improving the detection of retinal neurodegeneration in glaucoma: The role of medically relevant features extracted from electroretinograms in machine learning models

Authors: M. K. GAJENDRAN, ***P. KOULEN**;
Ophthalmology, Univ. of Missouri - Kansas City, Kansas City, MO

Abstract: In this study, we performed a comparative analysis of machine learning models for early-stage glaucoma detection. The comparison examines models using engineered and extracted statistical and wavelet-based features against models that also incorporate medically relevant features from electroretinograms (ERGs). These ERG features include A-wave amplitude, B-wave amplitude, A/B ratio, and the implicit times of the A and B waves.
The dataset included ERG signals from eyes of DBA/2 mice (15 four-months old and 15 eleven-months old male DBA/2 mice). The DBA/2 mouse model of pigmentary glaucomatous optic neuropathy resulted from spontaneous mutations and is characterized by chronic retinal neurodegeneration that increases in severity with age. These signals were subsequently grouped based on age and intraocular pressure. From these signals, we extracted medical features, we engineered and extracted statistical and wavelet-based features which were then utilized to train various machine learning models. Their performance was evaluated based on their capacity to classify the ERG signals.
The comparison of the model performances indicated that models integrating medically relevant ERG features significantly improved in their performance. Specifically, one machine learning methodology (ensemble bagged method) displayed superior effectiveness in classifying ERG signals in both binary and multiclass setups when trained with the enhanced feature set.
In conclusion, our study demonstrates that the inclusion of specific medically relevant ERG features into machine learning models greatly enhances their performance in early-stage glaucoma detection. This novel approach presents significant potential for identifying functional

deficits across varying stages of glaucoma in mouse models of glaucoma and potentially also in human patients.



Disclosures: **M.K. Gajendran:** None. **P. Koulen:** None.

Poster

PSTR546. Optic Diseases and Neuropathies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR546.13/EE5

Topic: D.06. Vision

Support: Retina France

Title: Modulating Glycogen Synthase Kinase 3 to preserve photoreceptors from retinal degeneration

Authors: ***J. E. ROGER**[1], C. HOTTIN[1], J. AGOSTINONE[2], R. RODUIT[2], M. PERRON[1]; [1]CNRS, Saclay, France; [2]Univ. de Lausanne, Lausanne, Switzerland

Abstract: Glycogen Synthase Kinase 3 (GSK3) is a master regulator of cell signaling processes from development to degeneration of the central nervous system. For decades GSK3 has been a target of interest for the treatment of brain disorders such as Alzheimer's disease. In the retina, recent works showed that GSK3 inhibition with pharmacological compounds preserves photoreceptors from degeneration, although their clinical use might show some limitations. In this context, the goal of this study is to decipher the neuroprotective mechanism of GSK3 inhibition to identify new therapeutic candidates to delay retinal degeneration. To this aim, we took advantage of a conditional mouse line allowing retinal-specific deletion of GSK3 (*Gsk3α* and/or *Gsk3β)* in retinal progenitors. During photoreceptor degeneration, we observed that GSK3 is significantly inactivated in three degeneration models, including *rd10* mice. After induction of photoreceptor death (*ex vivo* and *in vivo*) in our *Gsk3α*$^{f+}$*β*^{ff} *α-Cre* mice, we observed a significant reduction in apoptotic cells compared to the controls, suggesting that GSK3 inhibition has a significant neuroprotective role. To understand the mechanism underlying this survival, we first performed transcriptomic analysis on whole retinas. We identified 350 deregulated genes (DEGs), including those involved in inflammation and cell chemotaxis with several secreted. Among upregulated secreted factors, some are known to confer neuroprotection (*Fgf2, Gdnf, Pgf, Spp1, Vgf⋯)*. Proteomic analysis identified 79 deregulated proteins (DEPs). We focused on

upregulated DEPs with unchanged or downregulated transcripts. The rationale is that GSK3 is known to regulate the stability of its targets through phosphorylation leading to degradation. Such analysis led to the identification of 76 putative direct candidates of GSK3 (*Dlg3, Grin2b, Mapk6, Papola, Prune2⋯)*. One of them, ERK3 (*Mapk6*), was identified with putative GSK3 phosphorylation sites. MNU injection in ERK3 KD mice led to an increase in photoreceptor cell death suggesting its role in neuroprotection. These findings provide potential therapeutic candidates for retinal diseases, and ongoing functional validation is being conducted to assess their neuroprotective effects.

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Poster

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Program #/Poster #: PSTR546.14/EE6

Topic: C.10. Brain Injury and Trauma

Support: T32 EY013360
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Research to Prevent Blindness
Alcon Research Institute

Title: Homeostatic Ca2+ levels in retinal ganglion cells are differential at baseline and predict survival in neurodegeneration in-vivo

Authors: *S. MCCRACKEN[1], M. J. FITZPATRICK[2], Z. WANG[1], A. HALL[1], D. KERSCHENSTEINER[2], J. MORGAN[2], P. R. WILLIAMS[2];
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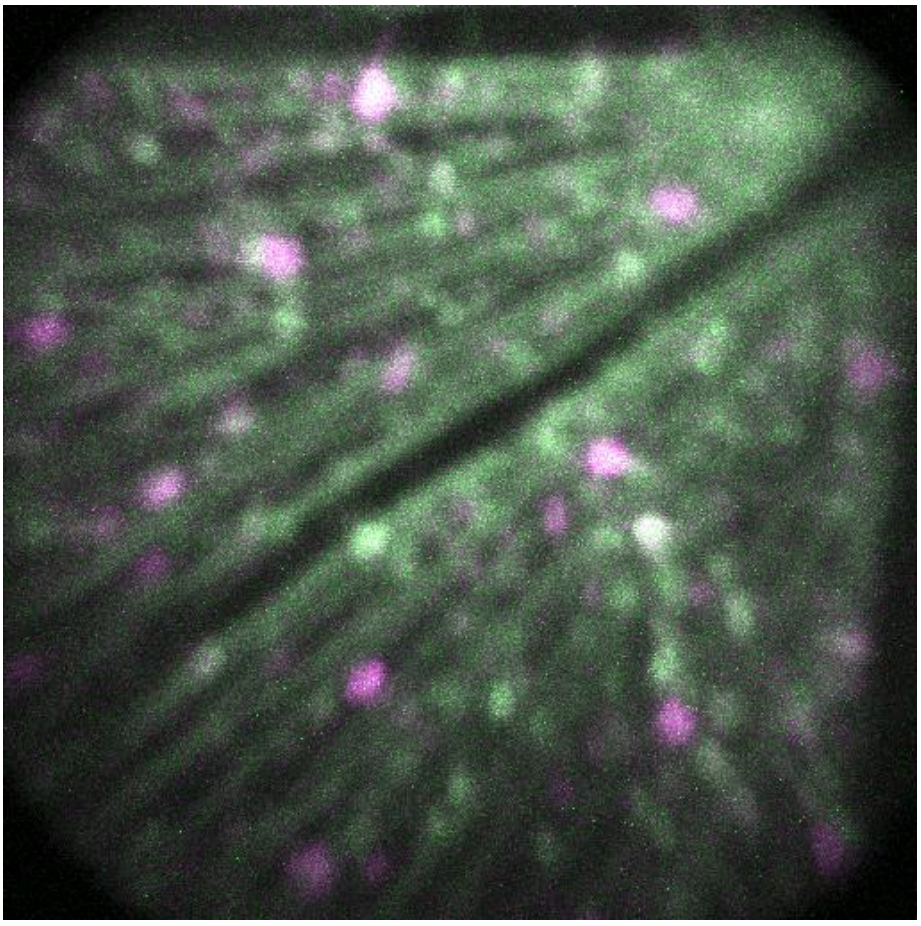
Abstract: Retinal Ganglion cells (RGCs) are the sole projection neurons from the retina to the brain. RGCs are susceptible to injury and disease and show a partial degeneration in disease and injury models. We recently discovered that cytoplasmic Ca^{2+} set-points are differential across RGCs within individual mouse retinas and hypothesize that this baseline Ca^{2+} differential corresponds with differential survival to robust injury. We visualized Ca^{2+} levels in mouse RGCs at single-cell resolution with two-photon microscopy. The ratiometric Ca^{2+} sensor, Twitch-2b, was expressed in RGCs using an intersectional approach consisting of VGlut2-Cre transgenic

mice and a Cre-dependent AAV2 expression vector. Baseline Ca^{2+} was measured in RGC somas, and optic nerve crush was then performed. RGCs were tracked longitudinally for two weeks to identify the relationship between Ca^{2+} and survivability. We evaluated both acute and chronic Ca^{2+} responses and found that high Ca^{2+} levels predicted survival, and that disturbing these levels by reducing calcium reduced RGC survival. We also identified a relationship between RGC subtypes and Ca^{2+} levels. Using cell-type specific targeting strategies, we found that well-surviving RGC subtypes have higher baseline Ca^{2+} levels than those that are more susceptible to injury. However, even within these well-surviving RGC subtypes, we still observed that higher Ca^{2+} levels were predictive of survival. Together, our results identify a novel heterogeneity in RGC Ca^{2+} levels that determine their survival after injury, implicating that homeostatic Ca^{2+} levels generally may be an important target or effector in treatments for neurodegenerative disease.

Figure 1: *In-vivo,* two-photon image of homeostatic Ca^{2+} levels in RGCs of a single mouse retina. A max projection of a z-stack is shown, with the YFP channel pseudo colored in pink and the CFP channel pseudo colored in green (YFP/CFP=R, R~Cytoplasmic Ca^{2+} levels). Heterogenous Ca^{2+} levels are observed across the RGC population.



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Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR547.01/EE7

Topic: D.06. Vision

Title: Neuromodulatory receptor co-expression patterns suggest that neurons act as state signal integrators

Authors: *A. M. BRIGANDE, A. A. DISNEY;
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Abstract: Neuromodulators are often studied in isolation, but neuromodulatory systems interact, and their joint action likely contributes to specifying brain state. Neurons respond to modulatory signals via receptors on the cell membrane, so a neuron's responsiveness to a given neuromodulator depends on its receptor expression. Not all neurons express neuromodulatory receptors, and receptor expression varies across brain regions and cell types. If brain states arise – at least in part – from the interaction of modulatory systems, then multiple signals must be integrated within local circuits to enable brain state specification. We hypothesize that individual neurons act as state signal integrators, based on which receptors they express. Using publicly available single-cell transcriptomics datasets from mouse and human cortex, and focusing in the first instance on G protein-coupled receptors, we explored patterns of receptor co-expression for dopamine, norepinephrine, acetylcholine, histamine, and serotonin. We found evidence for highly selective expression of receptors, suggesting integration of particular neuromodulators by identifiable transcriptomic cell types. Some neurons are narrowly responsive, expressing receptors for a single neuromodulator, sometimes through only one receptor subtype. Other neurons appear to selectively integrate specific modulatory signals through particular receptor types. Still other neurons are broadly responsive, expressing a wide range of receptors and integrating across all modulatory signals. At the highest level, these observations hold for both mouse and human cortex, but important differences do exist, raising the question of the extent to which species similarity exists at the receptor and transcriptomic cell type level, versus the algorithmic level.

Disclosures: A.M. Brigande: None. **A.A. Disney:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Program #/Poster #: PSTR547.02/EE8

Topic: D.06. Vision

Support: NIH Grant 1U01MH114824-01
NIH Grant 1RF1MH128778-01

Title: Automated reconstruction of local and long-range morphology of genetically defined neurons

Authors: *O. GLIKO[1], M. MALLORY[1], R. DALLEY[1], R. GALA[1], J. GORNET[2], Y. WANG[1], L. ALFILER[1], H. ZENG[1], S. SORENSEN[1], U. SÜMBÜL[1];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Computation and Neural Systems, Caltech, Pasadena, CA

Abstract: Correlating local and long-range morphology with gene expression and physiological function is key for understanding the brain organization and cell type diversity. The Patch-seq method can profile gene expression, electrophysiology and local morphology at the single cell level. Fluorescence micro-optical sectioning tomography (fMOST) can complement Patch-seq by sparsely labeling genetically defined populations of neurons using a combination of transgenic and viral tools and characterizing full neuronal morphology, including local morphology and long-range axonal projections. However, anatomical characterization remains a major throughput bottleneck for both methods, even with state-of-the-art semi-manual tools. Here, we develop an automated neuron reconstruction pipeline for light microscopy image data that produces digital representations of neuronal morphologies in swc format. We use manual traces to train deep convolutional network models to produce initial segmentation of neuronal arbors as well as secondary models for postprocessing including axon/dendrite labeling and correcting topological mistakes. We use this pipeline to reconstruct a large set of inhibitory neurons from brightfield image stacks in Patch-seq experiments and search for gene subsets that can predict the variation of laminar innervation within molecularly defined subclasses and types. In particular, we identify genes whose expression level can predict the amount of Layer 1 innervation in a transcriptomically defined subpopulation of Martinotti cells in the mouse visual cortex. In addition, we scale up the pipeline to enable reconstruction of full neuronal morphologies from the whole mouse brain imaged with fMOST microscopy. We find that, for sparsely stained brains, automated reconstruction followed by proofreading enables a 5-fold decrease in processing time as compared to manual reconstruction.

Disclosures: O. Gliko: None. **M. Mallory:** None. **R. Dalley:** None. **R. Gala:** None. **J. Gornet:** None. **Y. Wang:** None. **L. Alfiler:** None. **H. Zeng:** None. **S. Sorensen:** None. **U. Sümbül:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Program #/Poster #: PSTR547.03/EE9

Topic: D.06. Vision

Support: NIH Grant RF1MH125932

Title: Electron microscopy registration with patchseq data: a case study aligning vip expressing bipolar cells in the mouse visual cortex

Authors: *S. RATH[1,3], C. SCHNEIDER-MIZELL[3], C. GAMLIN[3], E. M. JOYCE[3], M. MALLORY[3], A. BODOR[3], L. ELABBADY[4,3], N. GOUWENS[3], B. LEE[2], T. JARSKY[3], C. REID[3], S. H. SEUNG[5], S. SORENSEN[3], F. COLLMAN[3], N. M. DA COSTA[3];
[1]Neural Coding, [2]Allen Inst., Seattle, WA; [3]Allen Inst. For Brain Sci., Seattle, WA; [4]Univ. of Washington, Seattle, WA; [5]Princeton Univ., Princeton, NJ

Abstract: The synaptic structure of neural circuits provides constraints on the nature of the computations in the brain, while gene expression provides constraints on how the structure is built and operates. Integrating these two fundamental properties of neuronal cell types is far from trivial and we still lack a basic understanding of something as important as the relationship between gene expression and synaptic structure of neuronal circuits. The recent release of large scale electron microscopy (EM)(1) and PatchSeq(2) data provides a timely opportunity to understand this relationship(3). While EM provides detailed information on the morphology and connectivity from single cells, PatchSeq includes morphology , electrophysiology , and RNA expression data collected together from single cells. Therefore, morphology can be used as a linking modality across datasets. However, due to technique-dependent systematic differences in their feature sets, the morphologies, although describing the same features, appear to exist in two separate feature-spaces, limiting the applicability of data-fusion techniques for coregistration. Here, we explore several feature alignment and data fusion techniques to bring the EM and PatchSeq morphological features into a common feature-space and evaluate the relevance of the models in situating EM cells with similar cells from Patch-seq. As a test for this alignment we use the well known Vasoactive Intestinal peptide (VIP) expressing bipolar cells in the mouse visual cortex as benchmark cells. We show that the distinctiveness of the morphology of this cell class as well as its specificity of connections towards other inhibitory cells makes it a robust test group in co-registration across modalities and therefore an ideal test case for feature alignment. We use the prevalent Seurat Canonical Correlation Analysis (CCA) protocol from transcriptomics analysis as a benchmark model to evaluate our protocol. We find that applying Kolgomorov-Smirnov (KS) metric minimization feature by feature, yielded the best results, matching the bipolar cells from EM data with VIP expressing cells measured from Patch-seq data. Connectivity data from the bipolar cells shows that they are inhibitory targeting neurons as expected.We believe that our feature-by-feature KS-metric minimization will help bring similar data from separate experimental modalities together and will find applicability beyond neural cell morphological coregistration. (1) bioRxiv, 2021.07.28.454025 (2021); (2) Cell 183, 935-953.e19 (2020); (3) bioRxiv,2023.03.22.533857 (2023)

Disclosures: S. Rath: None. **C. Schneider-Mizell:** None. **C. Gamlin:** None. **E.M. Joyce:** None. **M. Mallory:** None. **A. Bodor:** None. **L. Elabbady:** None. **N. Gouwens:** None. **B. Lee:** None. **T. Jarsky:** None. **C. Reid:** None. **S.H. Seung:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Zetta AI. **S. Sorensen:** None. **F. Collman:** None. **N.M. Da Costa:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR547.04/EE10

Topic: D.06. Vision

Title: Optogenetic control of inhibitory neurons in macaque visual cortex

Authors: *S. KHAN[1], A. ANDREI[2], M. SLAPIK[1], V. DRAGOI[2];
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Abstract: The brain's complexity arises from a finely tuned balance between excitation and inhibition, with inhibitory neurons playing an integral role in maintaining this equilibrium. Despite comprising only a small fraction of cells in the brain, inhibitory neurons contribute to several essential functions, including playing a key role in generating cortical rhythms in the gamma range (~35-100 Hz), which are typically observed to increase in power during active cognitive processing. However, the relationship between inhibitory neurons and gamma oscillations is still not well understood. One major obstacle in testing the role of inhibitory neurons lies in the ability to target and manipulate specific cell types in isolation, due to their diversity, complexity, and intermingling with other types of neurons in the brain. Recent advances in methodology pertaining to specific classes of neurons have opened new avenues for studying inhibitory neurons in awake, behaving non-human primates. We injected a viral construct (AAV1-mDlx-ChR2-mCherry-Fishell-3) in the macaque visual cortex to allow for optogenetic activation of inhibitory neurons. We trained monkeys to detect visual stimuli while we record population activity from visual cortex as we optogenetically manipulated spiking activity of inhibitory neurons. Our findings reveal that approximately 30% of neuronal activity exhibited an increase in responses upon optogenetic stimulation of inhibitory neurons. Additionally, we observed an enhancement in gamma coherence within the cortical network when inhibitory neurons were activated, most notable when neurons were stimulated at 35 Hz. Overall, this study will allow us to causally investigate the role of inhibitory neurons in the generation of gamma rhythm in macaque visual cortex.

Disclosures: S. Khan: None. **A. Andrei:** None. **M. Slapik:** None. **V. Dragoi:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Program #/Poster #: PSTR547.05/EE11

Topic: D.06. Vision

Support: National Science and Technology Innovation 2030 Major Program (2022ZD0204600)

Title: Good Gestalt uncrowds crowding in macaque V1

Authors: *C. CHEN[1], D. JIANG[2], S. TANG[3,2], C. YU[1];
[1]Sch. of Psychological and Cognitive Sci., [2]Ctr. of Life Sci., [3]Sch. of Life Sci., Peking Univ., Beijing City, China

Abstract: Crowding reduces the discriminability of visual features, especially in the visual periphery, when the latter are surrounded by similar stimuli. Previous studies by Herzog group discovered that the crowding effect can be reduced when the flankers are grouped separately from the target in different Gestalts (e.g., long flanking lines vs. a short target). Here we studied the neuronal mechanisms underlying this uncrowding effect in V1, V2 and V4 of awake, fixating macaques with two-photon calcium imaging. Our crowding stimuli consist of a parafoveal target line at 2-4° eccentricity, and a pair of same or longer lines flanking the target at different target-flanker distances. Compared with responses to the target line only, neuronal responses were significantly suppressed when flanking lines were added. However, when the flanking lines were longer than the target line, less suppression was observed in V1 at certain target-flanker distances in all three macaques, consistent with the uncrowding effect. The results were mixed in V2 as three macaques showed more, unchanged, or less suppression with longer flankers, respectively. In V4, for most of the distance levels, the neuronal responses were suppressed more strongly by the longer flankers, opposite to the uncrowding effect. These results suggest that the uncrowding effect occurs most likely in V1, may or may not in V2, and unlikely in V4. We suggest that flanking lines may suppress the responses to the target through surround modulation in V1, which could be reduced by longer flankers grouped into different Gestalts. In V4, the target and flanking lines are more likely fall into the same larger RFs, and longer flankers would induce stronger competition and suppression. The V1 correlates of the uncrowding effect suggest that V1 may also play a critical role in crowding, at least for simple stimuli.

Disclosures: C. Chen: None. **D. Jiang:** None. **S. Tang:** None. **C. Yu:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Program #/Poster #: PSTR547.06/EE12

Topic: D.06. Vision

Support: Simons foundation 543017
Gatsby charitable foundation GAT3708

Title: Generalized affine models explain stimulus-dependent correlated variability within and between V1 and V2

Authors: *J. XIA[1], A. I. JASPER[2], A. KOHN[3], K. D. MILLER[4];
[1]Columbia Univ., New York, NY; [2]Dominick Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; [3]Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Bronx, NY; [4]Columbia Unviersity, New York, NY

Abstract: Neuronal population responses often wax and wane together across trials despite identical visual stimulation. This synchronized trial-to-trial variability, known as "shared noise" or correlated variability, can be modulated by external stimuli. Understanding how shared noise is modulated by stimuli is crucial for unraveling their impact on stimulus coding. Previous work proposed three models of shared noise: multiplicative, additive, and affine; and found that affine models could explain how shared noise in visual cortex is modulated by variations in stimulus orientation. However, affine models may not capture changes in variability induced by other stimulus manipulations.Trial-to-trial variability is not only shared within one brain area, but also between areas. Recent studies have revealed that shared noise across areas has lower dimensionality compared with that within each area, facilitating flexible communication between brain regions. However, how this low-dimensional communication subspace depends on the stimulus remains largely unknown. Specifically, it is unclear if the previously proposed affine models can also capture stimulus modulation of shared noise across areas.To address these questions, we analyzed neuronal responses simultaneously recorded from monkey V1 and V2 under stimulation by drifting gratings. Affine models effectively accounted for stimulus-dependent shared noise within V1 and V2 when only orientations were varied. However, when both contrast and orientation were considered, the affine models needed modification to incorporate contrast-specific coefficients to account for the suppressive effect of increasing contrast on shared noise. Furthermore, the affine models can be modified to explain how the communication subspace between V1 and V2 depended on stimulus orientation. Additionally, we explored a generalized model allowing for arbitrary modulation forms for each stimulus. While this model slightly outperformed affine models given sufficient data, the differences were small.In summary, our work extends previous work in characterizing the nature of shared noise within a brain area to shared noise between brain areas. Importantly, we found evidence suggesting an alternative form of stimulus-dependent shared noise when varying multiple stimulus variables.

Disclosures: J. Xia: None. **A.I. Jasper:** None. **A. Kohn:** None. **K.D. Miller:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR547.07/EE13

Topic: D.06. Vision

Title: Influence of mixed-reality simulation on visual evoked potentials

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Abstract: Virtual reality technologies offer a powerful solution for on-the-ground flight training curricula. While this technology offers safer and cheaper instruction programs, it is still unclear how virtual reality impacts the brain. Indeed, virtual reality simulations engage students in a strange mix of incongruous visual, somatosensory and vestibular inputs. Characterizing brain dynamics during virtual reality simulation is important for understanding cognitive processes during virtual flight training. To this end, we studied the behavior of visually evoked potentials under mixed reality simulation. We used a Varjo XR-3, the mixed-reality headset that powers our flight simulator MUPUN-X, for our experiments. We recorded flash visual evoked potentials from 5 subjects under two conditions. First, we recorded normal visual potentials. Second, we recorded visual potentials triggered by the virtual representation of the flash used in the first experiment. The time of stimulation for the analog and virtual stimulus was recorded for EEG processing. All subjects had used the headset before and were familiar with the immersion experience. Our results show mixed-reality stimulation imposes an important processing delay in the visual cortex. The P2 component during mixed-reality stimulation was significantly delayed compared to the analog version, and the amplitude of the visually evoked response was also decreased. These results suggest that visual cognition during mixed-reality training is delayed, not only by the in silico processing delay of the headset, but also by an extra biological delay induced by the headset's limited visual performance. Flight training is a demanding task, and thus sources of cognitive latency are to be considered to understand the impact of virtual reality on flight instruction programs.

Disclosures: V. Hidalgo: None. **C. Bazaes:** None. **J. Letelier:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Topic: D.06. Vision

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Title: Response comparison of excitatory and somatostatin inhibitory interneurons in primate area V1 using two-photon microscopy

Authors: *J. PATTADKAL, B. V. ZEMELMAN, N. J. PRIEBE;
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Abstract: Inhibitory interneurons are an essential component of cortical circuitry but their function in the primate cortex has remained elusive, due to lack of approaches to target these cell types for measurement. We have previously described viral strategies to target different interneuron subclasses using intersectional methods (Mehta et al, 2019). Here we use this

targeting approach to target somatostatin (SST) interneurons for functional imaging in marmoset area V1. Specifically, we used an intersection of SST promoter and h56D promoter to express GCaMP6f and tdTomato in SST interneurons. In addition, GCaMP6f was also expressed in other cell types using pan-neuronal synapsin promoter. This allowed us to simultaneously measure responses of both SST and non-SST cells while detecting SST cells using the red label. We used in-vivo two-photon calcium imaging to record responses of both populations of cells in area V1 of awake marmosets. We characterized the orientation selectivity of cells using drifting gratings. We find several instances of orientation selective SST interneurons. We do not observe any differences in orientation selectivity of SST and non-SST populations ($p = 0.58$, KS test on orientation selectivity index distributions of SST vs non-SST populations). Similarly, no differences were observed in orientation preferences as well as direction selectivity distributions of both groups ($p = 0.82$, KS test on orientation preference distributions, $p = 0.57$, KS test on direction selectivity index distributions). Both cell populations are spatially organized in cortical space based on orientation preference and the preferences of nearby SST and non-SST cells matched. Our current results suggest no differences in response properties to visual stimuli between SST and non-SST cells. Future work using these methods will allow us to target and interrogate the function of these and other cell types in awake primate cortex shedding light on how multiple circuit elements together to implement circuit functions.

Disclosures: J. Pattadkal: None. **B.V. Zemelman:** None. **N.J. Priebe:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Topic: D.06. Vision

Support: NIH R01EY028657

Title: Quantifying signal and noise in V1 under rod and cone mediated vision

Authors: *R. O'SHEA[1], X.-X. WEI[2], N. J. PRIEBE[2];
[1]Dept. of Psychology, Univ. of Texas At Austin, Austin, TX; [2]Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Every day, the mammalian visual system undergoes a remarkable transformation whereby the peripheral receptors alternate between the rods and the cones as the sun rises and sets. This causes well-defined changes in the spatiotemporal response properties of RGCs *in-vitro*. These changes could limit the fidelity of encoding of visual features in regions downstream of the retina. Yet mammals can perform a wide array of visually guided tasks in both the scotopic and photopic regimes. It remains unclear to what extent these changes at the periphery impact downstream computations. We focus here on a recent result in rodent retina showing an increase in the magnitude of within-class RGC noise correlations in the scotopic versus photopic

state (Ruda et al., 2020). Importantly, scotopic light adaptation limits the accuracy of an RGC population decoder when the change in noise correlation magnitude is not considered. To determine whether this change in noise correlations in the retina impacts population activity downstream in LGN and V1, we recorded from neural populations while awake mice viewed repeated presentations of natural movies at scotopic and photopic light levels. Using the neuropixels probe to densely sample LGN neurons, we find a modest increase in the magnitude of noise correlations for cells separated by less than 300 microns in the scotopic (mean correlation= .032, std=.061) vs photopic state (mean correlation= .024, std=.049). This result is consistent with the LGN population inheriting the noise correlation structure from the retina. However, downstream in V1, we find that noise correlations do not increase in the scotopic (mean correlation= .048, std=.049) relative to photopic state (mean correlation= .057, std=.052). Using both 2-photon calcium imaging and electrophysiology, we find a remarkably consistent relationship between signal and noise correlations in V1 cells tracked across light adaptation states. In order to explain the invariant V1 population response despite changes in the peripheral input, we developed a simple feedforward model of the visual pathway to show that V1 noise correlations can remain invariant to changing noise structures at the periphery in a regime of dense afferent convergence. Our results show how the visual system can accomplish the goal of maintaining a consistent representation of the visual world across a vast range of luminance levels.

Disclosures: **R. O'Shea:** None. **X. Wei:** None. **N.J. Priebe:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Topic: D.06. Vision

Support: Simons 542999
NIH RF1 NS127107
NIH T32 GM007288

Title: Pulvinar interactions with visual areas V1 and V2 of macaque monkeys

Authors: *A. XU[1], A. I. JASPER[1], A. KOHN[1,2,3];
[1]Dominick P. Purpura Dept. of Neurosci., [2]Dept. of Ophthalmology Visual Sci., [3]Dept. of Systems and Computat. Biol., Albert Einstein Col. of Med., Bronx, NY

Abstract: How brain areas communicate is central to brain function, as nearly all functions are distributed across multiple areas. In visual cortex, areas communicate via cortico-cortical (CC) connections, but they might also relay signals to each other via the pulvinar, a thalamic nucleus with extensive connections from and to visual cortex. Though this cortico-pulvino-cortical (CPC) pathway is thought to contribute to signaling between visual areas, this possibility has received

limited experimental exploration. We assessed the moment-to-moment relationship between pulvinar and visual cortical activity to determine the functional connectivity between cortex and pulvinar and to test how CPC and CC signaling differ. We recorded neuronal population spiking activity simultaneously in the ventrolateral pulvinar and in areas V1 and V2 of anesthetized macaques. We presented drifting grating stimuli that covered the receptive fields of neurons in all three structures. Most pulvinar cells were visually responsive and many were tuned for orientation, selectivity for which suggests an involvement in early visual processing. To assess signaling between V1-V2 and between these areas and the pulvinar, we fit linear regression models to predict trial-to-trial fluctuations of activity in one area using activity in another. Visual cortical activity was predictive of activity in the pulvinar, and the quality of this prediction was highest when the receptive fields of the cortical and pulvinar neurons were aligned. Cortical activity was not predictive of activity in other visually responsive subcortical non-thalamic structures. The predictive performance of cortico-pulvinar models was similar to that of cortico-cortical models. The linear mapping relating cortical and pulvinar activity was low dimensional, suggesting that cortico-pulvinar interactions utilize a communication subspace, as previously observed for cortico-cortical interactions. The presence of a communication subspace indicates that only some dimensions of cortical activity are relayed to the pulvinar; the remaining dimensions of activity remain private within cortex. To probe the relative timing of activity between pulvinar and cortex, we cross-correlated pulvinar and cortical responses. This analysis revealed that pulvinar activity often led V2 spiking, perhaps indicating a role in relaying signals to V2. Our results suggest that the strength and structure of CPC interactions are similar to CC interactions, supporting the possibility that the pulvinar plays a role in relaying information across stages of cortical processing.

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Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Title: Estimating neuronal population encoding via image reconstruction

Authors: *A. A. DEHAQANI, B. WANG, C. R. PONCE;
Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Visual cortex neurons represent objects and places in the natural world. While individual neurons show tuning for attributes such as color, form, and texture, overall,

information of the natural world is distributed across populations. However, it remains unclear how and which of these attributes are actually encoded in population response patterns. To investigate the encoded visual information present within population response patterns, we recorded from neurons in V1, V4, and IT using chronic arrays. We presented photographs ("target images") to the monkeys and measured the population response patterns of neurons to each image. To quantify the retained information in these response patterns, we conducted closed-loop image synthesis experiments; specifically, we “threw away” the photograph and attempted to recover it using image generators (generative adversarial networks) combined with adaptive search algorithms. The objective was to synthesize new images evoking response patterns that best matched the target-image population response pattern. We entertained multiple hypotheses, including that the neuronal populations retained all necessary information for image reconstruction or only select attributes, such as color and overall shape. In parallel, we also used convolutional neural networks (CNNs), to estimate the kinds of visual information that can be retained in the middle layers of a feedforward architecture. We found that it was possible to create images that evoked population response patterns similar to that of the target images. Moreover, there was a noticeable increase in the visual similarity between the reconstructed and target images across the synthesis process, as estimated objectively using CNNs. We conducted parallel experiments using CNN unit populations (within given layers), and found that visual information was sparsely distributed and more easily recoverable through the more strongly activated units. Specifically, we found that increasing the population size resulted in better image reconstructions despite a concurrent decrease in the ability to replicate the target population response pattern. However, we also discovered that relying on units that were most activated by the target image resulted in better reconstructions than relying on units that were randomly sampled from the same layer. This suggests that in a multidimensional activity space, strongly activated units provide more information, consistent with a sparse representation. Next, we are investigating how this computational result translates to the ventral stream.

Disclosures: **A. A. Dehaqani:** None. **B. Wang:** None. **C.R. Ponce:** None.

Poster

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DFG (RTG 2416 ”MultiSenses-MultiScales”)
CNRS Multidisciplinary Exploratory Projects initiative

Title: Neural manifolds change with top-down communication from V4 to V1

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Abstract: High-dimensional brain activity is often organized into lower-dimensional neural manifolds that can encode for many behavioral variables. However, neural manifolds in the visual cortex of primates remain understudied [1, 2]. In addition, top-down communication from V4 to V1 is known to mediate visual attention for figure-ground segregation and contour integration in macaques [3]. Moreover, computational modeling shows that such signals may also influence neural manifolds by rotating them in a context-dependent manner [4]. However, whether top-down signals modulate neural manifolds in vivo remains to be shown.
Here, we study the neural manifolds of macaques (Macaca mulatta, N=3) in V1 during the resting state, using extracellular recordings with multi-electrode arrays (Utah array) [5]. Our analysis reveals two distinct neural manifolds in macaque V1 that are strongly correlated with eyes-open and eyes-closed conditions, even though the macaques were sitting in a dark room. The eyes-open manifold had a significantly higher dimensionality, primarily due to lower noise correlations.
We hypothesize that cortico-cortical communication, estimated from LFP coherence and Granger causality, induces these changes. We find that top-down signals from V4 to V1 are significantly stronger during the eyes-open periods, and that they primarily target the foveal region, in agreement with tract-tracing data [6]. Spectral analysis further reveals reduced alpha power in the eyes-open condition, consistent with alpha blocking found in EEG studies. Finally, we show in a small balanced spiking neuron model that top-down signals can induce multiple neural manifolds, suggesting a causal link between our experimental observations.
Taken together, the data analysis and simulations suggest that V4-to-V1 signals actively modulate neural manifolds in the visual cortex of the macaque. We postulate that the top-down modulation during the eyes-open periods prepares V1 for fast and efficient visual responses, resulting in a visual stand-by mode.
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[4] Naumann et al. 2022. eLife 11, 76096 (doi.org/10.7554/eLife.76096)
[5] Chen et al. 2022. Scientific Data 9 (1), 77 (doi.org/10.1038/s41597-022-01180-1)
[6] Wang et al. 2022. bioRxiv (doi.org/10.1101/2022.04.27.489651)

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Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Title: Evaluating differential correlations in macaque V4 during task learning and task engagement.

Authors: *S. LIU[1], A. PLETENEV[1], A. C. SNYDER[1], R. M. HAEFNER[2];
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Abstract: The spiking responses of sensory neurons are correlated, with important consequences for their ability to carry stimulus information. Recent theoretical work identified "differential correlations" - population response fluctuations mimicking a real signal - as critical for limiting information in the population (Moreno-Bote et al. 2014).
The implications of information-limiting correlations depend on how they are generated in the brain, which is currently unknown. Feedforward sources predict that stronger correlations are associated with worse perceptual performance. However, another important potential source could be task-relevant feedback signals (Haefner et al. 2016, Lange & Haefner 2022). Under the assumption that sensory neurons represent posterior beliefs, task-related beliefs propagate down to task-relevant neurons, and the fluctuation of such belief signals injects shared variability among neurons that provide congruent information, inducing differential correlations. This theoretical framework predicts the strength of differential correlations to be higher during active task engagement and associated with better behavioral performance.
We used a Utah array to record neural responses from V4 populations in a macaque monkey while it learned and performed two orientation-discrimination tasks. We evaluated the task-specific Fisher information in V4 populations in the original and shuffled data and computed the difference between them. Shuffling trials removed the original covariance structure, therefore this difference reflects the influence of noise correlations on population coding.We found (1) Fisher information was usually higher in shuffled data, indicating noise correlation structures impaired information coding in the population. (2) The magnitude of this difference was strongest at the end of the trial, consistent with the belief feedback framework.Relating the strength of this difference to behavior, we found these interesting trends: (3) It was larger during task performing than during passive viewing, suggesting that at least part of it was contributed by active task engagement. (4) Behavioral performance was positively correlated with stronger information differences between original and shuffled data, indicating stronger differential correlations in higher performance sessions.
This paradox suggests that only considering the impact of noise correlation in a purely feedforward framework is not sufficient. At least part of the information-limiting correlation appears to stem from sources that are consistent with top-down beliefs as predicted by approximate hierarchical inference.

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Poster

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Title: Spatial and temporal extents of spatiotemporal population receptive fields progressively increase across visual hierarchies.

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Abstract: The ability of the visual system to process information across the visual field and time is crucial for perceiving and interacting with the world. This is achieved by computations in spatiotemporal receptive fields (stRFs), which have mostly studied with invasive methods such as microelectrode recordings and electrocorticography (ECoG) because the temporal resolution of fMRI (seconds) is thought to be too slow to capture the temporal aspects of stRFs (milliseconds). Thus, stRFs in multiple human visual areas have not been fully characterized. Here, we used our recently-developed computational framework to estimate spatiotemporal population receptive fields (stPRFs) in voxels of human visual cortex in visual degrees and milliseconds (Kim, bioRxiv 2023). We collected fMRI data from 10 participants who viewed spatially and temporally varying stimuli and estimated stPRFs of individual voxels across multiple visual areas: V1, V2, V3, hV4, VO1/2, LO1/2, TO1/2, V3AB, and IPS. Additionally, we tested whether the hemodynamic response function (HRF) impacts the estimated stPRF parameters by comparing the results obtained using a canonical HRF with those obtained using voxel-wise optimized HRFs. We find that (i) the HRF alone does not explain the temporal variations across voxels, and (ii) the estimated stPRF parameters are similar across different HRF choices. When comparing stPRFs across visual areas, we find that both spatial and temporal processing windows progressively increase across the ventral, lateral, and dorsal visual streams, and that the spatial and temporal windows of stPRFs co-vary. Notably, our temporal estimates of stPRFs fall within the published distributions of electrophysiological data in macaques and ECoG in humans, while the responses in humans are about 18ms slower than macaques. Overall, the current framework not only enables precise mapping of stPRFs using fMRI but also presents opportunities for (i) optimizing experimental designs to better estimate stPRFs with high precision and (ii) comparing stPRFs across species and modalities. Moreover, this approach opens new research directions beyond the visual system, allowing deeper understanding of how the brain processes dynamic sensory and cognitive information.

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Poster

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Title: Regional Structural Changes in the Visual Cortex of Individuals with Psychotic Disorders

Authors: *H. TURKOZER[1], V. ZENG[2], D. HOANG[2], J. SRITHARAN[2], N. ISKA[2], E. I. IVLEVA[3], B. A. CLEMENTZ[4], G. D. PEARLSON[5], S. KEEDY[6], E. GERSHON[6], C. TAMMINGA[3], M. S. KESHAVAN[2], P. LIZANO[2];
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Abstract: The visual system is a significant locus of pathology in psychotic disorders. However, there is a limited amount of research investigating the morphology of a large proportion of the human visual cortex (VC) in this population. Using data from the Bipolar-Schizophrenia Network on Intermediate Phenotypes consortium (BSNIP-1 and BSNIP-2 Studies), we examined VC structural measures in psychosis probands and healthy individuals. Additionally, we explored the associations between these measures and clinical variables, cognitive markers, and childhood trauma scores. Cortical thickness and surface area of five VC subregions (hOc1, hOc2, hOc3v, hOc4v, MT) were quantified using FreeSurfer Version 7.1.0 in psychosis probands (n=1211) and healthy individuals (n=734). The ComBat package was utilized to mitigate scanner effects. The Positive and Negative Syndrome Scale (PANSS) was used to assess symptom severity. The Brief Assessment of Cognition in Schizophrenia (BACS) was used to assess general cognition. Childhood trauma scores were assessed in a subset of participants using

Childhood Trauma Questionnaire (CTQ). Psychosis probands demonstrated lower surface area in hOc1 and hOc2, and lower thickness in all five VC subregions compared to healthy participants. Thickness reductions in hOc1, hOc4v, and MT were regionally specific. Lower MT thickness was associated with higher PANSS Total scores, along with higher Positive and General PANSS subscores in probands. MT area, as well as hOc3v, hOc4v, and MT thickness measures were correlated with BACS Total z-scores. Reductions in hOc4v and MT thickness measures were correlated with higher total CTQ scores. Our results demonstrate significant impairments in visual cortex subregions in individuals with psychotic disorders, which are associated with higher symptom severity, cognitive decline, and higher childhood trauma scores. The distinct patterns of area and thickness changes, along with the association with childhood trauma scores, suggest potential developmental contributions to visual cortical alterations seen in psychotic disorders.

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Poster

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Title: Volumetric organization of visual and non-visual signals across the mouse visual cortex

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Neurotechnology and Biophysics, [4]Kavli Neural Systems Inst., Rockefeller Univ., New York, NY

Abstract: Neurons in the mouse visual cortex encode both high-dimensional sensory information and lower-dimensional behavioral variables. Here we asked how signals from these two streams are organized across the cortical volume, and specifically whether they follow distinct organizations.
We used Light Beads Microscopy (Demas et al, Nature Methods 2021) to functionally image Ca2+ transients at cellular resolution from large volumes of cortex (4 x 4 x 0.5 mm^3) in transgenic mice expressing GCaMP6s. To accurately segment individual cells and extract their activity from the fluorescence movies, we developed a novel volumetric cell extraction pipeline by accelerating and extending to three dimensions the Suite2P algorithms (Pachitariu et al, bioRxiv 2017).
First, we confirmed that the mouse visual cortex has a weak columnar organization of orientation preference (Kondo et al, Nature Comm 2016; Ringach et al, Nature Comm 2016). Cells within a column of width 10 um the x-y plane and of depth up to 60 um in V1 had more similar orientation preferences and responses to natural images. Next, we investigated the organization of non-stimulus activity through noise correlations and correlations during spontaneous behaviors. We found that the tight columnar structure was preserved for spontaneous and noise correlations. Furthermore, pairwise correlations showed a long-range laminar structure extending over 1 mm: cells at the same laminar depth were more likely to be more correlated, even if they were laterally separated by hundreds of microns. Correlations also depended on the borders between visual areas: a cell in V1 was likely to be more correlated with another cell in V1 than with a cell at a similar distance in a higher visual area.
The spatial structure of cortical correlations thus depends on three determinants: columnar, laminar and areal. Short-range columnar structure is present in both sensory and behavioral signals, while long-range laminar structure is only present in behavioral signals. Our findings can aid in understanding how sensory and state information is combined in the mouse visual system.

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Poster

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Title: Decoding of visual information from ECoG signals in mouse visual cortex

Authors: *S. YAMADA[1], H. ITO[2];
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Abstract: Brain-Machine Interface including the artificial vision system has advanced recently with the aid of nano- and AI- technologies. However, current artificial vision systems have difficulties in a long-term use and have not yet been optimized for congenital visual impairments. Attempting to overcome these issues, we fabricated own made ECoG electrode with biocompatible materials and tested its recording performance for a long-term use. ECoG electrodes have six recording sites of 2 by 3 layout with an interspacing of 1.25mm and were implanted to the visual cortex of two mice. We presented visual stimuli on the monitor placed in front of the left eye of Isoflurane anesthetized mice and examined whether we could decode stimulus information from LFP signals recorded at multiple sites. For the visual stimuli, a white horizontal rectangle was presented in three different locations on the monitor: presenting at the top, center, and bottom. In each recording trial, four stimuli including no stimulus presentation were presented in a random order and the LFP signals were recorded over 2,000 trials per day (4 stimuli×500 sets). The LFP signals of the six channels were down sampled from 30kHz to 120 Hz. After excluding the outliers and filtering the LFP signals with the Hampel filter, the signals were normalized according to the maximum and the minimum amplitudes of the entire signal. We attempted to decode the stimulus from the spatiotemporal patterns of the LFP signals (1.0 sec duration after the stimulus presentation) with the aid of the convolutional neural network (CNN). The discrimination accuracies estimated by the cross-validation were up to 47.14% (average: 45.43%) for one animal and up to 63.37% (average: 60.27%) for the other. We confirmed that the dataset with randomly shuffled stimulus labels provided the discrimination accuracies of the chance level (25%). Large receptive fields of the neurons in the mouse visual cortex provide a vague spatial representation of the visual field. Further, the LFPs recorded by the ECoG electrodes provide rather lower spatial resolution of the cortical activities. Nevertheless, our results suggested that the spatiotemporal LFP signals could provide useful information on the stimulus locations, which can support the application of the ECoG recordings to the artificial vision. We are continuing the ECoG recordings to examine how long the recorded LFP can keep a signal quality for the stimulus discrimination. By using various shuffling methods, we are investigating what specific features of the LFP signal mainly provide the information for the stimulus discrimination.

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Poster

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Title: Visual sequence prediction in superficial visual cortex

Authors: *Q. ZHANG, C. STRINGER, M. PACHITARIU;
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Abstract: Humans and other animals can observe patterns in their environment and learn statistical regularities even in the absence of direct rewards. In the temporal domain, statistical regularities can be exploited to predict future events, which allows animals to react to unexpected events. Despite an abundance of theories, the neural mechanisms underlying such predictive computations remain largely unknown. Here we chronically tracked thousands of neurons in the superficial layers of primary visual cortex (V1) while mice ran through an abstracted sequence of natural image stimuli, repeated in the same order on every trial. We distinguish two classes of neurons: those that responded transiently to a stimulus transition and those that responded in a sustained way. After several days of exposure, we found plasticity in the sustained but not in the transient neurons. The sustained responses became anticipatory for the next stimulus, ramping up over the course of the 1,2 or 3 second intervals between stimulus transitions. We then broke the regularity of the trained sequence in multiple ways and observed a variety of changes in neural responses. First, replacing a stimulus with a gray screen or with another stimulus from the sequence resulted in a large, population-wide response across all stimuli but only in the transient neurons, which we denote as a “mismatch” response. Second, replacing a stimulus with a completely new stimulus resulted in an even larger mismatch response. Finally, changing the transition intervals between stimuli did not produce a mismatch response, suggesting that mismatch computations are invariant to stimulus durations. The presence of a mismatch response dominated neural activity, but we also found smaller, more selective neural changes. These changes were consistent with a model in which the superficial layers of V1 provide the internal prediction necessary to calculate sensory mismatch elsewhere. In future work, we plan to test these models by recording in other neural populations and performing direct manipulations of the neural activity.

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Poster

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Title: Cortical readout of retinal motion signals is essentially perfect, but limited in space and time

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Abstract: In the primate visual system, most computations underlying complex visual behaviors are based on relatively simple signals from the retina. However, the factors determining how efficiently the brain reads out retinal signals are unknown. We measured the responses of neurons in the retina and visual cortex to compare the precision with which they encode stimulus speed, using the speed estimation performance of human observers as a benchmark. In all three measurements, the stimuli were moving bars matched in size, speed, duration, contrast, intensity, and retinal eccentricity. We used large-scale multielectrode recordings from complete populations of ON and OFF parasol retinal ganglion cells in 3 isolated macaque retinas ex vivo and multisite recordings of isolated MT neurons in 4 anesthetized macaques. We analyzed how optimal decoders of speed from neural activity in the retina and MT performed compared to human observers. When tested with spatially and temporally extended moving bars, the speed estimates obtained from human observers and MT neuron populations were much less precise (standard deviation ~10%) than those directly decoded from retinal activity (SD ~1%), suggesting central limits to the efficiency of motion computations. To probe the cortical limits of motion computation, we also measured the limits of spatial and temporal integration in cortical neurons with random- dot displays in which the dots were parametrically varied in their spatial and temporal separation, and took the limits of integration to be the largest dot separations in space and time that supported directionally selective responses. Restricting retinal readout to these limits reduced precision roughly 10-fold, thereby bringing retinal, cortical, and behavioral speed estimates into alignment. These findings reveal that under suitable conditions, the cortical readout of the retinal motion signal is nearly noiseless, but cortical limits to the integration of visual information in space and time limit the fidelity of motion sensing.

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Poster

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Title: Bayesian decoder predicts disparity for correlated but not anti-correlated stereograms

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Abstract: Neurons in primary visual cortex (V1) integrate input from both eyes. The horizontal displacement of the eyes provides each eye with a slightly different view of the environment. To use this information, the brain must solve the correspondence problem of determining which points in one retinal image correspond to the same points in the other retinal image. The difference between these retinal images is known as the disparity and can be measured using random dot stereograms (RDS). The disparity energy model posits that the goal of binocular neurons is to enhance signals for correct matches and suppress false matches in order to solve the correspondence problem. To uncover whether the responses of V1 populations can be used to estimate depth, we measured calcium signals using two-photon microscopy to both correlated and anti-correlated RDS. To determine the reliability of V1 depth signals, we applied a Bayesian decoder to the population activity for both correlated and anti-correlated stimulus conditions. If depth signals can be reliably read out from V1 population activity and are consistent with the disparity energy model, the decoder should have strong, correct predictions in response to correlated RDS stimulation and weaker, incorrect predictions in response to anti-correlated RDS stimulation. We find that disparity can be reliably predicted from correlated stereogram stimulation, with high likelihood that the disparity is near when the stimulus is near (mean 64.7% SD 10.0), zero when the stimulus is zero (mean 73.5% ± 24.2), and far when the stimulus is far (mean 68.2% ± 13.9). The decoder estimates were dramatically degraded for anti-correlated stimulation. Estimates were often inverted, such that very near or very far disparity stimuli generated predictions of zero disparity and were weaker (mean 36.8% ± 17.7) compared to correlated stimulation. These results indicate that binocular V1 neurons in mice may still be responding to false matches at very near and very far disparities, but with less certainty, which deviates from the disparity energy model to better solve the correspondence problem.

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Poster

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Title: Sex-specific development of cortical circuits supporting sensory context processing

Authors: *J. M. ROSS, M. L. ROBINSON, J. P. HAMM;
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Abstract: In natural environments, organisms must be able to process information within the context of previously experienced stimuli. Past work in humans and rodent models demonstrates that primary sensory cortices and their principal neurons exhibit reduced responses to repetitive stimuli but augmented responses to rare stimuli differing from contextual regularity (i.e. deviants). The latter has been referred to as “deviance detection” (DD). This ability to processes stimuli in context requires both feedforward and feedback mechanisms in cerebral cortex and, at least in humans, biomarkers of sensory context processing (i.e., mismatch negativity) continue to develop well into adulthood. While sensory context processing has been studied in adult animal models, this same process is understudied during postnatal development, which may hold clues to the underlying neurobiology given neural circuits develop and mature throughout adolescence. To assess DD development across adolescence, we employed two-photon calcium imaging of excitatory pyramidal cells (PYRs) in L2/3 of primary visual cortex (V1). We assessed the stimulus evoked activity of PYRs during a classic visual oddball sequences and a many standards control sequence to awake mice while imaging neural responses at distinct ages relevant to postnatal brain development: early adolescence, late adolescence, and adulthood. This design allows the direct comparison of responses to the same visual stimulus in different contexts - when the stimulus is expected (redundant), when it deviates from expectation (deviant), and when it is neither expected nor deviates from expectations (control). At the population level, DD is evident in females early during adolescence and persists into adulthood; however, does not manifest in males until adulthood, suggesting that the developmental trajectory of sensory context processing in V1, and possibly the underlying mechanisms producing DD, differs between males and females. In adult mice, prefrontal input to V1 is required for DD and previous research demonstrates several cellular and subcellular changes within prefrontal cortices during adolescence that may impact that ability to generate DD signals during this period. Ongoing work is aimed at tracing prefrontal input to V1 during these windows of adolescent development to determine the extent to which input changes with age or differs by sex. Altogether, this work may inform our understanding of how postnatal brain development contributes to sensory context processing and DD and highlight sexual dimorphisms that may be relevant to neuropsychiatric conditions related to aberrant sensory processing.

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Poster

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Title: Relating natural image statistics to patterns of response covariability in primary visual cortex

Authors: *A. FARZMAHDI[1], A. KOHN[1,2,3], R. COEN-CAGLI[1,2,3];
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Abstract: Understanding how the brain encodes information requires comprehending the structure of cortical activity. This structure includes both the variability of individual neurons and their shared covariability. Despite extensive research on single neurons in visual cortical representations of natural scenes, our understanding of the precise role of covariability in natural visual processing remains limited. Here we present a theory proposing that the trial-to-trial covariability structure within the primary visual cortex (V1) plays an important role in the optimal representation of natural visual inputs, establishing an important connection between V1 population activity structure and visual scene statistics. We consider V1 activity as representing probabilistic inferences using the neural sampling hypothesis and a well-established generative model of image statistics. In the model, we found that when the spatial receptive fields of neurons overlap and share a global feature, such as image contrast, pairwise 'noise' correlations are reduced as the image is made larger by adding spatial context. This is because the spatial context reduces uncertainty about the shared global feature, resulting in reduced covariability. Conversely, correlations increase between neurons with less overlapping receptive fields for the same image manipulation. To test our model predictions, we analyzed recordings from anesthetized macaque monkeys presented with natural image patches of different sizes. We identified visually responsive neurons and grouped them based on their spatial receptive fields, as either being centered on the stimulus or off-centered. The analysis revealed distinct modulations of noise correlations based on the proximity of the neurons' spatial receptive field. Centered pairs demonstrate decreased noise correlations for large images, whereas centered versus off-centered pairs show increased noise correlations, as predicted by the model. We verified that this result cannot be explained as a firing rate effect. In conclusion, our study elucidates the covariability structure of visual cortex responses to natural scenes using a generative model, assuming responses reflect samples from an inferred probabilistic inference. By integrating these factors, we establish a significant connection between covariability patterns and spatial context, shedding light on the underlying mechanisms of natural visual processing in the visual cortex.

Disclosures: **A. Farzmahdi:** None. **A. Kohn:** None. **R. Coen-Cagli:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR547.23/FF2

Topic: D.06. Vision

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Biotechnology and Biological Sciences Research Council studentship

Title: Neural population dynamics stabilise faster during active behavioural states in mouse V1

Authors: *E. A. B. HORROCKS, F. R. RODRIGUES, A. B. SALEEM;
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Abstract: The demands on the visual system can vary depending on an animals' behavioural goals. As an animal locomotes in an environment, visual inputs can change rapidly and may require faster neural processing for more immediate behavioural responses. Whilst locomotion is associated with a number of changes in the mouse visual system, it is not known if the temporal dynamics of neural responses are altered, which may be an important mechanism for speeding up the real-time encoding of visual inputs. To determine this, we performed electrophysiology recordings in mouse primary visual cortex (V1) and compared responses to moving dot fields while mice were either stationary or locomoting.

We performed acute recordings in 5 mice using Neuropixel probes inserted into V1. We recorded a total of 1,583 neurons (range: 235-464/session). We presented moving dot field stimuli in the contralateral visual field (black and white 2° diameter dots) at 6 different visual speeds (0, 16, 32, 64, 128, 256°/s) in the naso-temporal direction. Stimuli were presented for 1s with a 1s grey screen inter-stimulus interval.

Locomotion reshaped single neuron response dynamics, primarily by reducing transient stimulus onset responses. The reshaped response dynamics enabled tuning for visual speed to emerge twice as quickly and persist for twice as long (Median tuning onset: stationary = 180ms, interquartile range (IQR): 60-440ms; locomotion = 100ms, IQR: 50-210ms. Median tuning duration: stationary = 465ms, IQR: 180-900ms; locomotion = 955ms, IQR: 535-1130ms; sign-rank test: $p<0.001$ for both comparisons; n = 344 cells tuned in both stationary and locomotion trials).

Population temporal dynamics reorganised during locomotion such that population activity stabilised faster. Neural correlations stabilised faster and increased encoding capacity by reducing noise correlations in the direction of signal correlations. Latent population dynamics made more direct transitions between baseline and stimulus steady-states (Mean distance ratio: stationary = 8.73+-1.59 (mean+-sem); locomotion = 2.69+-0.13; repeated-measures ANOVA: $p = 0.01$, $F_{(1,20)} = 20.69$), and exhibited reduced tangling, indicating a dampening of the oscillatory-like dynamics present in stationary trials. Functionally, visual speed decoding was more accurate and stabilised faster during locomotion.

Our results reveal that the mouse visual system adapts to behavioural state by altering temporal response dynamics. During locomotion, faster stabilisation of responses enables the rapid encoding of new visual motion inputs which may serve altered perceptual requirements and behavioural goals during this state.

Disclosures: E.A.B. Horrocks: None. **F.R. Rodrigues:** None. **A.B. Saleem:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Title: Adaptation shapes the representational geometry in V1 to encode the statistics of the environment

Authors: *M. DIPOPPA[1,3,6], R. NOGUEIRA[4], S. BUGEON[6], Y. FRIEDMAN[7], C. B. REDDY[6], D. L. RINGACH[2], K. D. MILLER[8], S. FUSI[5], M. CARANDINI[6];
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Abstract: Neural responses dynamically change as a function of previously presented stimuli, a phenomenon known as sensory adaptation. Some of these changes can profoundly impact our perception while others can maintain invariant representations of certain sensory features. While adaptation has been mostly studied at the level of single neurons, to understand its impact on the neural code it is key to study it at the level of neural populations. We, therefore, asked how the geometry of the neural representations adapts to environments with different sensory statistics and what are the computational benefits of these adaptation effects. We addressed these questions experimentally and theoretically.
We recorded the responses of thousands of neurons in the mouse's primary visual cortex to sequences of oriented gratings drawn from uniform or biased distributions. To understand changes in representational geometry we considered several measurements, including the discrimination performance between any pair of stimuli. To our surprise, we discovered that, in the biased sequence, the distance between the representation of the adaptor and the other stimuli increased, leading to better decoding of the adaptor stimulus even though responses of neurons tuned to the adaptor decreased. A low-dimensional representation of the data, based on the preferred orientation of neurons, was able to capture these and several other geometric properties.
We adopted an efficient coding approach to understanding what population geometry is optimal under different environment statistics. We thus trained autoencoder models with metabolic constraints to reproduce the stimuli under uniform or biased statistics. In this way, in the training under biased statistics, the autoencoder would be penalized more when misclassifying the

adaptor's orientation. Within a wide hyperparameter region, the changes in tuning curves and population geometry in the model's hidden layer were consistent with our experimental observations.
In conclusion, adaptation-induced changes in the neuronal populations are orchestrated in such a way that the decoding of overrepresented stimuli increases even though responses to those stimuli decrease in magnitude. As our model suggests, these changes allow the brain to improve the representation of frequently presented stimuli while keeping the metabolic cost under control.

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Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

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Program #/Poster #: PSTR547.25/FF4

Topic: D.06. Vision

Support: Simon Foundation Grant 543027
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Title: The geometry of representation in mouse visual cortex follows a broken power law

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Abstract: Signal correlation, defined as the correlation of tuning between two neurons, is highly relevant to how populations of neurons represent stimuli and has been central to many studies of neural coding. Fundamental to this structure is the signal eigenspectrum, the ordered eigenvalues of the signal covariance matrix. The eigenspectrum precisely quantifies the dimensionality of the population code: if all eigenvalues are equal (a “flat" eigenspectrum) then each neuron encodes an orthogonal visual features, whereas if there is only one non-zero eigenvalue all neurons encode the same visual feature. Recent work made a theoretical argument that the eigenspectrum of the primary visual cortex should decay no more slowly than a power law with slope of approximately one (Stringer et al., 2019). This was argued to balance efficiency with smoothness of the population code. With a novel method for estimating the signal eigenspectra, cvPCA, they determined that the mouse primary visual cortex both follows a power law and is at the critical limit of decay. Yet these two empirical claims rest on the accuracy of the cvPCA estimator. We find cvPCA is substantially biased in simulations that match the distribution of the original experiment. Thus it is unclear if mouse visual cortex follows a power law and whether the power law decay is of the slope they claim. Here we address this issue. We first derive a consistent estimator of the signal eigenspectra that we demonstrate is less biased than cvPCA. We then re-analyse the original mouse V1 data from (Stringer et al., 2019) using our estimator. We find that

the signal eigenspectrum systematically deviates from a power law and is far better explained by a broken power law, with an initial segment in which eigenvalues fall off slowly and a second in which they decay more rapidly, with a power law exponent of approximately 1.2. This form of the eigenspectrum reflects a higher-dimensional representation with approximately ten dominating features that drive the dimensionality up, accounting for ~30% of neural variation. We sought to characterize how these dominant dimensions encode stimuli and made a surprising discovery: they are often well characterized by linear receptive fields in striking contrast to more non-linear individual neurons. Furthermore we find evidence that inhibitory neurons' contribution to these dominant dimensions tend to be larger and more uniform than the rest of the population.

Disclosures: D.A. Pospisil: None. **J.W. Pillow:** None.

Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

Location: WCC Halls A-C

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Program #/Poster #: PSTR547.26/FF5

Topic: D.06. Vision

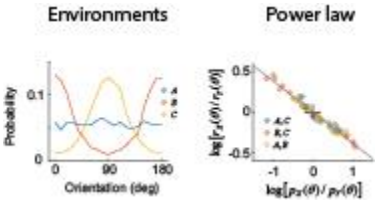
Support: NS116471
NS116377
NS123716

Title: A power law of cortical adaptation in neural populations

Authors: *D. RINGACH, E. TRING, D. ROWLEY, P. CHEN, M. DIPOPPA, J. COUTO, A. HUK, A. K. CHURCHLAND;
UCLA, Los Angeles, CA

Abstract: How do neural populations adapt to the time-varying statistics of sensory input? To investigate, we used two-photon imaging and Neuropixels recordings to measure the activity of neural populations in primary visual cortex adapted to different environments. Each environment was associated with a distinct probability distribution over the orientation domain. Within each environment, a stimulus sequence was generated by independently sampling form its distribution and presented at a rate of 3 frames per second. We find that two properties of adaptation capture how the population responses to a given stimulus, viewed as vectors, are linked across environments. First, the ratio between the response magnitudes in different environments is a power law of the ratio between the corresponding stimulus probabilities. Second, the response directions are largely invariant across environments. We show how these rules can be used to predict how cortical populations adapt to novel environments. Finally, we demonstrate how the power law implies that the cortex preferentially signals unexpected or novel stimuli, adjusting the metabolic cost of its sensory representation to the entropy of the environment. Namely, predictable environments are coded will a lower number of spikes than unpredictable ones,

consistent with theories of efficient sensory representation. We are currently exploring if these rules of adaptation hold across cortical areas and in different species.



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Poster

PSTR547. Visual Cortex: Neuronal Population Activity and Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR547.27/FF6

Topic: D.06. Vision

Title: From Spheres to Tori to Klein Bottle--the Topology of Neuronal Representations for Visual Images

Authors: L. CHIZHOV[1], A. SHAHIDI[2], L. VAN ROSSEM[3], L. MEYEROLBERSLEBEN[2], L. BUSSE[4], ***M. B. STEMMLER**[5];
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Abstract: Neurons in visual cortex respond to different combinations of image features. A key question is whether the dynamics of population activity across visual cortex is structured and lies on a low-dimensional manifold. The simplest such non-trivial manifold would be a circle, but many other manifolds are possible. Topological data analysis (TDA) and cohomological decoding can discover circular variables (such as a population encoding of visual stimulus orientation) without prior knowledge of the input space. Cohomological decoding of higher-dimensional manifolds is fraught with difficulty, however.Local patches from natural images exhibit the structure of a Klein bottle, a non-orientable, two-dimensional surface composed of two Möbius strips glued together. The Klein bottle is also the space spanned by the orientations and spatial phases of flashed visual gratings. We hypothesized that a population of simple cells in primary visual cortex would lift the Klein bottle continuously to a covering space, possibly in a one-to-one fashion. First, we devised a hierarchical method to decode the Klein bottle using cohomology and investigated the feasibility of such a decoding in a standard model of simple cells with Poisson spike statistics. We found that we could reconstruct the Klein bottle with as

few as 25 Poisson neurons. In experiments on mice, we flashed sine-wave gratings with 20 orientations and 20 spatial phases 50 times for 80 ms each, but in a random order. The population activity signatures from 41 isolated units were consistent with a Klein bottle, with a surprisingly clear signal that the spatial phase had a circular representation. To compare, we performed TDA on visual neuron responses to natural images based on publicly available data. The most prominent loop component found in TDA was strongly correlated with local orientation. Natural images and gratings, therefore, lead to a comparable topology of population activity

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Poster

PSTR548. Plasticity of the Visual System

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR548.01/FF7

Topic: D.06. Vision

Support: Fitzgerald Translational Neuroscience Fund

Title: Studying the spatiotemporal dynamics of organotypic visual thalamocortical slices by utilizing a 3D-multi electrode array

Authors: *J. T. LIM[1], M. KHANTAN[2], E. ANGELOPOULOS[1], N. SHAWKI[1], I. OBEID[4], A. NAPOLI[1], D. CULLEN[5], M. SERRUYA[3];
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Abstract: Introduction: The mammalian visual circuit is a well-suited model for investigating mechanisms of plasticity, information processing and learning. Stimulus-selective response plasticity demonstrates a durable change in V1 electrophysiological signatures in response to novel stimuli. Despite the visual circuit's extensive literature *in vivo*, there are no *ex vivo* 3D tissue models that investigate the electrophysiological properties of organotypic brain slices on a 3D multi-electrode array (MEA). We aim to record within tissue and implement chronic patterned stimulation to study experience-dependent plasticity. To validate this novel approach, we aim to characterize the spatiotemporal activity of thalamocortical circuitry within an organotypic brain slice.
Methods: We partnered with Neuronexus to build an *ex vivo* setup for extracellular potential recordings on tissue where the 3D MEA was secured to the bottom of the dish. Recordings and electrical stimulation were performed by an Intan acquisition system. We sectioned organotypic brain slices 500um thick at a 55 degree angle to preserve the visual circuitry from adult rodents. Slices were cultured on the 3D probe for over 14 days *ex vivo* inside a customized incubator-

physiology rig with automated media exchange to minimize mechanical disruption of the tissue-electrode relationship. Daily recordings were performed for post-hoc analysis with custom Matlab scripts.
Results: The structural phenotype of thalamocortical slices were validated via immunohistochemical staining and anterograde tracing from LGN to V1. Spontaneous multi-unit and field potential activity were observed in the recorded regions of the slice. Longitudinal recordings were made possible by having the electrophysiology rig integrated directly into the incubator. We aim to observe increased peak firing rates in V1 driven by electrical stimulation of LGN. Spontaneous activity decreased over time.
Conclusion: Our findings demonstrate the ability to longitudinally record field potentials and multi-unit activity from organotypic brain slices with a 3D MEA ex vivo. Specifically, we were able to target the LGN-V1 thalamocortical circuitry within the slice. Focal stimulation of LGN is expected to lead to an increased peak firing rate in layer IV visual cortex. Neural activity was confirmed via pharmacological interventions by selectively modulating glutamatergic activity and blocking sodium channels. This novel experimental approach shows the feasibility of recording from the same target region over time, where cultures can be stimulated with chronic structured patterns of activity to study mechanisms of plasticity.

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Poster

PSTR548. Plasticity of the Visual System

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Program #/Poster #: PSTR548.02/FF8

Topic: D.06. Vision

Support: R21-EY034297

Title: Mouse thalamic visual prosthesis model for studying cortical plasticity in blindness

Authors: *L. MESIK[1,2,3], J. U. KANG[4,3], H.-K. LEE[5,3];
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Abstract: Neural prostheses hold the promise of restoring lost sensory function, yet their adoption is hindered by multiple challenges. One of these is the reduced plasticity potential of adult neuronal circuits. We set out to develop a mouse model of a visual prosthesis that would allow us to test different methods of enhancing the ability of neural circuits to adopt to neuroprosthetic stimulation. Our approach is to express the excitatory opsin ChRmine in the visual thalamus (dLGN) of enucleated mice, implant a GRIN lens to gain optical access to a subset of these dLGN neurons, and perform pattern optogenetic stimulation using a digital micromirror device coupled to the GRIN lens through fiber bundle. At the same time, the mice

are implanted with a cranial window over the primary visual cortex, allowing us to perform 2-photon calcium imaging of neuronal activity during optogenetic stimulation. We train the mice to perform visual detection and discrimination in a go-nogo task with optogenetic stimuli. Most mice learn to detect these stimuli without trouble, although so far only about third of these mice was able to also successfully proceed to stimulus discrimination. We are currently also in the process of recording and analyzing calcium imaging data to gain understanding of how much the neuronal representations of optogenetic stimuli in V1 can change at the baseline level without any pharmacological intervention.

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Poster

PSTR548. Plasticity of the Visual System

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Topic: D.06. Vision

Support: International Max Planck Research School for Neuroscience (JS)
CRC 889 (SL)
Dorothea Schloezer-Programm (CS)

Title: Orexin/hypocretin knockout causes precocious closure of critical period for ocular dominance plasticity in mouse primary visual cortex

Authors: *J. SATHIYAMANI[1,2,3], T. S. NAIR[4,3], S. LÖWEL[4,2], C. SCHÖNE[4,2];
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Abstract: Previous studies have highlighted that lateral hypothalamic orexin/hypocretin (OH) circuits drive both physical and cortical arousal by increasing locomotion and cortical gamma power during wakefulness. In turn, physical activity and cortical gamma activity are key physiological variables affecting the gain and plasticity of neuronal responses in the primary visual cortex (V1). OH also directly activates neurons in the dorsolateral geniculate nucleus and layer 6b of V1. Given the links between OH and visual circuits, we tested if OH signaling plays a role in experience-dependent V1 plasticity during the critical period (CP) for ocular dominance (OD) plasticity (ODP) in juvenile orexin knockout (KO) and wildtype (WT) mice (both sexes). Using intrinsic signal optical imaging (OI), 4 days of monocular deprivation (MD) were sufficient to induce an OD-shift towards the open eye in binocular V1 of age-matched WT and orexin KO mice in the early (P21-P23 at OI) and middle CP (P24-27; OD-index (ODI), WT/KO: -0.15±0.04/0.03±0.04, n=10/11). In contrast, during the late CP (P28-P35 at OI), orexin KO mice failed to show ODP, unlike WT mice (ODI, WT/KO:0.03±0.03/0.23±0.04, n=6/7; $p<0.001$, 2-

way ANOVA with Holm-Sidak's test for multiple comparisons): the previously closed contralateral eye continued to dominate V1 in orexin KOs, while V1 was about equally well activated by visual stimulation of both eyes in orexin WT mice. Notably, preliminary data indicate that a longer MD period (7 days) during late CP recovered OD-shifts in orexin KO mice. Without MD, ODIs were similar between genotypes (ODI (P21-35), WT/KO:0.30±0.03/0.27±0.02, n=15/13). To ensure that basic spatial vision was similar between groups, we measured the spatial frequency threshold (SFT) of the optokinetic reflex and its enhancement following MD. Baseline SFT (SFT [cyc/deg], WT/KO:0.38±0.002/0.39±0.002, n=24/18) and its enhancement on day 4 (WT/KO:0.46±0.005/0.46±0.003, n=14/11) were similar in orexin KO and WT mice during late CP ($p>0.05$, two-way ANOVA with multiple comparisons), suggesting that the subcortical circuits mediating this reflex and their plasticity were unaffected. Finally, preliminary data indicate that total PV^+ cell numbers in V1 were not modified by orexin KO. Together, our data show that OH neuropeptides are important for experience-dependent functional plasticity in mouse V1, with a KO strongly impairing OD-shifts in late CP for ODP. *Acknowledgments: Funding support by International Max Planck Research School (IMPRS) for Neuroscience and CRC 889 project B5 (to SL), Göttingen, Germany*

Disclosures: J. sathiyamani: None. **T.S. Nair:** None. **S. Löwel:** None. **C. Schöne:** None.

Poster

PSTR548. Plasticity of the Visual System

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Program #/Poster #: PSTR548.04/FF10

Topic: D.06. Vision

Support: CRC 889 project B5 (SL)
IMPRS for Neuroscience (JS)
Dorothea Schlözer-Programm (CS)

Title: Ocular dominance plasticity positively correlates with amount of running in individual mice, but running cannot rescue plasticity in adult orexin/hypocretin KO mice

Authors: *C. SCHÖNE[1], J. S. SATHIYAMANI[2], M. GURANDA[3], S. LÖWEL[2];
[2]Dept. of Systems Neurosci., [1]Univ. of Goettingen, Göttingen, Germany; [3]Univ. Med. Ctr. Göttingen, Göttingen, Germany

Abstract: One week of running wheel (RW) exposure during monocular deprivation (MD) is sufficient to restore ocular dominance plasticity (ODP) in the primary visual cortex (V1) of adult mice (>postnatal day 110, Kalogeraki et al 2014). Here, we investigated which properties of RW enrichment promote ODP and if arousal and locomotion promoting orexin/hypocretin circuits play a critical role for this plasticity. To this end, we developed a novel "gated"-RW cage (gRW) in which group-housed RFID chipped mice were tracked to quantify individual RW activity during MD. The gRW consisted of a standard rat cage (43cmx27cm) and a separate RW

compartment, separated by a seesaw that automatically blocks the entrance for other mice. gRW data was registered using a raspberry pi4 and custom python scripts. Average running distance per mouse/day was consistent with previously published data (1.3±0.3 km/d, n=21). To quantify ODP, we visualized V1 activity 7 days after MD using intrinsic signal optical imaging and calculated an OD index (ODI, Cang et al 2005). Wildtype (WT) female adult (>P110) gRW mice showed a significant OD-shift after 7 days of MD towards the previously deprived (contralateral) eye (ODI, no MD/MD: 0.24±0.04/0.06±0.04, n=10/10, $p<0.01$ unpaired t-test). Our data suggest a striking correlation between the total time and distance run by each mouse over 7 days with ODI after MD: animals that ran more had larger OD-shifts, i.e. lower ODIs. Running speed and number of running bouts did not influence ODIs. Interestingly, running during the first day of MD was the strongest predictor for ODI measured 7 days later, as running distance on this day alone already correlated with ODI (Pearson correlation of ODI with running distance on day 0: $p<0.05$, other days: $p>0.05$). Mouse locomotor activity is correlated with high gamma activity and increased arousal - brain states associated with learning and plasticity, potentially promoting ODP. Since orexin neurons drive both locomotion and high gamma wakefulness, we tested ODP and running behaviour in orexin KO mice. RW did not rescue ODP in adult (>P110) orexin KO mice after 7-day MD, unlike in WT controls (ODI WT/KO: 0.10±0.03/0.31±0.06, n=17/4, 2-way ANOVA, $p<0.01$, single housed RW males and gRW females for each genotype). The absent rescue of ODP in orexin KO mice may be partially explained by reduced running of orexin KO compared to WT mice (WT/KO: 1.8±0.4/0.9±0.4 km/d, n=17/4, 2-way ANOVA, $p<0.05$). Together with our accompanying poster (Sathiyamani et al. 2023), our data strongly support orexin's involvement in experience-dependent ODP in mouse binocular V1, the mechanisms of which remain to be elucidated.

Disclosures: **C. Schöne:** None. **J.S. Sathiyamani:** None. **M. Guranda:** None. **S. Löwel:** None.

Poster

PSTR548. Plasticity of the Visual System

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Program #/Poster #: PSTR548.05/FF11

Topic: D.06. Vision

Support: NEI R00EY028964

Title: Sleep is required for experience-dependent sequence plasticity in mouse primary visual cortex.

Authors: *N. HOSAMANE[1], J. P. GAVORNIK[2], M. S. SIDOROV[1];
[1]Children's Natl. Med. Ctr., Washington, DC; [2]Boston Univ., Boston Univ., Boston, MA

Abstract: The brain is constantly being remodeled, both structurally and functionally, in response to experience. This phenomenon, known as experience-dependent plasticity, has been studied extensively using the visual system. Previous work has demonstrated that repeated

exposure to a sequence of four visual stimuli presented in the same order elicits “sequence plasticity” in mouse primary visual cortex (V1). Sequence plasticity is expressed through increases in the magnitude of V1 visually evoked potentials (VEPs) and is specific to the familiar visual sequence, as opposed to the same stimuli presented in an unfamiliar order. Prior studies have found that sleep promotes V1 plasticity in response to repeated exposure to a single familiar stimulus; however, little is known about the role of sleep in V1 sequence plasticity. We hypothesize that sleep is required for sequence plasticity in V1. Using *in vivo* electrophysiology, we recorded V1 VEPs in awake, head-fixed mice viewing a monitor presenting oriented grating stimuli. Using a between-subjects longitudinal design, we recorded responses at baseline and used gentle handling techniques to sleep deprive mice. After six hours, mice were exposed to the familiar sequence and responses were recorded. Subsequent recordings were done once a day with *ad libitum* sleep. Mice were between P60-P100 and both males and females were tested. There was no difference between VEP magnitudes at baseline and after six hours of sleep deprivation, however, subsequent sleep resulted in VEP magnitudes comparable to the control sleep condition. Overall, we conclude that sleep deprivation inhibits plasticity in V1, indicating that sleep is necessary for V1 sequence plasticity.

Disclosures: **N. Hosamane:** None. **J.P. Gavornik:** None. **M.S. Sidorov:** None.

Poster

PSTR548. Plasticity of the Visual System

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR548.06/FF12

Topic: D.06. Vision

Support: F31EY033649-01
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SmartNets 860949

Title: The synaptic mechanisms behind somatic orientation matching in the binocular visual cortex

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Abstract: During critical periods in postnatal development, cortical circuits undergo significant plasticity and refinement due to experience. This is evident in the rodent binocular visual cortex (bV1), where visual experience from p22 to p34 is critical for rewiring binocular circuits to align visual information from the ipsi and contra eye. As a result, the preferred orientation of bV1 neurons becomes matched between the two eyes. While the maturation of bV1 neurons is well

understood at the somatic level, no studies have thus far explored whether somatic orientation matching is linked to changes at the synaptic level and which plasticity mechanisms facilitate this alignment. We hypothesized that Hebbian and heterosynaptic plasticity regulate somatic orientation matching during the critical period by modifying synaptic inputs correlated with the postsynaptic neuron or with synaptic neighbors, respectively. To test our hypothesis, we used in vivo two photon calcium imaging to track the eye-specific and binocular visual responses of neurons and their dendritic spines. At the somatic level, we found that responses to the ipsi eye become gradually aligned with the soma's binocular preference over development, whereas contra eye responses are already matched at p22. At the synaptic level, we found that most dendritic spines exhibit dynamics in their structural and functional properties from p22 to p34. To determine whether Hebbian and heterosynaptic plasticity impact the structural turnover of dendritic spines, we quantified the signal correlations between the tuning properties of dendritic spines and the soma, and the trial-to-trial correlations between dendritic spines and their neighbors. We found that retained spines are more correlated to somatic tuning preferences than those that have been added or lost, indicating a role for Hebbian-like mechanisms. However, trial-to-trial correlations to spine neighbors are not significantly different among retained, lost, or added spines, suggesting that heterosynaptic interactions may not affect spine turnover. To quantify the contribution of Hebbian and heterosynaptic plasticity in driving somatic orientation matching, we built a computational model that simulates the turnover of dendritic spines from p22 to p34. On simulated data, we find that Hebbian plasticity alone cannot drive the alignment of orientation matching. We are currently testing whether our model can recapitulate the final state of synapses we observe in vivo using the initial functional and structural properties of dendritic spines. Overall, our research provides insights into how synaptic mechanisms shape the refinement of sensory circuits.

Disclosures: K. Tsimring: None. **C. Cusseddu:** None. **K. Jenks:** None. **G.R. Heller:** None. **J.P. Ip:** None. **M. Sur:** None.

Poster

PSTR548. Plasticity of the Visual System

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Topic: D.06. Vision

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Title: Primary visual cortex plasticity after optic tract transection in the rat

Authors: *B. MOLINA[1], M. OLOARTE FLORES[2], M. RODRIGUEZ MERUELOS[3], O. LARA-GARCÍA[2], M. LARA GARCIA[3], P. PACHECO[1];
[1]UNAM, Xalapa-Enríquez, Ver., Mexico; [2]Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; [3]Univ. Veracruzana, Xalapa, Mexico

Abstract: Optic tract (OPT) has been analyzed through behavioral, histological, and electrophysiological studies. From histological studies, it is known that OPT axons reach the dorsal region of lateral geniculate nucleus (LGN) and from here, primary connections to visual cortex arise. On the other hand, it has been mentioned that acute or chronic central nervous system lesions induce or stimulate a neuronal hypersensitivity state known as neuronal unmasking. Hence, in the present study we explored in adult male Wistar rats primary visual cortex electrophysiological activity related to lateral geniculate body cells connected to their corresponding OPT. Simultaneous monopolar electrical activity of both right and left primary visual cortex was obtained by binocular or monocular photic stimulation from intact animals and with acute or chronic unilateral OPT transection. When binocular stimulation was applied, the intact animals recordings presented short and long latency components in both cortices. These components were predominant in contralateral cortex, with a lesser amplitude depending upon the monocular eye stimulated. In acute left OPT lesioned animals, a reduced amplitude in short and long latency components was observed in the contralateral cortex to the lesion, while these components were absent in ipsilateral cortex to the lesion after binocular stimulation. Interestingly, monocular eye stimulation produced short and more long latency components in contralateral cortices, while these were absent in ipsilateral cortex. In chronic left OPT lesioned animals, shorter latencies, higher amplitudes as well as more components were obtained in contralateral cortex after binocular or monocular stimulation; meanwhile, ipsilateral cortex recordings were similar to those observed in intact animals, albeit a reduced basal activity was noticed. Our results showed that visual pathway organization possess a bilateral modulation on primary visual cortex. This modulation is modified after OPT transection, which induces a neuronal unmasking or hypersensitivity effect. It is likely that an OPT reconnection process is present, as ipsilateral cortex recordings in chronic lesioned animals presented intact-like evoked potentials.

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Poster

PSTR548. Plasticity of the Visual System

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Program #/Poster #: PSTR548.08/FF14

Topic: D.06. Vision

Title: Transient loss of visual inputs affects microglial density and phenotypic variation

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Abstract: Microglia have putative roles in mechanisms critical to neural functioning such as synaptogenesis, brain reorganization, and neuroinflammation. Microglia can assume different morphologies indicative of their response to the neural environment and their functionality,

however, the stimuli underlying such changes to microglia are not fully understood. The goal of this study was to determine whether transient loss of visual inputs impacts microglial density or phenotypic representation in different layers of the visual cortex. Adult microglia-EGFP (Cx3cr1) mice were subjected to either 21 days of complete darkness (dark-housing; N=19) or 12:12 light:dark cycle (control; N=18) beginning at postnatal day 90. At the completion of the 21-day manipulation, all animals were tested for depth perception in a lit room using two depth perception tasks (visual depth test and visual cliff). Animals were then euthanized with sodium pentobarbital and transcardially perfused. Brains were harvested, flash frozen, and cryostat-sectioned (at 40 microns) for histological analyses. Digital montages of entire sections were captured using fluorescence microscopy. Microglial density in superficial, middle, and deep layers of the primary visual cortex and other control cortical regions (primary auditory and entorhinal cortices) was quantified. Microglia were also categorized into different phenotypes based on morphological parameters. We report that transient loss of visual inputs caused deficits in the depth perception of dark-housed animals. Control animals made significantly more entries than dark-housed animals ($p < 0.05$) into “safe” areas of the visual cliff. Visual depth test data validated the disruption of depth perception in that dark-housed animals took longer to detect an emerging platform ($p < 0.05$). Ramified and dystrophic microglia were the most and least common phenotypes observed, respectively, in control animals. The profile of microglia phenotypes changed substantially after the dark-housing manipulation. Furthermore, while no difference was detected in overall microglial density in gray matter ($p > 0.05$), layer-specific differences were observed for both microglia density and phenotypic variation. We conclude that short-term loss of visual inputs is sufficient to cause changes in the density and phenotypic profiles of microglia. These observations may provide insight into mechanisms important for the reorganization of circuits in the visual cortex.

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Poster

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Topic: D.06. Vision

Support: Max Planck Society
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Title: Two-timeframe monosynaptic rabies tracing reveals changes in neuronal connectivity contributing to ocular dominance plasticity

Authors: D. K. PAYNTER[1], A. A. HENNRICH[2], K.-K. CONZELMANN[2], T. BONHOEFFER[1], M. HÜBENER[1], *P. M. GOLTSTEIN[1];

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Abstract: Synaptic plasticity allows the brain to adapt to environmental changes. For instance, temporarily depriving one eye of its input via monocular deprivation (MD) shifts the responses of neurons in mouse binocular visual cortex towards the open eye. This functional change is accompanied by changes in synaptic strength and increased formation of new dendritic spines. However, the presynaptic partners of the new dendritic spines have not yet been identified. Thus, it remains unclear whether the effect reflects an increase in synapse number between already connected neurons, or whether it entails the formation of connectivity with novel presynaptic cells.

To identify newly formed presynaptic inputs, we developed a technique for two-timeframe monosynaptic rabies virus tracing: A helper construct is expressed in starter cells in the brain of a tdTomato reporter mouse. Then, we infect the starter cells with a newly-designed N2c strain rabies virus carrying eGFP and inducible Cre recombinase, tracing existing inputs to the starter cells. After labeling the initial inputs, we systemically inject 4OHT to induce expression of tdTomato, thus creating a "snapshot" of all rabies-infected neurons. Inputs to starter cells continue to form after 4OHT has been metabolized. Finally, we read out two sets of input cells: those infected before 4OHT injection (timeframe 1), expressing eGFP and tdTomato, and those infected after 4OHT injection (timeframe 2), which express only eGFP.

We employed this method to search for new connections formed during ocular dominance (OD) plasticity in adult mice. The experiment was arranged such that timeframe 1 inputs reflected pre-MD inputs to neurons in binocular V1 contralateral to the closed eye, while timeframe 2 captured newly-labeled (and thus presumably novel) inputs. Shifts in OD index were confirmed with intrinsic optical signal (IOS) imaging. Preliminary results show that MD caused an overall increase in the fraction of new inputs (46.0%, n=6 mice) compared to control mice that had not undergone MD (34.2%, n=8 mice). A brain wide search revealed that several regions, particularly V1 as well as higher visual areas and thalamus, contributed to this trend of increased novel connectivity. Crucially, we found that a substantial fraction of new inputs originated in the opposite visual cortex (MD: 52.6%, Control: 34.9%), which largely represents the eye that had been left open during MD. These results lay the foundation for using two-timeframe tracing to investigate the role of novel neuronal connectivity in OD plasticity, and point to callosal connections as a prominent source for providing strengthened open eye inputs to the visual cortex after MD.

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Poster

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Topic: D.06. Vision

Support: NEI IRP

Title: Seasonal Neuroplasticity within the Primary Visual Cortex of Hibernating Thirteen-lined Ground Squirrels

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Abstract: Evidence of seasonal neuroplasticity has been seen in the brain anatomy of hibernating and seasonally breeding animals. Many of the neuronal differences in volume, dendritic length, spine density, and soma area that arose during hibernation and/or the winter season were often reversed once the animal's body temperature increased (i.e. gentle handing to induce arousal or the coming of spring). Pronounced seasonal neuroplasticity in hibernating ground squirrels has been observed in the hippocampus, thalamus, and somatosensory cortex. Here, we focus on the visual pathway of thirteen-lined ground squirrels to see if similar neuroplasticity arises with hibernation. We looked at ground squirrels in three different states (4 animals per group): hibernation, interbout arousal, and awake. Hibernating animals were in torpor and had a body temperature below 35 °F, while awake animals had not entered hibernation and had an average body temperature of 100 °F. Interbout arousal ground squirrels were aroused from torpor using gentle handling and had body temperatures ranging from 96.2 °F to 103 °F. All samples were collected in March to exclude potential seasonal differences unrelated to hibernation. We use a Golgi-staining technique to compare the dendritic length and branching of layer 2 and 3 pyramidal neurons in the primary visual cortex (V1). Experimenters were blind to the group. Neurons in awake ground squirrels had longer basal dendrites and more branches closer to the soma compared to both interbout arousal ($p=0.0167$, $p=0.0077$) and hibernating ground squirrels ($p=0.0028$, $p=0.0003$). In contrast, these features were not different between the interbout arousal and hibernating groups (all $p>0.5$). We also found no differences in apical dendrites regarding dendritic length and branching between any of the groups (all $p>0.1$). Our findings show that that there are neuronal differences in the dendritic length and branching of layer 2 and 3 V1 pyramidal cells in hibernating ground squirrels compared to awake and interbout arousal ground squirrels, consistent with findings in other brain areas, suggesting that seasonal plasticity extends to the visual pathway.

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Poster

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Title: Pharmacological deactivation of the cortex reveals how the cortical reorganization that results from the early loss of vision in the short-tailed opossum (Monodelphis domestica) is associated with behavioral and kinematic adaptations in sensory-guided ethologically relevant tasks

Authors: *C. R. PINEDA, L. KRUBITZER;
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Abstract: Early sensory input dramatically impacts neocortical organization and behavior. In humans, congenitally blind adults adopt new strategies to navigate and acquire new means of written communication, such as Braille reading which has been shown to be functionally represented in the occipital cortex. These behaviors are supported by the spared senses which blind adults use in to generate adaptive behavior. However, despite the importance of these compensatory behaviors to the lives of congenitally blind humans, it is not known how the reorganized occipital cortex contributes to such behavioral compensation. Studies in our laboratory in short-tailed opossums (*Monodelphis domestica)* that are bilaterally enucleated at post-natal day 4 (EB), before the formation of retinogeniculate and thalamocortical pathways, reveal drastic changes to the behavior and cortical organization of EB opossums. For example, EB opossums have lower texture discrimination thresholds than sighted controls (SC) and lower error rates in complex navigation tasks, which are accompanied by postural differences when compared to SC opossums (Englund et al., 2020; Rammamurthy et al., 2021). These differences in behavior co-occur with the functional reorganization of the visual occipital cortex (rV1) of EB opossums. Studies show that the cortical area usually devoted to vision in SC opossums is reorganized and represents somatosensory and auditory stimuli in EB opossums (Kahn & Krubitzer, 2002). To examine how EB opossums compensate for blindness in an ethologically relevant context, we trained EB and SC opossums in a skilled reaching and ladder-rung walking tasks. Video recordings allowed us to extract pose kinematics in three dimensions using DeepLabCut, a deep learning algorithm (Mathis et al., 2018). Results show from tests that EB opossums compensate for the lack of vision under light and dark conditions, and both EB and SC rely heavily on tactile and olfactory information. Kinematic differences accompany the compensation in performance accuracy. To examine what compensatory function the reorganized occipital cortex imparts to the performance of EB opossums, the occipital and somatosensory cortex of EB and SC opossums was pharmacologically deactivated during reaching and grasping, and ladder-rung walking tasks. Characterizing the effects of the deactivation of both S1 and rV1 allowed us to determine how the behavioral contributions of rV1 compare to that of S1 in both ethologically relevant behavioral tasks. These data contribute to the growing body of knowledge about the impact of cortical reorganization on the behavioral compensations of congenitally blind individuals.

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Poster

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Title: Late-postnatal development of complex natural scene processing does not disrupt the representation of simple visual features in primary visual cortex

Authors: T. FUCHS[1], B. B. JEON[1], M. MOSSO[1], A. L. BARTH[1], ***S. J. KUHLMAN**[1,2]; [1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Physiol. and Biophysics, Univ. at Buffalo, Buffalo, NY

Abstract: The majority of neurons in the primary visual cortex (V1) respond reliably to simple oriented bars of light, commonly referred to as grating stimuli. In the adult, after the closure of the classic critical period for ocular dominance plasticity and spatial acuity, these responses are resistant to drift in baseline conditions over the course of weeks to months and do not require continuous visual input to remain stable. Recent evidence demonstrates that although most V1 neurons are driven by grating stimuli, grating stimuli are not the preferred stimuli of adult V1 neurons. For example, stimuli containing complex features, such as natural scenes, elicit higher amplitude responses. To address whether complex natural scene stimulus responses follow a similar developmental trajectory to that of simple grating stimulus responses, we recorded excitatory neuron activity in the V1 of mice presented with both grating and natural scene stimuli using 2-photon calcium imaging, from 4 to 8 weeks of age. Stimulus encoding of natural scenes, assessed using classifiers, improved during this time window. Increased selectivity and reliability of natural scene responses could account for the improvement. In contrast, response amplitudes for grating stimuli decreased slightly during this same time period. Two weeks of dark exposure, initiated at 4 weeks of age, interrupted the trajectory of natural scene response development, whereas grating responses were resistant to dark exposure at this age. Notably, during normal development, despite the slight loss of response amplitude to grating stimuli, improved natural scene processing did not appear to disrupt the representation of grating stimuli in V1, assessed using classifiers. Our results indicate that the circuit elements required for natural scene processing continue to mature past the closure of the classically defined critical period. Despite these changes, grating stimulus encoding remains intact as the system gains new functionality. We are currently examining the role of somatostatin neurons in facilitating the acquisition of new functionality without perturbing existing sensory processing, and are using the development of the visual system as a model to study continual learning.

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Poster

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Title: Neural correlates of natural forgetting of a visual recognition memory

Authors: *S. NIRAULA[1], N. PRITCHETT[1], A. HOLT[1], A. ROUSE[2], J. SUBRAMANIAN[1]; [1]The Univ. of Kansas, Lawrence, KS; [2]Neurol., Univ. of Kansas Med. Ctr., Kansas city, KS

Abstract: Neural correlates of natural forgetting of a visual recognition memory
Suraj Niraula[1], Nick Pritchett[1], Austin Holt[1], Adam Rouse[2], and Jaichandar Subramanian[11]Department of Pharmacology and Toxicology, University of Kansas, Lawrence KS 66045, USA[2]Department of Neurosurgery, University of Kansas Medical Center, Kansas City, KS 66103
Many long-term memories are forgotten over time. The natural decay of memories due to the turnover of proteins and interference from related memories have been proposed as contributing factors to forgetting long-term memories. Here, we test how everyday visual experience may contribute to forgetting long-term visual recognition memory. We studied visual recognition memory (VRM) of a grating stimulus of specific orientation in C57BL/6 mice. At the cellular level, we found that repeated experience of a grating stimulus reduces the fraction of neurons responsive to that stimulus but increases it for natural images. Furthermore, the similarity between familiar grating stimulus-evoked and spontaneous activity increases. We tested whether the forgetting time period is associated with the reversal of all aforementioned neural correlates of familiarity. We also determined the role of everyday visual experience in mediating forgetting.

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Poster

PSTR549. Representation of Faces and Bodies

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Title: Neuronal responses to Faces, Objects and Pareidolia stimuli in the macaque AM and AF face patches

Authors: *W. ROBISON[1,2], K. W. KOYANO[2], J. TAUBERT[3,4], E. N. WAIDMANN[5,2], D. A. LEOPOLD[2,6];
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Abstract: Primates are social animals that rely heavily on the visual analysis of faces in their daily interactions. This ability is supported by specialized face-processing areas across the primate brain, most notably in the inferior temporal cortex. In macaques, these areas, known as face patches, contain a high proportion of neurons that respond more to face images than to object images. How would face-selective neurons in these areas respond to ordinary objects that, by happenstance, resemble faces? This phenomenon of face pareidolia has previously been tested in neuroimaging studies, with pareidolia stimuli eliciting more robust fMRI responses in some face patches than their non-illusionary counterparts. In the present study, we recorded responses of individual neurons to real faces, pareidolia stimuli, and non-face objects from anterior medial (AM) and anterior fundus (AF) face patches, which are thought to be involved in the identification of faces. We found that the responses to pareidolia stimuli in both patches more closely resembled the responses to non-face objects than to real faces. In the AM face patch, some neurons responded more robustly to the pareidolia stimuli than to the corresponding non-face objects; however, these responses were still smaller than those evoked by real face images. These findings suggest that the striking face-like appearance of pareidolia stimuli does not stem from a strong engagement of anterior face patch neurons. These results align with recent electrophysiological findings showing that AF and AM face patch neurons are driven by less by the configurational geometry of facial elements, thought to be an important factor in the illusion of face pareidolia, and more by details of the local facial features themselves.

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Poster

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ANR-20-CE37-0005

Title: Comparing face-selectivity in fMRI-defined face patches of macaque frontal cortex

Authors: *E. MERGAN[1,2], Q. ZHU[1,2,3], X. LI[1,2], R. VOGELS[1,2], W. VANDUFFEL[1,2,4,5]; [1]KU Leuven, Leuven, Belgium; [2]Leuven Brain Inst., Leuven, Belgium; [3]CEA DRF/JOLIOT/NEUROSPIN, Univ. Paris-Saclay, Gif-sur-Yvette Cedex, France; [4]Radiology, Harvard Med. Sch., Boston, MA; [5]Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Face-selective patches have been described in the macaque ventrolateral prefrontal cortex and orbitofrontal cortex, but a detailed fMRI-guided electrophysiological characterization of the cells across all these patches is currently lacking. We performed an fMRI-guided electrophysiology study comparing category selectivity, spatial sensitivity, and face-selective response latencies across these areas. In addition, we aimed to identify stimulus features driving these face-selective frontal neurons. Specifically, 3 rhesus monkeys passively viewed face and non-face images, while we recorded with a linear 16-channel V-probe from a large number of cells in 2 face patches in the orbitofrontal sulcus (POa and POp), 1 face patch near the arcuate sulcus (PA), and as a comparison, 1 in anterior inferotemporal cortex (face patch AM in 1 subject). Next, based on the recorded single unit activity, we trained an artificial neural network-based encoding model to accurately predict responses of these face patches and screened their responses to a large set of novel images. Moreover, by applying the Bubbles technique to the predicted responses on the preferred stimuli, we revealed the image fragments which may drive these neurons. We found that face patches POp and AM contained the highest fraction of face-selective cells, followed by POa and PA. The latencies of responses to faces were remarkably fast. The vast majority of face-selective cells in the 2 orbitofrontal face patches showed (face-selective) latencies that were as fast as those in face patch AM. Surprisingly, cells in frontal face patch PA were even faster than the cells in inferotemporal patch AM. In all frontal face patches, face-selective neurons also responded to non-face stimuli with an overall roundish shape that had some smaller round textures within the object (e.g. buttons). The Bubbles technique further revealed that local image fragments in these non-face stimuli that resembled eyes or ears might drive these neurons. The same holds true for the face images, in which the eyes most often drive the face-selective neurons. Besides category and feature selectivity, the neurons showed tuning for face location in the visual field with a bias towards the fovea and contralateral hemifield. Overall, our results suggest that cells in frontal face patches are not just processing high-level and invariant facial information, but may be also driven by lower-level image information. Moreover, the surprising differences in face-selective response latencies suggest that at least some face-information is processed much faster in frontal cortex compared to anterior inferotemporal cortex.

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Poster

PSTR549. Representation of Faces and Bodies

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Title: Brain-like Familiar Face Representation in Convolutional Neural Networks

Authors: *H. DENG, S. YUE, N. LIU;
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Abstract: Abstract: Faces are a critical visual stimulus for humans, serving as a rich source of identity-specific information necessary to identify known individuals - a crucial aspect of face processing in social species. Recently, computer vision has undergone a transformative shift with the advent of Convolutional Neural Networks (CNNs), which have exhibited human-like and, at times, even higher accuracy in face recognition. Notably, face representations in CNN models align loosely with human ones. This study aimed to investigate the similarities and differences between the performances of CNN models and humans in face recognition, offering insights into potential enhancement avenues for artificial neural networks and their application in visual research. We examined the representations of both unfamiliar and familiar faces in the VGG-face model and compared a series of face recognition abilities observed in humans, which typically show enhanced performance for familiar faces. Our results showed that CNN models, akin to humans, could recognize faces in low-resolution images, rely heavily on both high-frequency and low-frequency details, and treat eyebrows the most significant facial feature for face recognition. Additionally, texture and color cues played important roles in the face representation of the VGG-face model. Besides, the VGG-face model exhibited robustness in terms of illumination and compression changes, with the latter being less effective than human capability. Moreover, our results showed that the VGG-face model exhibited a processing preference for familiar faces, mirroring human behaviors. Importantly, we found that the VGG-face model contained neurons sensitive to familiarity, displaying characteristics akin to human brain neurons. These findings suggest that the VGG-face model mirrors human behaviors in familiar face representation, indicating the presence of familiar face representation in neural networks. Our results may potentially provide valuable guidance for the enhancement of neural networks and their implementation in visual research.

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Poster

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Title: Category-specificity and relative timing of gamma band activity recorded from subdural electrodes in human occipitotemporal cortex

Authors: D. CALBICK[1], A. D. ENGELL[2], ***G. MCCARTHY**[1];
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Abstract: Stimuli such as faces, objects, and words evoke a sequence of evoked potentials from subdural electrodes in ventral and lateral occipito-temporal cortex in humans. Prior studies from our lab and others have shown that faces evoke a prominent negative-voltage deflection (N200) in discrete regions of the fusiform gyrus and lateral posterior cortex with a modal latency of ~180 ms. These evoked potentials are associated with gamma band activity. However, the relative timing of gamma band activity for different categories of stimuli has not been thoroughly assessed across different brain regions. Here we re-investigated the category-specificity and relative timing of gamma band activity by examining the single trial spectra from &gt 6000 subdural electrodes from 59 subjects who were monitored for epileptic seizures. Nearly all cortical regions were sampled. A linear classification was applied on a training set consisting of labeled single-trial spectra corresponding to trials in which faces, scrambled faces, tools, or letter-strings were presented. Prior to transformation into the frequency domain, the average evoked potential for the category from that electrode was subtracted from that single trial. The spectra were analyzed in 20 ms steps beginning 200 ms prior to the stimulus with each step representing the average spectra over a 52 ms window. The classifier was performed individually across all electrodes for each subject and was then applied to a hold-out sample of single trials from that same subject. In the hold-out sample, above-chance levels of between-category discrimination were obtained for each of the four categories tested. Each category was tested against the remaining three categories and the pre-stimulus control period. Faces, scrambled faces, and letterstrings were all significantly discriminable at &lt 100 ms post-stimulus while tools were discriminable from the other categories between 100-200 ms. Face trials obtained the highest accuracy of ~60% while tools had the lowest classification rate at ~40% accuracy. Within electrodes, we found that for faces, significant discrimination occurred at ~50-70 ms in posterior occipital cortex. This preceded significant discriminability for faces in the more anterior ‘fusiform face area’ by 20-50 ms, suggesting a sequence of information flow within the face network.

Disclosures: D. Calbick: None. **A.D. Engell:** None. **G. McCarthy:** None.

Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR549.05/FF24

Topic: D.06. Vision

Support: Human-Centered Artificial Intelligence Institute of Stanford University Grant
Big Ideas Grant

Title: Spatio-temporal dynamics of category-selective responses in human visual cortex

Authors: *Y. D. CHEN, X. YAN, K. GRILL-SPECTOR, A. M. NORCIA;
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Abstract: Category-selective cortical areas have long been identified in ventral and lateral human visual cortex using fMRI. While much is thus known about the spatial organization of human category selectivity, less is known about the dynamics of category-selective responses. Here we use a fast periodic oddball approach coupled with Reliable Components Analysis (RCA) to identify multiple category-selective processes with different spatial topographies and response dynamics. High-density EEG (128 channels) was recorded in adult participants (N=19) who were presented with rapid natural image sequences with periodic oddball faces, cars, corridors, limbs, and characters. The latter four category images were presented randomly as the remaining images in the image stream. Two image update frequencies were used (4.286 Hz and 6 Hz, 233 msec/image and 167 msec/image, respectively). RCA identified at least two category-selective components per image category. Both topography and dynamics were largely stable across the two image update rates within a category. The RC1 response was right lateralized in occipito-temporal areas for face images and left-lateralized for character images. RC2 was generally right lateralized in midline occipital area electrodes. Response dynamics differed strongly across categories and components, being most temporally broadband for the face oddball images. The results are consistent with a traditional sensor space analyses that has shown that faces and nonface objects have distinct spatio-temporal distributions of neural activity (Jacques et al., 2016, NeuroImage). Extending this result, RCA identifies multiple neural sources for each category, each with their own topography and dynamics.

Disclosures: Y.D. Chen: None. **X. Yan:** None. **K. Grill-Spector:** None. **A.M. Norcia:** None.

Poster

PSTR549. Representation of Faces and Bodies

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR549.06/Web Only

Topic: D.06. Vision

Support: TUBITAK 3501 GRANT 119K654

Title: Prior information shapes temporal dynamics of biological motion perception: evidence from EEG and temporal generalization analysis

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[1]Neurosci., [2]Psychology, Bilkent Univ., Ankara, Turkey; [3]Aysel Sabuncu Brain Res. Ctr. and Natl. Magnetic Resonance Res. Ctr. (UMRAM), Ankara, Turkey

Abstract: Biological motion perception is essential for survival, communication, and interaction. Although neuroimaging studies have identified a dedicated brain network for processing biological motion, the influence of top-down processes on biological motion remains largely unexplored. This study aims to elucidate the impact of prior information on the neural mechanisms and dynamics of biological motion perception.
To this end, we conducted an EEG experiment using a cued individuation paradigm. Participants (N=17) performed a task in which each trial started with a presentation of a cue symbol, which predicted the action type in the subsequent biological motion stimulus (Kick or Walk). After the cue symbol, an intact and scrambled version of a point-light biological motion stimulus was presented on the left and right sides of the screen relative to central fixation. The cue was congruent with the action depicted by the biological motion target in 60% and incongruent in 20% of the trials. In the remaining 20% of the trials, the cue was not informative (neutral). Participants were required to locate the position of the biological motion stimulus. Thus, the information provided by the cues and the task was independent. To enhance task difficulty, stimuli were embedded in noise dots. Our analysis focused on the impact of congruent and incongruent prior information on biological motion perception relative to neutral prior information. To this end, we utilized temporal generalization analysis (King & Dehaene, 2014), enabling us to track the evolution of representations in the brain over time in relation to the congruency of prior information. Our findings revealed that prior information significantly influences the temporal dynamics of biological motion processing. Temporal generalization matrices indicated that correct prior information expedites biological motion perception, hinting at a quicker formation of the relevant representations (~100ms for the location of biological motion and ~400ms for action type the biological motion in the congruent condition, while ~1000ms in the incongruent condition, relative to stimulus onset). Interestingly, we observed a sequence in these representations: location information precedes action-type information in biological motion perception. These findings shed new light on the progression of neural processing during biological motion perception and underscore the need for computational models of biological motion perception to account for predictive processes and their temporal aspects. They also support the broader applicability of predictive models, extending from low-level to higher-level stimuli.

Disclosures: H.O. Elmas: None. **S. Er:** None. **B.A. Urgen:** None.

Poster

PSTR549. Representation of Faces and Bodies

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The European Union's Horizon 2020 Framework Program for Research and Innovation under Grant Agreement No 945539 (Human Brain Project SGA3)
KU Leuven C14/17/109 and C14/21/111

Title: Mesoscale functional organization of macaque face and body patches in inferior temporal cortex

Authors: *X. LI[1,2], Q. ZHU[1,2,3], I. POPIVANOV[1,4,5], R. VOGELS[1,2], W. VANDUFFEL[6,2,1,7]; [1]Res. Group Neurophysiology, KU Leuven, Leuven, Belgium; [2]Leuven Brain Institute, KU Leuven, Leuven, Belgium; [3]Cognitive Neuroimaging Unit, CEA DRF/JOLIOT/NEUROSPIN, INSERM, CEA, Univ. Paris-Saclay, Gif-sur-Yvette Cedex, France; [4]Dept. of Cognitive Sci. and Psychology, New Bulgarian Univ., Sofia, Bulgaria; [5]Clin. of Neurology, Univ. hospital "Alexandrovska", Sofia, Bulgaria; [6]Radiology, Harvard Med. Sch., Boston, MA; [7]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Early visual areas are characterized by segregated mesoscale anatomo-functional networks conveying highly specific signals. Well-known examples include color-processing channels formed by reciprocally connected blobs in area V1 with thin stripes in V2. Although scant, previous evidence also revealed small functional or anatomical subdomains in extrastriate visual areas. It is largely unknown, however, whether such mesoscale domains 1) are just idiosyncratic structures in some areas, or whether these are abundantly present and form fundamental building blocks of extrastriate cortex; and 2) whether domains of the same type also form mesoscale anatomo-functional networks. We started to address these specific questions taking advantage of our sensitive sub-mm fMRI methods. Specifically, we aimed to prove that category-selective areas consist of mesoscale functional units (MFUs) that can be distinguished from each other based on functional criteria. Second, if MFUs exist, we aimed to prove that they also have distinctive functional connectivity profiles. Third, we aim to reveal the neuronal underpinnings of fMRI defined MFUs. To achieve these goals, we first identified category selective patches of IT cortex using traditional category-selective localizers and based on the stimulus category evoking the largest response across 3 contrasts and across days. Next, we selected all the 0.216 mm^3 voxels belonging to face patch ML and body patch MSB and performed unsupervised cluster analysis on the fMRI tuning curves of all voxels to infer functional clustering. We show that voxels within both patches can be reliably segregated into 3 different subpopulations (= functional clustering). Moreover, these 3 functionally-defined groups of voxels were surprisingly well clustered in space (= anatomical clustering). Furthermore, independent high-resolution resting-state fMRI data revealed that these sub-patch clusters (MFUs) were characterized by distinct interhemispheric functional connectivity. Finally, we showed that single cell responses, recorded from fMRI-defined body patch MSB in a different monkey, can also be grouped into 3 functional clusters which correspond surprisingly well with the average high-resolution fMRI responses of the 3 MSB clusters. Overall, these sub-patch clusters displayed functional and connectional characteristics mimicking that of well-established mesoscale functional units and networks in early visual cortex. Therefore, we propose that MFUs

form mesoscale functional networks in extrastriate visual cortex. By extension, these may be fundamental architectural features of neocortex in primates.

Disclosures: X. Li: None. **Q. Zhu:** None. **I. Popivanov:** None. **R. Vogels:** None. **W. Vanduffel:** None.

Poster

PSTR549. Representation of Faces and Bodies

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Program #/Poster #: PSTR549.08/GG1

Topic: D.06. Vision

Support: ERC 2019-SyG-RELEVANCE-856495
FWO-G0E0220N

Title: Largely separate body and face networks in the macaque cortex revealed by a data-driven approach

Authors: *B. LI[1], A. BOGNÁR[2], R. RAMAN[2], G. RENS[2], R. VOGELS[2], B. DE GELDER[1]; [1]Univ. Maastricht, Maastricht, Netherlands; [2]KU Leuven, Leuven, Belgium

Abstract: Understanding the body movements of others is a critical skill for humans and for non-human primates. Previous studies have defined several body patches in the temporal cortex of macaques, but it remains unknown how these body patches are involved in the whole-brain dynamics underlying body perception. Here we used 3T functional magnetic resonance imaging (fMRI) in macaques to investigate brain activity related to body perception at the network level. Brain activity was measured from two macaques during the presentation of naturalistic videos and static images of monkey bodies and faces. Moving and static objects were also presented as controls. The functional data were analyzed with a fully data-driven approach. Seventy-five networks were extracted for each monkey using spatial Independent Component Analysis (ICA). Body and face selectivity was tested using the general linear modelling of the network time courses and the contrast analysis against the object condition. After multiple comparison corrections, we observed two networks in both monkeys showing significant selectivity for either bodies or faces. A significant preference for dynamic conditions was found in the body network but not in the face network. The two networks overlapped around the superior temporal sulcus (STS), the ventro-lateral prefrontal area (45b & 46v) and the anterior inferotemporal cortex (IT). However, by comparing the network weights at each voxel, dominance of the body or the face network was found in several adjacent clusters along the entire STS. These clusters showed a coarse medial-to-lateral organization, with the body clusters being located more medially than the face clusters. Other body clusters were found around the lateral intraparietal area (LIP), whereas face clusters were found in the anterior IT. These results extend previous findings obtained with electrical micro-stimulation of two separate networks for body and face processing (Premereur et al, Curr. Biol., 2016).

Disclosures: **B. Li:** None. **A. Bognár:** None. **R. Raman:** None. **G. Rens:** None. **R. Vogels:** None. **B. de Gelder:** None.

Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

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Program #/Poster #: PSTR549.09/GG2

Topic: D.06. Vision

Support: ERC 2019-SyG-RELEVANCE-856495
HFSP RGP0036/2016

Title: Norm-referenced encoding of facial expressions facilitates transfer learning to novel head shapes

Authors: M. STETTLER[1,2], ***A. LAPPE**[1,2], R. SIEBERT[1], N. TAUBERT[1], P. THIER[1], M. GIESE[1];
[1]Hertie Inst. for Clin. Brain Res., Tuebingen, Germany; [2]IMPRS-IS, Tuebingen, Germany

Abstract: Humans can easily recognize facial expressions displayed on non-human head shapes such as cartoon characters or animals. It is unclear how the brain achieves this strong generalization across head shapes. State-of-the-art computational models from the computer-vision literature fail to perform such domain transfers unless trained with significant amounts of data from novel domains. We demonstrate that norm-referenced encoding, which has been shown to be relevant for the representation of facial identity in area IT (Leopold et al. Nat. **442**, pages 572-575, 2006), helps to account for such spontaneous transfer of expression recognition to novel head shapes.Methods: We developed a deep-neural-network model for facial expression recognition that is based on norm-referenced encoding. The model can recognize facial expressions on previously unseen head shapes using just a single additional training example. This is achieved by storing a single reference vector for each observed head shape (domain), and domain-independent tuning vectors for each facial expression. Thus, the model assumes that facial expressions can be recognized as deviations from a reference face that are preserved across domains. For a new domain introduced after training, only the reference vector needs to be created to perform inference. We trained the model parameters using a novel, tailored optimization procedure on the tuning and reference vectors. This method allows for simple geometric interpretations of the training progress and the inner workings of the model. Results: We evaluated our model on two datasets containing facial expressions on humans, rhesus macaques and cartoon characters. It is more data efficient than models from the computer-vision literature even when using a heuristic, example-based optimization procedure. Our novel optimization procedure further improves classification accuracy across all levels of data availability. Training on only one head shape still yields high performance on other head shapes, demonstrating the model's transfer ability. Further, the model's output to facial expressions resembled human behavioral perception more closely than standard computer-vision models.

Conclusion: Norm-referenced encoding might not only play a role in the neural encoding of facial identity, but might also underlie the neural representation of facial expressions. At the same time, it may support efficiency of learning by supporting transfer of learned expressions to novel heads shapes with minimal training data.

Disclosures: **M. Stettler:** None. **A. Lappe:** None. **R. Siebert:** None. **N. Taubert:** None. **P. Thier:** None. **M. Giese:** None.

Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

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Program #/Poster #: PSTR549.10/GG3

Topic: D.06. Vision

Support: National Science and Technology Innovation 2030 Major Program (grant 2021ZD0203703)

Title: Spatiotemporal integration of sensory evidence during saccadic sampling for face and object categorization

Authors: ***Z. ZHENG**, J. HU, G. OKAZAWA;
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Abstract: Natural objects, including human faces, consist of multiple parts (e.g., eyes and mouth of a face), each of which could be informative for recognition. Saccadic eye movements can play a crucial role in collecting sensory evidence from these parts. However, the extent to which saccadic sampling of object parts contributes to face and object recognition is poorly understood. The present study used quantitative behavioral paradigms (Okazawa et al., Cell 2021; J Neurosci 2021) to examine whether and how humans integrate information across saccades during face categorization and whether saccade patterns depend on decision-making processes.Twelve human participants viewed a noisy face stimulus (morphed from two prototype faces, e.g., happy vs. sad) and reported which prototype the stimulus more closely resembled. During stimulus presentation, the morph levels of facial features (eyes and mouth) randomly fluctuated rapidly (~100ms), enabling us to test which facial features participants used to make a decision during saccadic sampling (i.e., psychophysical reverse correlation). Participants either freely made saccades within a face stimulus (eye-to-mouth distance = 5 visual degrees) during judgment (free saccade condition) or were guided to make a saccade from one feature to another by a changing fixation point (guided saccade condition). In the free saccade condition, participants often made saccades from one facial feature to another, and accumulated sensory evidence across these saccades. Furthermore, even when they fixated on one facial feature, the other unfixated features still had a significant influence on their decisions, indicating continuous integration of evidence across spatial features, despite that fixation patterns appeared to scan information locally from

each feature. We also found that the patterns of saccades did not largely change as a function of stimulus difficulty. In the guided saccade condition, both fixated and unfixated features influence participants' choice, consistent with the findings from the free saccade condition. We also found that participants integrated evidence even when they maintained fixation on a quickly moving face stimulus, mimicking the change in visual input by a saccade. Thus, the active process of saccades is not necessary for integrating information across features.Overall, humans continue to integrate spatiotemporal evidence regardless of saccadic patterns, and saccadic patterns also do not depend on the ongoing decision-making processes. These findings suggest object (face) recognition and oculomotor control can be modeled as largely independent processes.

Disclosures: Z. zheng: None. **J. Hu:** None. **G. Okazawa:** None.

Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

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Program #/Poster #: PSTR549.11/GG4

Topic: D.06. Vision

Support: 111-2423-H-002 -012 -

Title: The interaction effects of the race and spatial configuration on the ERP components for facial expression perception

Authors: T.-T. HSIUNG, *C.-C. CHEN;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: We considered two face perception related effects: (1) the other-race effect (ORE), or it is more difficult to discriminate faces with different ethnic backgrounds from an observer, and (2) the face inversion effect (FIE), or it is more difficult to recognize an inverted face than upright ones. We investigated how the event-related potential (ERP) for facial expression was affected by the interactions of ORE and FIE. We recorded the event-related potential (ERP) in 20 Asian and 20 Caucasian participants. The stimuli were either upright or inverted frontal view images of 15 Asian and 14 Caucasian models displaying one of the seven expressions: neutral, happy, sad, fearful, angry, surprised, and disgusted. We used a fast event-related design in which each trial included a fixation period of 100ms, followed by a 400ms stimulus presentation, and a 1000ms response interval. The task of the observer was to indicate the perceived expression for each image. Scalp potentials were recorded from 128 hydro-cell electrodes with a 500Hz sampling rate. The Asian participants had greater difficulty in identifying fearful expression on Caucasian faces than Asian faces, while the Caucacian participants had more problem with surprised expression in Asian faces than Caucasian ones.The N170 component of the time-locked ERP waveform showed a strong FIE for both Asian and Caucasian faces. However, the effect was culture-dependent. The Asian participants showed greater negative potential in inverted than upright faces only for Asian faces while Caucasian participants had it for both. For

both Asian and Caucasian participants, the N170 components showed a more negative potential for other race faces. Similar ORE was also found in P200. However, while Asian participants showed ORE for both upright and inverted faces, Caucasian participants only exhibited it for upright faces. This result suggests that the Caucasian participants are more susceptible to facial configuration effects than Asian participants in facial expression judgements. Our result is inconsistent with the universality theory for facial expression as facial configuration interacts with the ORE differently while perceiving different facial expressions.

Disclosures: **T. Hsiung:** None. **C. Chen:** None.

Poster

PSTR549. Representation of Faces and Bodies

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Program #/Poster #: PSTR549.12/GG5

Topic: D.06. Vision

Support: ERC 2019-SyG-RELEVANCE-856495
FWO-G0E0220N

Title: Posture and viewpoint preference of body-patch neurons assessed with a large set of monkey avatar stimuli.

Authors: *A. BOGNÁR[1], A. MUKOVSKIY[3], G. GHAMKHARI NEJAD[1], N. TAUBERT[3], M. STETTLER[4], L. MARTINI[3], A. LAPPE[3], R. RAMAN[2], M. A. GIESE[5], R. VOGELS[2];
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Abstract: Using fMRI we mapped an extensive network of body-selective patches in the macaque temporal visual cortex (Bognár at al., 2023). In order to investigate the relationship between the anatomical organization of body-responsive regions and the complexity of coded features across the visual hierarchy, we designed a stimulus set comprising 720 stimuli. The set is based on a selection of OpenMonkeyStudio data, representing 9 action classes: climbing, cornered, eating, hanging, jumping, laying, sitting, standing and walking. The stimuli featured the same monkey avatar depicted in 45 different body postures (5 variations of each class). These poses were rendered from both horizontal and top views and captured from 8 different viewing angles. We recorded neural activity from the mid-Superior Temporal Sulcus (STS; MSB) and anterior-STS body patches (ASB) using 16-channel V-probes in two male macaques (200 units per monkey per region). In the targeted regions, we found a high proportion of body-responsive multi-units using the same videos of bodies, faces, and objects as in the fMRI study throughout the 11 and 12 recording sessions in MSB and 6 and 8 recording sessions in ASB in monkey 1 and monkey 2 respectively. Videos of acting monkeys elicited stronger average responses overall. Thereafter the electrodes were left in the same position, and the static avatar stimuli were

presented for 200 ms during passive fixation. Data from the confirmed body-responsive channels were then sorted and analyzed offline. Although the latency difference of the response onset between the two regions was clearly present in only one of the two subjects, we found that the view-independent pose information could be decoded earlier from MSB than from ASB using a correlation classifier in both monkeys. Examining the neuronal responses, we observed that MSB units in each monkey exhibited strong view selectivity, with a preference for top camera views, particularly frontal and nearby views. Additionally, poses with less extended limbs, such as sitting and eating postures, were found to be less preferred in this region. In contrast, ASB units showed lower view selectivity and did not exhibit a clear preference for horizontal or top views, nor for any particular poses. However, it did demonstrate a preference for profile views, and in one subject, we observed generalization across mirror-symmetrical profile views. In conclusion, body patch neurons at different hierarchical levels encode both body pose and view, although emphasizing different properties, suggesting a distributed representation of pose and viewpoints across body patches.

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Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR549.13/GG6

Topic: D.06. Vision

Support: HHMI

Title: Understanding how the primate brain composes visual representations

Authors: *K. MOHAN[1], J. LU[2], D. TSAO[2];
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Abstract: Our visual experience is remarkably rich and structured - we instinctively parse a visual scene into its core elements, understanding who or what is in the scene, what objects are there, and how they interact. Indeed, humans can imagine new experiences by combining visual concepts they have seen before. How does the brain construct an abstract, meaningful code of our online experience? We hypothesize that the brain encodes a complex scene by breaking it down into parts and representing how these parts relate to each other through a graph-like structure, akin to how words connect in a sentence. We explore this idea by designing rich, naturalistic, 3D videos that depict a “sentence” in action, like “subject-verb-object” (for example, a monkey throwing a fruit). Using a whole brain mapping approach, we identified brain areas that preferentially respond to these sentence-like videos while monkeys watched these videos in a fMRI scanner. We found robust activations in a distributed network of brain areas, including

the inferotemporal cortex, ventrolateral prefrontal cortex, and the orbitofrontal cortex. To further pinpoint the neural population codes underlying scene understanding, we used fMRI-guided electrophysiology to target these brain areas using Neuropixels probes. Our early explorations have revealed a robust representation of individual parts of speech - subject, verb, object - in both inferotemporal and prefrontal cortex. Linear decoding results have further revealed that subject and object representations are transient, with strong subject decoding early in the trial and strong object decoding late in the trial. In contrast, verb representations were sustained and lasted throughout the trial, suggesting that causal interactions may bind objects over time. We test the hypothesis that population-level neural dynamics represents the ingredients of a visual scene - different objects and how they interact - through an abstract, compositional representation with separate slots for subjects, verbs, and objects which are dynamically filled with context-specific details. Our findings provide a starting point for understanding how the brain extracts semantic meaning from visual scenes.

Disclosures: **K. Mohan:** None. **J. Lu:** None. **D. Tsao:** None.

Poster

PSTR549. Representation of Faces and Bodies

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Program #/Poster #: PSTR549.14/GG7

Topic: D.06. Vision

Support: HHMI
NIH R01EY030650

Title: Face cell population responses contain contextual information

Authors: *D. BI[1], Y. SHI[2,1], F. LANFRANCHI[3], J. HESSE[1], D. TSAO[1,4];
[1]UC Berkeley, Berkeley, CA; [2]Caltech, Pasadena, CA; [3]Caltech/UC Berkeley, Berkeley, CA; [4]HHMI, Chevy Chase, MD

Abstract: Primate brains contain specialized neuronal populations in inferotemporal (IT) cortex, called face cells, which fire selectively to faces present in visual stimuli. Previous studies from our lab have shown that face cells' tuning properties can be compactly represented by axes in a 50-dimensional face feature space. Recently, we also found that IT cortex population responses can be parameterized by a more general object space. However, the question of what encoding scheme face cells use--whether there exists a face detection step prior to face cells, or if face cells directly represent visual features present in the image--remains largely unanswered. More generally, the representations that enable face cells to simultaneously distinguish between faces and non-face objects, but also between different facial identities, remain elusive.
To answer these questions, we designed a novel stimulus set consisting of faces superimposed on natural scene contexts--if a face detection step exists before face patches, for example, then we should observe response invariance between trials with different background contexts. We

recorded extracellular potentials with high-throughput Neuropixels probes from face patches ML and AM in the presence of these stimuli, and found that there exists some invariance in population responses to both face and background scene identity, i.e., correlation between population response vectors was elevated for both "same face" and "same scene" trials. Moreover, we found that face and context information were represented by subsets of face cells with different face selectivity indices (FSI); in particular, decoding accuracy for context identity was higher in units with low FSI while decoding accuracy for face identity was higher in units with high FSI. Finally, we compared face patch population responses to the latent representations obtained from artificial neural network (AlexNet) units, and found a distinct representational difference between real and artificial neurons, wherein artificial unit responses could consistently decode context information more readily, and higher decoding accuracy for face identity was found in low-FSI units, in contrast to our observations in biological units. Taken together, our results suggest the possibility of neural subpopulations within face patches that distinctly encode face and context information--which may be understood through a framework of variance decomposition into face detection and identification components--and provide new insights into the neural coding mechanisms of faces in natural settings.

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Poster

PSTR549. Representation of Faces and Bodies

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Program #/Poster #: PSTR549.15/GG8

Topic: D.06. Vision

Support: Simons
HHMI
FQxI

Title: Interleaved representation of conscious perception and physical stimulus in binocular rivalry

Authors: *J. K. HESSE[1], F. LANFRANCHI[2], Y. SHI[3], D. Y. TSAO[4];
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Abstract: Consciousness is arguably the most important reason why it matters to us whether we are dead or alive. Yet, the neural mechanisms of conscious perception remain unknown. To reveal the mechanisms of how new conscious percepts are generated and coordinated across different levels of the cortical hierarchy, we used newly developed primate Neuropixels probes to simultaneously record from thousands of neurons across different face patches in macaque IT cortex during a binocular rivalry paradigm. In binocular rivalry, a constant visual input evokes

spontaneous changes of conscious perception, enabling dissociation of neural representations of conscious percept and physical input. We employed a novel no-report binocular rivalry paradigm that allowed us to infer conscious percept from eye movements. We find that throughout IT, including in the most anterior face patch AM, neural activity during spontaneous perceptual switching in rivalry differs dramatically from activity when physically alternating an unambiguous stimulus. First, in contrast to physical alternation, where only the unambiguous consciously perceived stimulus is encoded, during rivalry cells in face patches encoded not only the consciously perceived but also the suppressed stimulus. Second, cells appear to temporally multiplex conscious perception and the physical stimulus: within each dominance epoch, face patches appear to oscillate on the scale of 100 ms between a representation that resembles the conscious percept, and a different representation that allows decoding of the suppressed stimulus. Taken together, these results suggest that the brain rapidly alternates between two modes, one of feedforward processing of physical input, interleaved with a recurrent mode, which interprets the input. Our results are consistent with the intriguing possibility that conscious perception is discrete, and stitched together from distinct epochs of time.

Disclosures: **J.K. Hesse:** None. **F. Lanfranchi:** None. **Y. Shi:** None. **D.Y. Tsao:** None.

Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR549.16/GG9

Topic: D.06. Vision

Support: HHMI
NIH

Title: Generating something out of nothing: an exploration of mental imagery at single neuron resolution in the human brain

Authors: *V. WADIA[1], C. M. REED[2], J. M. CHUNG[2], L. M. BATEMAN[2], A. N. MAMELAK[3], U. RUTISHAUSER[3,4], D. Y. TSAO[5,6];
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Abstract: As human beings interact with the world, we sample it, build representations of its underlying structure, and subsequently use that knowledge for future reasoning. This ability to quickly and reliably compute low dimensional structured representations of the world from the deluge of high dimensional sense data relies on an active interaction with our environment, i.e., *generative* hypothesis testing. This capability lies at the heart of many of the complicated behaviors we seek to understand in ourselves and endow our machines with -including efficient planning, inference under ambiguity, and creativity itself. However, the neural mechanisms of

such generative processes have proven to be elusive. In the visual domain, our ability to generate visual percepts without external stimulation is the basis of our memory for experiences, as episodic memories are simply a subset of all possible visual scenes we could imagine. Here we present findings on the neural mechanisms of visual imagery. We approached deciphering visual imagery by first laying out coding principles for object perception and then directly comparing responses during viewing to subsequent imagery of those images. We recorded 384 visually responsive neurons in inferotemporal (IT) cortex of 12 epilepsy patients as they viewed and subsequently visualized from memory carefully parametrized visual objects. We verified that neurons in IT cortex represent visual objects by encoding specific axes that span a high dimensional object feature space (i.e. via an 'axis code'). 223/384 visually responsive neurons (~58%) were axis tuned, and the axis model explained more variance than other models tested. We demonstrate robust reactivation of individual neurons across the brain (~35% of neurons across the brain and ~50% of neurons in IT cortex) and a recapitulation of viewing stimulus preference during pure visual imagery in IT. By closely examining how brain-wide recall events are coordinated at single neuron resolution we present evidence for generative processes in the human brain.

Disclosures: V. Wadia: None. **C.M. Reed:** None. **J.M. Chung:** None. **L.M. Bateman:** None. **A.N. Mamelak:** None. **U. Rutishauser:** None. **D.Y. Tsao:** None.

Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR549.17/GG10

Topic: D.06. Vision

Support: Simons
HHMI
FQxI

Title: Probing feedforward and feedback contributions to visual perception using electrical microstimulation

Authors: *F. L. LANFRANCHI[1], J. K. HESSE[2], Y. SHI[3], D. Y. TSAO[4];
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Abstract: Many visual functions rely on the coordination of feedforward and feedback computations within a network of brain areas, including visual search, visual attention, and visual inference informed by high-level priors. However, the distinct contributions of feedforward versus feedback pathways for different visual computations are not well understood. Here, we explore two exciting new possibilities for identifying feedforward and feedback pathways and assaying their function, leveraging recently developed NHP Neuropixels probes

combined with simultaneous electrical microstimulation. We focused our efforts on understanding pathway-specific computations in the macaque face patch system, a network of regions in IT cortex specialized for processing faces, which are known to be bidirectionally connected from anatomical tracing studies.First, we performed antidromic stimulation to identify feedforward projection neurons from face patch ML to AM, by recording with a Neuropixels probe in ML and simultaneously electrically stimulating in AM. To facilitate this approach, we developed a novel microstimulation protocol that allows recording with extremely short electrical artifacts. Second, we reversed the geometry, placing the Neuropixels probe in ML and the stimulation probe in PL, to identify feedback projections neurons from ML to PL. In both cases, we obtained a yield of ~1-3 antidromically-activated neurons per session, much higher than without a Neuropixels probe.Second, we recorded from face patch ML, while electrically microstimulating a higher-level face patch, and a lower-level face patch PL, respectively, while monkeys were in the dark or viewing stimuli, to directly compare the effects of feedforward versus feedback input on visual representations. We found that microstimulation in both face patches AM and PR could drive activity in ML, suggesting a possible mechanism to support top-down-driven functions including mental imagery.Together, these new approaches give us a remarkable new experimental opportunity to explore how feedforward and feedback projections shape perception.

Disclosures: **F.L. Lanfranchi:** None. **J.K. Hesse:** None. **Y. Shi:** None. **D.Y. Tsao:** None.

Poster

PSTR549. Representation of Faces and Bodies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR549.18/GG11

Topic: D.06. Vision

Support: HHMI
NIH Grant R01EY030650

Title: Differential encoding axes for faces and non-face objects in face cells

Authors: *Y. SHI[1,2], D. BI[2], J. GAO[2], P. BAO[3], X. DAI[4], F. LANFRANCHI[1,2], J. HESSE[2], Y. MA[2], D. TSAO[2,5];
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Abstract: Face cells within the primate inferior temporal (IT) cortex are known for their role in face encoding. Yet, these cells also manifest varied responses to non-face objects. Both encoding schemes follow axis coding. A long-standing question has been whether face cells utilize the same axis to encode both face and non-face object categories. A shared encoding axis would enable predicting a face cell's responses to faces using the axis derived only from non-face objects. Prior attempts to compare the encoding axes for face and non-face objects have been

hampered due to their distinct, non-overlapping distributions within the common feature space — an issue known as the Out-of-Distribution (OOD) problem.
To circumvent this issue, we created a collection of non-face objects that occupy the same distribution within the feature space as faces. This was achieved through controlled image masking or by identifying objects that share the same principle features as faces. We presented these stimuli to the monkeys during extracellular recordings from the anterior medial (AM) face patch — the most anterior face patch within IT cortex. Despite overlapping feature space distributions, our results showed significant divergence in the encoding axes. To further explore how the two axes guide the cell's responses to both categories, we tested the cell's response to "optimal" objects generated at the extremes of the respective axes. We employed real-time image generation coupled with high-throughput Neuropixels recording to produce optimal images for each cell on the fly. However, we were generally unable to find a non-face object capable of triggering a cell firing response as strong as that to an optimal face. Based on these results we hypothesize that early in development, a general encoding axis for non-face objects may be formed. As time progresses, face cells develop a preferential encoding schema for faces — potentially facilitated by internal connections within face cells — leading to a differential encoding approach for face and non-face objects.

Disclosures: **Y. Shi:** None. **D. Bi:** None. **J. Gao:** None. **P. Bao:** None. **X. Dai:** None. **F. Lanfranchi:** None. **J. Hesse:** None. **Y. Ma:** None. **D. Tsao:** None.

Poster

PSTR550. Cerebellum: Beyond Motor Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR550.01/GG12

Topic: E.02. Cerebellum

Support: Simons foundation
NIH Grant R01NS119519

Title: A timing adaptation task for probing the role of the primate cerebellum in non-motor learning

Authors: *G. M. STINE, B. ZHENG, M. JAZAYERI;
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Abstract: The cerebellum plays a critical role in predictive processing by forming internal models that predict the sensory consequences of actions. Such models allow the cerebellum to correct ongoing movements based on its predictions and update the model when predictions fail repeatedly. An intriguing hypothesis is that the cerebellum plays a similar role in cognitive function by correcting ongoing internal processes based on the predictions of non-motor internal models. We developed an interval timing task with non-motor and learning components designed to test this hypothesis directly. The task requires subjects to initiate an eye movement after a

target interval of time. The non-motor component involves a visual stimulus that is briefly flashed on the screen during the timing epoch. The timing of this “flash” is fixed relative to the target interval such that the subject can use it to improve its motor timing behavior. The learning component involves uncued, persistent changes in the flash time and target interval. The ratio between the flash time and the target interval are kept constant, motivating subjects to use an internal model to efficiently adjust their motor timing behavior. Critically, the flash only occurs on a random half of the trials, which allows us to assess how this internal model affects timing behavior even when the flash does not occur.
Here, we present behavioral data from three humans and one monkey performing this task. We hypothesized that subjects would (1) form an internal model that predicts the timing of the flash and (2) use prediction errors to correct their motor timing and update their internal model. Consistent with this hypothesis, we found that the flash decreased timing variability in both species and that changes in the flash time induced immediate shifts in timing behavior within a single trial. In contrast, adaptation on subsequent trials without a flash was slower, revealing the gradual update of subjects’ estimates of the current target interval. The data are explained by a model that integrates temporal prediction errors to adjust the speed of ramp-to-threshold dynamics. Such adjustment allows for fast, online correction of time estimation and slower adaptation to persistent changes in the temporal environment. These results reveal the application and adjustment of an internal model that is highly reminiscent of those used by the cerebellum for motor control, but operates on a covert, non-motor process. We plan to probe the neural underpinnings of this non-motor internal model in the dentate nucleus with future neurophysiological experiments.

Disclosures: **G.M. Stine:** None. **B. Zheng:** None. **M. Jazayeri:** None.

Poster

PSTR550. Cerebellum: Beyond Motor Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR550.02/GG13

Topic: H.08. Learning and Memory

Support: KAKENHI 21H00181
JST PRESTO JPMJPR21S4

Title: Cognitive signals in the cerebellar dentate nucleus contribute to the associative learning

Authors: *Y. AKIYAMA[1], H. YAMADA[2], M. MATSUMOTO[2], J. KUNIMATSU[2];
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Abstract: It has recently been reported that the cerebellar dentate nucleus (DN) which has strong connections with the prefrontal cortex (PFC), is involved in higher cognitive functions such as action strategy and movement timing (Kunimatsu et al., 2016, 2018). However, these studies have only reported phasic activity before movements, but not sustained activity that reflects

cognitive signals found in PFC. If DN mediates cognitive function through its interaction with PFC, it should also exhibit sustained signals. To address this question, we recorded the neuronal activity in monkey DN during visuomotor associative learning in which is known to involve the delayed-period sustained activity in PFC (Histed et al., 2009). In the associative learning task, one of two fractal objects (A or B) was presented in the center of the monitor for 500 ms. After that, the fractal object was disappeared, then two identical saccade target points were presented on left and right hemifields. If the monkey made a saccade to one of the directions that associated with the fractal object (e.g. A-right, B-left), they got a liquid reward.
We recorded the activity of 536 DN neurons in two conditions: learning condition in which novel fractal objects were used and over-trained condition in which well-learned fractal objects were used. We found that 78 of the 536 neurons (15%) showed sustained activity for the period of 200-400 ms after the fractal objects were presented, and it was greater in the learning condition than in the over-trained condition. Interestingly, the enhancement of sustained activity was diminished when the correct direction was randomly determined, even though the correct rate was equivalent to the early phase of the learning condition. These sustained activities may reflect the behavioral context, as they were only observed when the monkey was aware of the need to learn the visuomotor association. Next, we examined the changes in sustained activity during learning to investigate its contribution to the learning process. As a result, these sustained activities were changed depending on the saccade direction associated with the fractal objects. Notably, the direction selectivity of the sustained activity was enhanced when the previous trial was correct. However, this direction selectivity disappeared in the over-trained condition. These results suggest that the sustained activity of DN neurons mediates the visuomotor associative learning, but not retrieval and retention. Thus, the sustained activity in DN works as cognitive signals which reflect the behavioral context and the signal may interact with PFC during visuomotor associative learning.

Disclosures: **Y. Akiyama:** None. **H. Yamada:** None. **M. Matsumoto:** None. **J. Kunimatsu:** None.

Poster

PSTR550. Cerebellum: Beyond Motor Function

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Program #/Poster #: PSTR550.03/GG14

Topic: E.02. Cerebellum

Support: Institute for Social Science Research (ASU Internal Funding)
Arizona Alzheimer's Disease Consortium
Institute for Mental Health Research

Title: Deep cerebellar nuclei perineuronal nets reveal sex-dependent guidance of reversal learning and social preference in adolescent mice

Authors: *T. LYLE, D. CHAMBERS, J. L. VERPEUT;
Psychology, Arizona State Univ., Tempe, AZ

Abstract: Autism spectrum disorder (ASD) clinical evidence has found atypical connectivity between the cerebellum and distal cortical regions associated with cognition and sociability. The most common atypical brain structure in ASD, the cerebellum, has multisynaptic connections through the deep cerebellar nuclei (DCN) and thalamus to cognitive- and social-associated brain regions (Pisano et al., 2021; Verpeut et al., 2023), yet formation of these pathways are not fully understood. Moreover, DCN perineuronal nets (PNNs), specialized extracellular matrix structures whose appearance is associated with the end of a critical period of plasticity, may shape cerebellar-cortical circuits via intermediate thalamic nuclei. However, the extent to which DCN PNNs influence adolescent behavior is currently unknown. We hypothesized that typical maturation of DCN PNNs is required to shape the cerebello-neocortical circuit, which is essential for flexible learning and social preference. In the following experiments, DCN activity was manipulated in adolescent mice using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to enhance (AAV2-hSyn-hM3D(Gq)-mCherry) or reduce (AAV2-hSyn-hM4D(Gi)-mCherry) cerebellar output. Males (n=20) and females (n=17) were exposed to the DREADD receptor ligand, clozapine-N-oxide (CNO; 10 mg/kg), in drinking water from postnatal day 21-35. Post-CNO, learning and reversal was analyzed using a pairwise visual discrimination task. Social behavior was assessed using a classic three-chamber assay and analyzed using SLEAP (SLEAP Estimates Animal Pose). DCN PNN intensity was examined to understand sex differentiated relationships between neural development and behavior. We found DCN manipulation did not alter learning the visual discrimination task compared to controls. Yet, reversal learning was found to be sex-differentiated where DCN excitation in females improved reversal ($p<0.05$) and female controls completed more trials ($p<0.01$), initiated more trials ($p<0.001$) and responded to trials faster ($p<0.05$). Whereas DCN inhibition improved reversal learning ($p<0.01$) in males. In the three-chamber task, both DCN inhibition and excitation resulted in indifference in social preference ($p<0.05$) in males. PNN intensity negatively correlated with reversal performance ($R^2 = -0.48$, $p<0.01$), yet positively correlated with social preference ($R^2 = 0.17$, $p<0.01$). These results suggest reducing PNNs in the DCN improves flexible cognition, but may negatively impact social behavior. These findings can guide clinical applications targeting development in individuals with neurodevelopmental disorders, such as ASD.

Disclosures: **T. Lyle:** None. **D. Chambers:** None. **J.L. Verpeut:** None.

Poster

PSTR550. Cerebellum: Beyond Motor Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR550.04/GG15

Topic: E.02. Cerebellum

Support: NIH grant 1R01OD030496-01

Title: Sex Difference in the Cerebellar Vermis due to Gonadal Sex but not Sex Chromosomes

Authors: I. WRIGHT[1], E. POPESCU[2], *W. GRISHAM[4], A. HERNANDEZ[2], C. SCHAAF-GRISHAM[2], X. CHEN[3], M. R. DWINELL[5], A. M. GEURTS[5], A. P. ARNOLD[3]; [1]Neurosci., Lafayette Col., Easton, PA; [2]Psychology, [3]Integrative Biol. & Physiol., UCLA, Los Angeles, CA; [4]UCLA Chapter, Los Angeles, CA; [5]Physiol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The cerebellum is generally not included in lists of sex differences in the brain. Nonetheless, sex differences due to both gonadal sex or sex chromosomes have been detected in cerebellar vermis using MRI scans in a 4-core mouse model (Corre et al., 2014). Here we 1) extend research on the cerebellar vermis to a rat model, 2) look for differences at a cellular level, & 3) describe Purkinje cells across lobules and see if they differ with either gonadal sex or sex chromosomes. The density and size of Purkinje cells in the cerebellar vermis were compared among XY male rats (n = 5), XX female rats (N = 10), and transgenic XX male rats (N = 6), who had testes due to the insertion of the Sry gene into an autosome. Forty-micron cerebellar sagittal sections were thionin stained, and between 2-6 pictures of a line of Purkinje cells spanning 330µm were taken of each lobule of the vermis. Cell counts (densities) were made blind and only included cells where the nucleus was not touching the edge of the field of view and a nucleolus was visible. Counts were averaged within a lobule for each animal. Also, 3 to 12 Purkinje cell sizes were measured in each lobule and averaged per lobule for each rat. Purkinje cell densities varied significantly across groups, $F(2,18) = 7.509$, $p < 0.01$ $\eta^2 = 0.455$. A Holm test revealed Female XX had fewer cells than Male XX ($p < 0.01$) or Male XY ($p < 0.05$). This result suggests that gonadal steroids are responsible for the difference. There was no interaction between lobule measures and genetic/gonadal sex, but we did find density differences across lobules, $F(10,170) = 7.776$, $p < .001$, $\eta^2 = 0.314$, as well as cell size differences $F(10,200) = 3.620$, $p < .001$, $\eta^2 = 0.153$. Posthoc Holm tests showed density differences were primarily due to lower densities in lobules 6b and 7; size differences were due to cells in Lobules 2 and 6b being smaller than those in Lobules 8 & 9. Our data show 1) Similar to the mouse model, there are gonadal sex differences in the rat cerebellar vermis 2) these differences exist at the cellular level, at least in Purkinje cell density 3) There are differences among lobules in the Purkinje cell density and size, which have not been well described before.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

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Program #/Poster #: PSTR550.05/GG16

Topic: E.02. Cerebellum

Support: NIH Grant AT010414

Title: The Cerebellum is a Neural Hub for Predictive Control of the Adrenal Medulla

Authors: *R. SEESE[1,2,3], A. C. BOSTAN[3], R. P. DUM[3], P. L. STRICK[3];
[1]Akron Children's Hosp., Akron, OH; [2]Pediatrics, Northeast Ohio Med. Univ., Rootstown, OH; [3]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Most neuroscientists believe that the cerebellum is largely concerned with the control and coordination of movement and balance, as well as with motor learning. This brain region is generally thought to construct "internal models" of the motor apparatus that move the eyes, trunk, and limbs. These internal models are updated based on sensory feedback and the consequences of motor actions. The cerebellum uses these models to enable predictive control of motor output and to generate feedforward command signals. In addition, there is a growing consensus that the cerebellum is involved in the regulation of cognition and affect. The uniformity of cerebellar circuitry suggests that it performs the same computational algorithms across all these domains. Here, we expand these concepts about cerebellar function to include predictive control and dynamic regulation of the adrenal medulla and, by extension, the brain-body connection.

This hypothesis arises from our studies using retrograde transneuronal transport of rabies virus to map the large-scale neural networks that regulate the adrenal medulla, our “first responder” in times of stress. We previously used this approach to define the regions of the cerebral cortex that influence the adrenal medulla in Cebus monkeys (Dum, Levinthal, & Strick, 2016, 2019). Here, we extend this approach to define the regions of the cerebellar cortex that influence the same organ. We injected rabies virus into the adrenal medulla of Cebus monkeys (n = 8). Neurons in the cerebellar nuclei were first infected after retrograde transport through a chain of four synaptically connected neurons. These infected neurons were located at specific sites bilaterally in all three cerebellar nuclei, with the highest density located centrally in the fastigial nucleus. Purkinje cells in the cerebellar cortex were first infected after retrograde transport through a chain of five synaptically connected neurons. Virus-infected Purkinje cells were located at specific sites bilaterally throughout the cerebellar cortex. The greatest density of infected cells was found in vermal lobules III, IV, V, and rostral VI. Additional groups of infected cells were in vermal lobules VIII and IX and scattered laterally throughout hemispheric lobules III through crus II. Thus, regions of the cerebellum that are interconnected with cortical and subcortical sites concerned with how we move, think, and feel have multi-synaptic influences over the adrenal medulla. This arrangement enables the cerebellum to act as a neural interface or hub that allows multiple processing streams to exert predictive control over the function of the adrenal medulla.

Disclosures: R. Seese: None. **A.C. Bostan:** None. **R.P. Dum:** None. **P.L. Strick:** None.

Poster

PSTR550. Cerebellum: Beyond Motor Function

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Topic: E.02. Cerebellum

Support: NIH Grant RO1NS106915
VA Grant BX003893

Title: Cerebellar endocannabinoid signaling mediates the extinction of associative fear memory

Authors: *R. KANCHIPURAM BHUVANASUNDARAM, M. FAROOQ, S. LIU;
Dept. of Cell Biol. and Anatomy, Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Fear extinction is a type of inhibitory learning that is used as behavioral therapy for patients with fear-related psychological disorders (e.g., post-traumatic stress disorder). The role of the cerebellum in associative fear learning and memory has been explored to some extent. However, its contribution to fear extinction remains poorly understood. This study addresses how Purkinje cells (PCs) in the cerebellum are involved in fear extinction, and its cellular mechanisms. We tested this in mice using chemogenetics, pharmacology, and an enzyme assay, combined with the fear conditioning (FC) and extinction behavioral paradigms. Mice aged 55-90 days underwent FC training with 8 tone and foot shock pairings and one extinction session with 20 tones in a new context 24 hours later, followed by an extinction memory retention test ("recall") the next day using a few tones. First, we injected L7-Cre/Gi-DREADD mice with clozapine N-oxide (CNO) to selectively silence cerebellar Purkinje cells (PCs) 30 minutes before extinction training. The freezing response to tone during recall was significantly higher in the CNO-injected mice than in the saline-injected control group. The results indicate that blocking PCs impaired extinction learning and memory formation. Next, since activation of PCs evokes the release of endocannabinoids, we examined whether the endocannabinoid system in the cerebellum plays a crucial role in fear extinction. To examine this, we micro-infused CB1 receptor blocker (NESS) into lobules V/VI of the cerebellar vermis before extinction training. CB1 receptors are highly expressed in the cerebellum, and 2-AG binds to these receptors and suppresses synaptic transmission. Our preliminary results demonstrate that blocking CB1 receptors during extinction learning impaired extinction memory. 2-AG is released from PCs and degraded by monoacylglycerol lipase (MAGL). We had previously discovered that FC accelerates the degradation of 2-AG via MAGL in lobules V/VI of the cerebellar vermis and this is critical for fear memory consolidation. We therefore hypothesize that fear memory extinction would suppress MAGL activity to elevate tonic 2-AG signaling. Our MAGL assay results demonstrate that extinction training reduces MAGL activity in lobules V/VI in wild-type (WT) mice, relative to conditioned mice. Overall, our results suggest that PC activity in the cerebellum is required for fear extinction learning and memory, and this is mediated by cerebellar endocannabinoid signaling.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

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Program #/Poster #: PSTR550.07/GG18

Topic: E.02. Cerebellum

Support: NIH DIR
BBRF YIA
BWF CASI

Title: Cerebellar granule cells and climbing fibers jointly acquire signals to learn reward timing

Authors: M. GARCIA GARCIA[1], A. KAPOOR[2], O. AKINWALE[2], L. TAKEMARU[2], T. H. KIM[3], C. PATON[5], A. LITWIN-KUMAR[6], M. J. SCHNITZER[7], L. LUO[4], ***M. WAGNER**[2]; [1]Natl. Inst. of Neurolog. Disorders and Stroke, Natl. Inst. of Neurolog. Disorders and Stroke, Rockville, MD; [2]NIH, Bethesda, MD; [3]Stanford Univ., Menlo Park, CA; [4]Stanford Univ., Stanford Univ., Stanford, CA; [5]Cornell, Cornell, NY; [6]Columbia Univ., New York, NY; [7]Stanford Univ. Dept. of Biol., Stanford Univ. Dept. of Biol., Stanford, CA

Abstract: The cerebellum helps animals learn to predict events and outcomes, most famously in sensory and motor contexts. More recently, cerebellar neural activity was found to encode reward. Yet it remains unclear whether or how the cerebellum learns to compute reward predictions. Cerebellar Purkinje cells (PkCs) learn to compute predictions based on the relative activity of two principal input pathways: granule cells (GrCs) and climbing fibers (CFs). Nevertheless, GrCs and CFs have never been recorded together. Here, we imaged simultaneous activity of populations of individual GrCs and CFs while animals learned to make forelimb movements for delayed water reward. As mice learned the reward timing, GrCs and CFs jointly developed reward expectation signals: many GrCs acquired sustained anticipatory activity to span the delay until reward, which triggered widespread time-locked CF spiking. Using known CF-dependent plasticity rules to predict GrC→PkC synaptic changes, we found that CF reward spikes were thus sufficient to grade the synaptic strengths of many GrCs by their anticipatory timing. PkCs could thereby continuously estimate time throughout seconds-long reward delay - a prediction borne out in PkC recordings. We thus detail the first cerebellar computation to learn to predict reward timing. By estimating reward time over substantially longer intervals than in classical cerebellar motor adaptation, this computation broadens possible roles for cerebellar learning.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

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Topic: E.02. Cerebellum

Support: NIDA Grant R01DA044761
NIMH Grant R01MH115604
Grass Fellowship (2018)

Title: The cerebellar projections to substantia nigra dopamine neurons convey subjective reward value information

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Abstract: Striatal dopamine release from the substantia nigra pars compacta (SNc) is important for movement initiation and vigor, sensory salience, and reward-based reinforcement learning. We recently characterized monosynaptic glutamatergic projections from the cerebellum to the SNc (Cb-SNc) and showed that this pathway is active during behavior and is capable of modulating dopamine release in the striatum. The cerebellum is traditionally recognized for its role in sensorimotor computations for precise motor control. Recent studies, however, have shown that the cerebellum may also be involved in reward processing. These findings raise an intriguing question: Does the cerebellum provide some component of the reinforcement learning reward signal to the SNc? Using fiber photometry in head-fixed mice, we have shown that the Cb-SNc activation correlated with locomotion, lever press and licking, indicating that the cerebellum can modulate striatal dopamine levels during movement. Interestingly, in a Pavlovian task that paired a sensory cue with a water (regular) or sweet water (high) reward, we also saw robust activation of Cb-SNc with reward detection in rewarded trials compared to when reward was omitted. These signals were also significantly larger in response to high vs. regular reward. To rule out that this difference was due to increased licking activity, we examined the correlation between the Cb-SNc signal and the number of licks in each trial. This analysis showed a significant increase in the Cb-SNc signal with reward and a significant increase with high vs. regular reward at the same lick frequencies. It is, however, possible that the Cb-SNc signal conveyed sensory information and not reward. To examine this distinction, we did a reward devaluation experiment where we compared the Cb-SNc signals when the animal was thirsty to when it was allowed to drink water before the session. These experiments showed that the reward-related Cb-SNc activity was significantly reduced following reward devaluation. Because a purely sensory signal should be independent of devaluation, this result strongly suggests that Cb-SNc conveys subjective reward value. All three deep cerebellar nuclei that form the output of the cerebellum project to the SNc. Interestingly, we find that the projection from each nucleus may have distinct levels and kinetics in response to motor activity and reward, indicating potential differences in their functional roles. These findings provide compelling evidence that the cerebellum conveys information regarding the subjective reward value to SNc dopamine neurons, thereby contributing to reinforcement learning computation by these neurons.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR550.09/GG20

Topic: E.02. Cerebellum

Support: NINDS Grant R01NS050808
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Title: Utilizing a novel CACNA1A mutation to characterize the contribution of the cerebellum to motor and cognitive impairment

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Abstract: Mutations in CACNA1A, the gene encoding P/Q type voltage gated calcium channels (Cav2.1), lead to the movement disorder Episodic Ataxia type 2 (EA2) as well as Familial Hemiplegic Migraine type 1 (FHM1). However, there is often considerable overlap of the symptoms between these two disorders. The mechanism responsible for motor dysfunction such as ataxia is attributed to the role that these channels play in cerebellar Purkinje cell pacemaking. Activation of calcium activated potassium channels (SK channels) in Purkinje cells alleviates motor impairment in both EA2 and FHM1 mouse models. More recently, patients with CACNA1A mutations have been identified who present primarily with cognitive symptoms such as intellectual disability and general developmental delay, and for whom ataxia is not the most significant symptom. Many of the mutations that these patients carry have not been rigorously characterized. Moreover, it is not known what mechanisms drive cognitive impairments rather than ataxia to be the most prominent characteristic symptom of these patients. Here, we use a novel CACNA1A mutation, D1634N to investigate the role of the Cav2.1 channel and the cerebellum in both motor and cognitive impairment. These patients affected by this mutation present mainly with cognitive symptoms, such as intellectual and verbal developmental delays, and mild ataxia. Utilizing immunocytochemistry and whole cell electrophysiology in human embryonic kidney (HEK293t) cells we found the D1634N mutation results in a decrease in the overall and surface expression of the Cav2.1 channel, as well as channel dysfunction. To better understand the effect of the changes in channel properties and the mechanism underlying patient symptoms, we used CRISPR to generate two knock-in mouse models. We induced the D1634N mutation in the mouse homologous site (D1585N), resulting in D1585N +/- heterozygous mice, and D1585N-L7PCPcre mice with the mutation only in cerebellar Purkinje cells. Utilizing slice electrophysiology and behavioral assays, we found an increase in the irregularity of Purkinje cell pacemaking activity and motor impairment in both models. We also found impairment in social interaction and short-term memory. Activation of SK channels resulted in improved Purkinje cell activity and motor performance, and also improved social interaction in both mouse models. This work begins to investigate the role of the cerebellum and the Cav2.1 channel in cognitive

impairments, and the utility of SK channel activators as an effective therapeutic treatment in these patients.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

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Program #/Poster #: PSTR550.10/HH1

Topic: E.02. Cerebellum

Support: NIH Grant R15CA271450

Title: Impact of lesion location on motor and cognitive outcomes in pediatric cerebellar tumors

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Abstract: Sixty percent of pediatric brain tumors are in the posterior fossa, including the cerebellum. Children with developmental cerebellar damage are at an increased risk for an array of adverse outcomes, including higher rates of autism diagnoses and long term cognitive and academic challenges. Traditionally considered a motor structure, extensive evidence now links the cerebellum to a wide range of behaviors, and atypical cerebellar structure and function has been reported in multiple neurodevelopmental and psychiatric disorders. One of the key features of cerebellar neuroanatomy is the existence of functional subregions that support sensorimotor and non-motor functions. This leads to the hypothesis that motor, cognitive, and behavioral outcomes should be predictable based on the location of developmental cerebellar disruption. To test this, we conducted lesion-symptom mapping in a cohort of 42 children (sex assigned at birth: 25 male, 17 female; average age at diagnosis 6.7±4.3 years) with a history of cerebellar tumor resection. Clinical MRI scans and neuropsychological assessment data were used to evaluate the impact of lesion location on motor (pegboard), cognitive (verbal comprehension, verbal fluency, working memory, processing speed) and behavioral control measures (BRIEF). Broad grouping of patients by lesion location (hemisphere involvement n=21, midline only n=21) revealed trends for lower processing speed scores in patients with midline lesions ($p=0.085$) and reduced semantic fluency in the patients with lesions impacting the cerebellar hemispheres ($p=0.084$). This coarse grouping, however, ignores the presence of multiple cerebellar functional subregions within the medial cerebellum and the lateral hemispheres. To address this issue, we are mapping individual lesions onto a standard template and will use support vector regression lesion-symptom mapping (SVR-LSM) to determine relationships between behavioral profiles and lesion patterns. SVR-LSM analyses will include covariates of age of diagnosis, lesion size, and treatment factors (radiation, chemotherapy). We predict that SVR-LSM will be more sensitive to the relationship between lesion location and behavioral profile than broad anatomical grouping

of lesions. These findings are of clinical interest for prognosis and management of patients with cerebellar tumor resection in childhood.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR550.11/HH2

Topic: E.02. Cerebellum

Support: NSF grant # 1539067

Title: Posterior Cerebellum Contribution to Development of Sensorimotor and Cognitive Functions

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Abstract: Effective sensorimotor processing in early childhood provides the foundation for healthy cognitive and behavioral development. The crucial brain pathway for integrating sensory processing from multiple modalities with motor planning and execution is the cerebellar-thalamic-cortical pathway (CTC; van Dun et al., 2016). While the functional significance of earlier maturing sensory thalamic nuclei (LGN, MGN, VPN) is well defined, the understanding of the later developing posterior cerebellum to the role of the CTC pathway in child's cognitive development awaits new studies (Dacre et al., 2021). We ask whether the age-dependent anatomical MRI morphometry changes in the earlier maturing I-V lobules of the cerebellar vermis and in the neocortical lobules VI-VII (Leiner, et al, 1989; Schmahmann,1997) correspond to changes in visual-sensorimotor and speech-motor/concept task proficiency in children. *Methods:* High-resolution structural 3T MR scans were obtained from typically developing children (TDC; n=33; age =9 -15) and healthy adult controls (HAC; n=31; age=18-28). Two independent raters used FReeSurfer computer-assisted algorithm to evaluate the volumetry (mm^3) of the cerebellar Vermis lobules I-V, VI-VII, and total brain (&gt 90% inter and intrarater reliability). Neuropsychological assessment was completed. We apply the subtests of WASI-II relying on visual-sensorimotor (*Block Design*) and motor-speech-cognitive (*e.g. Similarities*) functions. *Results:* ANOVA revealed no age-dependent differences in total brain volume, which lends validity to our cerebellar morphometry findings with reduced values in TDC: (i) HAC vs.

TDC (*M difference* =36.71mm^3 ±5.11) for lobules I-V; (ii) HAC vs. TDC (*M difference* = 21.68 mm^3 ±3,09) for lobules VI-VII. The ratio of lobules VI-VII to I-V remained constant across groups suggesting ongoing in parallel developmental process. The Pearson correlations showed: (i) Positive effect in TDC for VI-VII and BD (r = .393, p=.032) and approaching sign. for I-V and BD (r=.349, p=.059). The latter was more prominent in the younger (age 9 -11) children (r=.652, p=.016). Tasks relying on motor-speech/concept (*Similarity*) showed significant positive relation to morphometry VI-VII only in adolescents aged 12-15 (r=.545, p=.019). *Conclusion:* The posterior cerebellar age-related changes continue across the late childhood and adolescence with predominant relationship of visual sensorimotor to I-V and with motor-speech to VI-VII lobules. Increased understanding of the developmental cerebellar changes within the CTC pathway opens a field of identifying very early markers of developmental disorders and their prevention.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR550.12/HH3

Topic: E.02. Cerebellum

Support: NIH R21 NS125546

Title: A preliminary fMRI study of finger tapping and motor imagery in cerebellar ataxia

Authors: *J. BERENBAUM[1], J. M. LISINSKI[2], S. M. LACONTE[2], P. A. NADKARNI[1], J. E. DESMOND[1], R. S. SAEED[1], A. N. KUCHARSKI[1], L. S. ROSENTHAL[1], C. L. MARVEL[1]; [1]Neurol., Johns Hopkins Univ., Baltimore, MD; [2]Fralin Biomed. Res. Inst. at VTC, Virginia Tech., Roanoke, VA, VA

Abstract: BACKGROUND:Cerebellar ataxia (CA) is a movement disorder caused by progressive degeneration of the cerebellum. Motor imagery could be a potential therapeutic tool to improve motor dysfunction by “exercising” motor brain regions without the need for overt movement. To assess the feasibility of using a motor imagery approach to improving motor function in CA, this study combined functional MRI (fMRI) of finger tapping with motor imagery scales. METHODS:Participants with CA (n = 7, mixed subtypes) completed a finger tapping task in conjunction with functional MRI (fMRI) on a 3T Philips scanner using a 32-channel head coil. Participants were presented with a flashing cross and pushed a button, using their right hand, at the same frequency as the flashing cross. Tapping blocks consisted of 1Hz or 4Hz speeds, with rest blocks in between: all blocks = ~30 seconds; 3 blocks of each speed/run, 2 runs. Standard pre-processing steps were conducted using SPM12, followed by a computation of individual statistical maps for each subject. Random effects analysis was performed to map the

mean BOLD responses to tapping at each speed. Atlas-based regions of interest (ROI) related to the motor and pre-motor cortices (Mayka et al., 2006) were applied. BOLD values within these ROIs were correlated with scores on the Kinesthetic and Visual Imagery Questionnaire (KVIQ), using Spearman's Rho correlations. Behavioral performance was measured as the root mean square error (RMSE) between the target tapping speed (1 or 4 Hz) versus actual tapping speed. RESULTS:Preliminary results of the behavioral data showed that the CA participants tapped faster than the target at 1Hz (mean tap frequency = .84, SD = .14, p = .025) and slower than the target at 4Hz (mean tap frequency = .34, SD = .12, p < .001). ROI data revealed a negative correlation between the left primary motor cortex (LM1) and RMSE for tapping at 1Hz, r = -.929, p < .001 (greater activity was associated with better accuracy). There was no relationship between LM1 and RMSE at 4Hz. However, LM1 and right M1 activity at 4Hz tapping positively correlated with KVIQ kinesthetic scores, r = .775, p =.04 and r = .901, r = .006, respectively (greater activity was associated with more vivid kinesthetic imagery). This suggested that the faster tapping speed involved integration with what the tapping experience might "feel" like. KVIQ visual scores trended with M1 activity, p <.10. IMPLICATIONS:
These findings suggest that, within the context of CA, slower tapping speed (1Hz) relies on M1, but at faster tapping speeds, M1 may integrate with motor imagery. This suggests that motor imagery may be a feasible tool for improving motor function in people with CA.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR550.13/HH4

Topic: E.02. Cerebellum

Support: NIH MH111868
NIH MH125995

Title: Cerebellar TMS changes functional connectivity and behavior in a dose dependent manner

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Abstract: *Introduction:* Cerebellar neuromodulation, a promising intervention for treatment of neurological and psychiatric conditions has received increased recent interest as emerging evidence has extended cerebellar functions beyond traditional associations to motor functions.

However, most commonly transcranial magnetic stimulation is applied cortically, leaving cerebellar practitioners to adapt cortical designs to cerebellar stimulation. Investigators adapting cortical paradigms to cerebellum have considered changing intensity as principal measure to ensure efficient stimulation. We empirically tested the relationship between stimulation intensity and cerebellar-cortical network response. *Methods*:In n=26 healthy volunteers, we performed 3 separate sessions of fMRI-guided intermittent theta-burst TMS to the dorsal attention network node of the cerebellar vermis (VII). Each of three sessions was performed at 75%, 87.5% or 100% of active motor threshold. Task-based functional connectivity was assessed before and after each stimulation session. Cognitive performance was assessed using the gradual continuous performance task (gradCPT). *Results*: Higher intensity TMS resulted in functional connectivity increases and improved behavioral performance in a dose-dependent manner (standardized coefficient = -0.28, p =0.018). This improvement was associated with stronger cerebellar dorsal attention network to cortical frontoparietal network connectivity (standardized coefficient = -0.56, p = 0.008) and weaker cerebellar-cortical ventral attention network connectivity (standardized coefficient = 0.32, p = 0.006), controlling for model complexity. *Discussion:* We find that improvements in an attention task due to cerebellar stimulation come at the highest of stimulation intensities independent of individual motor threshold. Critically, network-response forms a crucial role in obtaining a desired cognitive improvement from cerebellar TMS, and must be included to predict performance. These findings suggest lower intensity stimulation, indexed by cerebral cortex excitability, will likely lead to understimulation of the cerebellum.

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Poster

PSTR550. Cerebellum: Beyond Motor Function

Location: WCC Halls A-C

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Program #/Poster #: PSTR550.14/HH5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH/NIMH grant R01 MH128278

Title: Phonemic fluency in a sample of controls and individuals with spinocerebellar ataxia is correlated with grey matter density in the cerebellum and the left middle frontal gyrus

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[1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]Dept. of Neurol. Div. of Cognitive Neurosci., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Spinocerebellar ataxia (SCA) refers to a group of rare, genetic disorders that cause cerebellar degeneration. Historically research on SCA has focused on the loss of motor coordination. Cognitive deficits associated with cerebellar atrophy have not been examined as intensively. As part of a study to quantify cognitive abilities in SCA, we administered the Cerebellar Cognitive Affective Syndrome (CCAS) scale (Hoche et al, Brain, 141:248, 2018). The phonemic fluency subitem of CCAS may be used as a litmus test for frontal lobe dysfunction. However, given the interconnections between the cerebellum and frontal lobe (Middleton and Strick, 2001), and the cerebellum's role in phonological fluency (Leggio, 2000), we hypothesized that impaired performance on this item may predict atrophy of the cognitive cerebellum as measured by voxel-based morphometry (VBM). Individuals with autosomal dominant SCA and controls were recruited for participation at the Johns Hopkins Medical Center. The CCAS phonemic fluency was measured by asking participants to list as many words as possible starting with a given letter in 1 minute. High resolution MPRAGEs were acquired for anatomical imaging of subjects. Subject grey matter maps were extracted using the CAT12 toolbox in SPM. These were smoothed prior to being entered into a linear regression analysis with the phonemic fluency CCAS sub-scores. All measures for the sample are reported as mean ± stdDev. For our preliminary exploratory analysis, we pooled data for a total of 5 ataxia subjects including SCA3 (2), SCA6 (2), and SCA8 (1) (age=64 ± 12) and 3 controls (age=67 ± 7). Ataxia subjects had a mean CCAS score of 86 ± 9.0 with a mean phonemic fluency score of 13.6 ± 4.6 words. Controls had a mean CCAS score of 101 ± 7 with a mean phonemic fluency score of 17.3 ± 1.5. Regression analysis revealed a positive relationship between phonemic fluency and grey matter density in clusters ($p<0.001$ unc., $k>=27$) located in the right middle occipital cortex, right cerebellar lobule X, right crus I of the cerebellum, left cerebellar lobule IX, left cerebellar lobule VIII, and the left middle frontal gyrus. Similar to findings in past studies, lower phonemic fluency was associated with lower grey matter density in the left middle frontal gyrus. However, low phonemic fluency also predicted lower grey matter density in cerebellar regions including right crus I, which has been associated with verbal working memory, word generation, and verbal reasoning. In the future, utilizing DTI scans, we are investigating whether the degradation of intermediate white matter tracts between regions of cerebellar degeneration and left frontal degeneration are associated with SCA.

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Poster

PSTR551. Basal Ganglia: Codes and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR551.01/HH6

Topic: E.03. Basal Ganglia

Title: Basal Ganglia neural dynamics during directional hand reaching task

Authors: *S. JAVADZADEH NO[1], M. ASADI[2], S. SEYYEDMOUSAVI[2], T. SANGER[3,4]; [1]BME, [3]EECS, [2]Univ. of California Irvine, Irvine, CA; [4]Children's Hosp. of Orange County, Orange, CA

Abstract: There has been extensive research on decoding movement parameters such as direction of reaching, from motor cortex activity in animals and human subjects. Whether or not Basal Ganglia (BG) and thalamic regions encode such parameters in their activity is less understood. The use of Deep Brain Stimulation (DBS) as a treatment for movement disorders such as childhood dystonia provides us with the rare opportunity to study the modulation of BG and thalamic neurons during movement. During the DBS procedure, several stereoelectroencephalography (sEEG) depth electrodes are implanted in various BG and thalamic nuclei. Recorded electrical activity from these temporary stimulation/recording electrodes can be utilized to understand the underlying dynamics of dystonia and motor control in general. For the purpose of this study, while recording the intracranial data, we asked the patient to perform a reaching experiment that included 8 trials (i.e., 4 trials per arm) with 5 minutes of rest period between each trial. Each trial consisted of 80 center-out reaching repetitions in 8 different directions ordered randomly. We employed a custom spike sorting pipeline in order to acquire neural activity from BG and thalamic regions on both the multi-unit and population scales. This pipeline was tailored for our data recorded from lower impedance electrodes compared to single-unit recording electrodes. Looking at single spiking unit activity, we found that, while there is directional tuning in the activity of movement-correlated units, this tuning is far less significant than what is reported in motor cortex neurons. The directional tuning is measured with firing rates from onset-synchronized reaching trials. To ensure that lack of directional tuning is not originating from inconsistent reaches, trials with non-similar reaching trajectories are removed. Moving to population level analysis, we have applied linear and nonlinear decoding algorithms to the population spiking activity measured with firing rates to decode the direction from basal ganglia activity. In contrast with prior results in cortex, we found that the encoding in basal ganglia does not show strong correlations with direction of movement, and linear decoding methods including population vector methods are not effective. Our results suggest the need for nonlinear decoding methods that reflect a complex encoding of movement parameters in deep brain nuclei.

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Poster

PSTR551. Basal Ganglia: Codes and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR551.02/HH7

Topic: E.03. Basal Ganglia

Title: Low Dimension Subcortical Structure Described by Nonlinear Mapping

Authors: *M. ASADI[1], S. JAVADZADEH NO[1], S. SEYYEDMOUSAVI[1], T. D. SANGER[1,2];
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Abstract: Despite extensive research on the low-dimensional representations of dynamics in motor areas in the cortex, our understanding of the basal ganglia remains limited. This study aims to investigate the latent dynamics of the basal ganglia and explore the possibility of a low-dimensional representation of these dynamics. In the cortex, JPCA methods have been capable of reducing dimensionality and demonstrating simple structures in population response. To obtain a low-dimensional representation, we applied JPCA method and simple nonlinear dynamic Networks to both local field potential and spike firing rate data. Our intracranial neural data is recorded from pediatric patients with dystonia, performing a point-to-point reaching task. Results showed that, in contrast to cortical regions, JPCA is less effective when applied to the basal ganglia and it yielded a poor approximation of data in lower dimensions. However, by employing nonlinear methods on our basal ganglia data, we observe higher levels of accuracy (approximately 20% to 30% higher preserved variance). Further research is needed, but our results indicate that deep subcortical structures are likely to have low-dimensional dynamics that can be uncovered through the use of nonlinear encoding and decoding methods.

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Poster

PSTR551. Basal Ganglia: Codes and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR551.03/HH8

Topic: E.03. Basal Ganglia

Title: Effects of Deep Brain Stimulation on Motor Performance and Activation Pattern of Task-Related and Task-Unrelated Frequency Content During a Cyclic Movement Task

Authors: *J. NATARAJ[1], R. SOROUSHMOJDEHI[2], M. KASIRI[3], S. A. SEYYED MOUSAVI[1], T. D. SANGER[4];
[2]EECS, [3]Biomed. Engin., [1]Univ. of California, Irvine, Irvine, CA; [4]UCI, CHOC, Redondo Beach, CA

Abstract: Pediatric dystonia is a movement disorder with clinical features of involuntary intermittent or sustained muscle contractions causing abnormal postures and repetitive movements. This disorder can be further classified as primary or secondary, depending on the etiology of the disease. Dystonia may involve reduced suppression of involuntary muscle activity, resulting in superposition of extraneous motor components on desired voluntary movements. Deep brain stimulation of basal ganglia and the substantia nigra pars reticulata (SNr) has shown efficacy in treatment of secondary dystonia and other motor disorders such as Parkinson’s disease, though the mechanism of action is unknown. We aim to characterize pattern

changes in frequency content of intracranial signals recorded during a cyclic motor task when stimulation is off, and during various stimulation conditions that are being evaluated for clinical efficacy. We expect that in a cyclic motor task, muscle activity will occur at task-related frequencies that match frequencies of motion. We hypothesize that upper limb muscles and intracranial motor control structures such as basal ganglia and thalamus will contain task-related frequency content, and that the distribution of power in both task-related and task-unrelated frequency bands will change with differing stimulation settings. Task performance under various stimulation conditions was also characterized using speed-accuracy tradeoff. The cyclic motor task was performed while the subject was in an inpatient Neuromodulation Monitoring Unit (NMU). During the NMU, ten temporary depth electrodes were placed bilaterally in basal ganglia, thalamus, SNr, and pedunculopontine nucleus. The subject was asked to perform the motor task while kinematic trajectories, intracranial data, and electromyographic signals were recorded. Individual repetitions of the task were scaled to matching durations offline, and power spectra of all signals were used to compare the frequency content under different stimulation conditions. When the motor task was performed without intracranial stimulation, the task-related components are not well-resolved, presenting as more wideband activation around task-related frequencies. This wideband activation is mitigated when stimulation is turned on, with power more centered around task-related frequencies. Additionally, the distribution of task-unrelated power up to 50Hz and the speed-accuracy tradeoff was altered in each of the different stimulation conditions. These changes could shed light on mechanisms of deep brain stimulation and may also be useful in identifying optimal stimulation targets.

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Poster

PSTR551. Basal Ganglia: Codes and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR551.04/HH9

Topic: E.03. Basal Ganglia

Support: DA 040701

Title: Striatal Direct and Indirect Pathway Neurons Play Complementary Roles in Specifying Action Parameteres

Authors: ***F. HONG**[1], I. FALLON[2], S. O. FERNANDEZ[1], B. LU[1], H. YIN[1];
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Abstract: The striatum, the primary input nucleus of the basal ganglia, has been implicated in motor control, compulsive behavior, and habit formation. Within the striatum, D1 and D2 expressing spiny projection neurons comprise the direct and indirect output pathways and are thought to be involved in the initiation and suppression of movement, respectively. However,

emerging evidence suggests coordinated activity between these two distinct neuronal populations is critical for generating actions. In this study, we employed a behavioral paradigm that require mice to perform a skilled movement in which they use their paws to grasp and drink water while recording the activity of both D1- and D2-SPNs at single-cell level resolution in the dorsal striatum. Our findings reveal that specific subpopulations of both direct and indirect pathway neurons are associated with distinct components of the action sequence, including forelimb extension, target touch, forelimb retraction, and paw licking. The temporal pattern of neuronal firing in relation to these movements also exhibits considerable plasticity. As mice became more proficient on this task, the timing of direct and indirect pathway neurons is shifted relative to contact with sucrose reward. When the location of the reward spout location was manipulated, the neuronal populations involved in reaching also changed. Optogenetic stimulation of the indirect pathway resulted in misses by causing ipsiversive deflections of the paw. The degree of deflection is not only determined by stimulation parameters, but quantitatively depends on the target position. Together these results showed that direct and indirect pathways play complementary roles in specifying action parameters. During skill learning, these parameters are modified as striatal populations undergo significant plasticity.

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Poster

PSTR551. Basal Ganglia: Codes and Behavior

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Program #/Poster #: PSTR551.05/HH10

Topic: E.03. Basal Ganglia

Support: NS094754
MH112883

Title: Deconstructing the reward prediction error hypothesis of dopamine function

Authors: *H. YIN[1], K. BAKHURIN[1], I. FALLON[1], Q. JIANG[2], M. HOSSAIN[3], B. GUTKIN[3]; [2]1085 Stillwell Dr, [1]Duke Univ., Durham, NC; [3]École Normale Supérieure, Paris, France

Abstract: According to a popular hypothesis, phasic dopamine (DA) activity encodes a reward prediction error (RPE) necessary for reinforcement learning. However, recent work showed that DA neurons are necessary for performance rather than learning. One limitation of previous work on phasic DA signaling and RPE is the limited behavioral measures. Here, we measured subtle force exertion while recording and manipulating DA activity in the ventral tegmental area (VTA) during stimulus-reward learning. We found two major populations of DA neurons that increased firing before forward and backward force exertion. Force tuning is the same regardless of learning, reward predictability, or outcome valence. Changes in the pattern of force exertion can explain results traditionally used to support the RPE hypothesis, such as modulation by reward

magnitude, probability, and unpredicted reward delivery or omission. Thus VTA DA neurons are not used to signal RPE but to regulate force exertion during motivated behavior.

Disclosures: **H. Yin:** None. **K. Bakhurin:** None. **I. Fallon:** None. **Q. Jiang:** None. **M. Hossain:** None. **B. Gutkin:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: NS094754

Title: Ventral tegmental area GABAergic neurons contribute to approach behavior

Authors: *Q. JIANG, K. BAKHURIN, R. N. HUGHES, B. LU, H. H. YIN;
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Abstract: The ventral tegmental area (VTA), a midbrain region associated with motivated behaviors, consists predominantly of dopaminergic (DA) neurons and GABAergic neurons, with a small population of glutamatergic neurons. While DA neurons have been extensively studied, the functional roles of VTA GABAergic neurons remain controversial. This study aimed to investigate whether the activity of VTA GABAergic neurons represents reward prediction, as previously hypothesized, or performance variables. To accomplish this, we chronically implanted electrode arrays with optic fibers into the VTA of VGAT-Cre mice and recorded the single-unit activity of GABAergic neurons using a wireless headstage. Concurrently, we measured behavioral variables by head-fixing the mice to a load-cell device capable of continuously measuring the force exerted by the animals. The water-deprived mice were trained using a stimulus-reward Pavlovian learning task. Applying a hierarchical clustering algorithm to the recorded data, we identified distinct populations of GABAergic neurons. Their activity did not encode reward expectancy but rather the forces exerted by the animals. We varied the position of the reward spout within the same sessions, placing it 2mm in front of or behind the animals' mouths. This manipulation generated equivalent levels of reward prediction but different movement directions. We observed that well-trained mice exerted forward or backward forces in accordance with the spout positions during Pavlovian tasks, while maintaining similar licking rates. The activity of GABAergic neurons also reflected these performance variables instead of reward prediction. Unlike dopamine neurons in the VTA, which represented changes in force exertion, GABAergic neurons, which often showed tonic firing activity, more commonly represented force. Different populations of GABAergic neurons exhibited unique activity patterns of excitation or inhibition during forward and backward movements. Notably, these patterns were not limited to in-task movements but were also observed during spontaneous movements. Changes in GABAergic neuron activity also accounted for force modulations during

learning, unexpected reward omissions, and aversive stimuli. Optogenetic manipulation of these neurons also generated movements. Overall, our findings demonstrate that the activity of VTA GABAergic neurons contributes to approach behavior rather than encoding reward expectancy. These results shed new light on the functional roles of GABAergic neurons within the VTA and provide a deeper understanding of the neural mechanisms underlying motivated behaviors.

Disclosures: Q. Jiang: None. **K. Bakhurin:** None. **R.N. Hughes:** None. **B. Lu:** None. **H.H. Yin:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: NS094754
DA040701

Title: The role of striatal neurons in counting behavior

Authors: *I. FALLON[1], M. ROSHCHINA[3], F. HONG[3], C. VIGNALI[2], S. FERNANDEZ[3], H. H. YIN[3];
[2]Neurobio., [1]Duke Univ. Sch. of Med., Durham, NC; [3]Psychology and Neurosci., Duke Univ., Durham, NC

Abstract: Nonverbal counting and timing of actions are highly conserved processes, yet their neural substrates are poorly understood. Here, we studied the activity of dorsolateral striatal neurons during a novel operant counting task, in which mice must perform an exact number of lever presses to earn reward. We employed *in-vivo* calcium imaging and optogenetics to record and manipulate the activity of direct and indirect spiny projection neurons (dSPNs and iSPNs) during counting. We found that often striatal activity (both dSPN and iSPN) represented proximity to the lever and the reward location, as well as the count number. Unilateral excitation of iSPNs caused a premature transition to the reward location and reset the next count, whereas inhibition of iSPNs prevented transition to the reward and prolonged the current count. Unilateral excitation of dSPNs prolonged the count and prevented transition to the reward location. In contrast, inhibition of dSPNs caused a premature transition to the goal and reset the next count. Collectively, our results reveal for the first time that dSPNs and iSPNs bidirectionally modulate counting behavior to optimize transition between the completion of an action sequence and the goal.

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Poster

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Topic: E.03. Basal Ganglia

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NS121253

Title: Segregated striatal populations represent the distinct actions organized into skilled sequences

Authors: K. BAKHURIN, J. PARK, ***B. LU**, H. YIN;
Psychology & Neurosci., Duke Univ., Durham, NC

Abstract: Skilled behaviors are comprised of an orderly sequence of actions required to achieve a specific goal. Extensive work has implicated the basal ganglia as playing a crucial role in the learning and execution of skilled behaviors. The neural mechanisms in the basal ganglia that mediate the coordination of the action components remain unclear. In this study, we recorded extracellular spiking activity of medium spiny neurons (n = 350) in the dorsal (DLS) and ventral (VLS) striatum while freely-moving mice (n = 4) perform a forelimb reaching task for drops of sucrose. Using high-speed video analyses, we identified three actions with distinct kinematic profiles that are sequenced to produce the reaching behavior: aiming for the water target, reaching with the forelimb, and drinking from the hand once the water is acquired. Distinct populations of MSNs in the striatum are related to either the aiming, reaching, and drinking components of the task, showing rough a rough spatial topography. If a given action was being performed the corresponding action-related population is active, while the other populations are inactive. During learning, initial attempts to reach the water with the tongue are rapidly suppressed as reaching becomes gradually inserted into the proper action sequence. Following the learning, the licking behavior, primarily mediated by VLS neurons, is only initiated upon the completion of the reach and the placement of the paw close to the mouth. Optogenetic stimulation of direct pathway neurons in the VLS targeting the licking-related populations resulted in persistent generation of the licking action directed at the water target, and akinesia of the hand for the duration of stimulation. Together these findings reveal that distinct action components are represented by distinct neuronal populations in the striatum. During skill learning, there is a development of a selective inhibition mechanisms whereby competing actions are suppressed at specific points in the sequence.

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Poster

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Topic: E.03. Basal Ganglia

Support: NIH NS094754

Title: External Globus Pallidus neurons show precise representation of movement kinematics

Authors: *A. D. FRIEDMAN, C. T. SHOEMAKER, H. H. YIN;
Duke Univ., Durham, NC

Abstract: The basal ganglia have long been implicated in the control of movement. Traditional models posit that the direct pathway initiates movement and the indirect pathway suppresses it. However, these models ignore evidence that indirect pathway stimulation creates movement, and they fail to explain how movement parameters are continuously adjusted. Previous work has shown that many neurons in the striatum and substantia nigra signal movement kinematics, and play a causal role in specifying movement parameters like velocity and position, but it is unknown exactly how the external globus pallidus (GPe), a key component of the indirect pathway, contributes to movement. In the current study, we recorded single unit activity from the GPe, while monitoring movement using 3D motion capture, while mice performed a whole-body pellet reaching task. We found distinct neuronal populations whose firing rates correlate with either instantaneous body length or body length velocity (derivative of body length). Optogenetic stimulation of the GPe also reliably altered movement kinematics. These results suggest that the GPe also plays a key role in regulating the detailed parameters of goal-directed movements, and that different GPe populations may be used in distinct computations for generating the required kinematics for movement control.

Disclosures: A.D. Friedman: None. **C.T. Shoemaker:** None. **H.H. Yin:** None.

Poster

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Support: NIH Grant NS094754

Title: Cholinergic interneurons in the dorsolateral striatum play a key role in movement generation

Authors: *J. KIM, F. HONG, H. H. YIN;
Duke Univ., Durham, NC

Abstract: Cholinergic interneurons (CINs) are the major source of acetylcholine in the striatum. Although they make up only 1-2% of striatal neurons, CINs function as the key regulator of striatal microcircuitry by modulating the excitatory and dopaminergic inputs to the striatum as well as striatal projection neurons. However, the role of CINs in behavior remains obscure. We recorded CIN activity in behaving mice using in vivo electrophysiology, a genetically encoded calcium sensor, and a fluorescent acetylcholine sensor while measuring behavior using a behavioral setup that measures force generated by movements (Hughes et al., 2020) as well as 3D motion capture. The continuous monitoring of CIN activity and behavior showed that acetylcholine dynamics reflect movements at all times and independently of learning. Furthermore, chemogenetic and optogenetic manipulations of CINs in the dorsolateral striatum suggest that these neurons play an important role in movement generation.

Disclosures: J. Kim: None. **F. Hong:** None. **H.H. Yin:** None.

Poster

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Topic: E.03. Basal Ganglia

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Title: Dorsal striatum subregions show distinct indirect pathway neuron firing during movement initiation.

Authors: *I. GONZALEZ MONTALVO, T. W. FAUST, M. DUHNE, K. KIM, L. PELATTINI, J. D. BERKE;
UCSF, San Francisco, CA

Abstract: Neural circuits in the dorsal striatum, and their modulation by dopamine, influence which actions we perform and how vigorously we perform them. Two subtypes of striatal spiny projection neurons (SPNs) form the direct pathway (dSPNs) and indirect pathway (iSPNs); these are hypothesized to facilitate and inhibit movement, respectively. However, this is now controversial since dSPNs and iSPNs have been found to be coactivated during movement in some tasks. dSPNs express dopamine D1 receptors (D1+) while iSPNs express dopamine D2 (and adenosine A2a; A2a+) receptors. We are using Cre-dependent expression of the opsin Chrimson, in freely moving transgenic knockin rats (D1-Cre, A2a-Cre) to record spiking of optogenetically-identified D1+ neurons and A2a+ neurons from the dorsomedial striatum (DMS; "associative") and dorsolateral striatum (DLS, "motor"). Rats perform a trial-and-error task in which they adjust their response vigor to a Go! cue according to recent reward probabilities. We find that, on average, A2a+ neurons in the dorsolateral striatum (n = 92) sharply decrease firing just prior to both contralateral and ipsilateral movements. By contrast, A2a+ neurons in

dorsomedial striatum (n = 107) increase firing during movement onset, selectively for contralateral movements. Our results are a step towards revealing the distinct contributions of SPN subpopulations within dorsal striatal circuits to specific aspects of motor control.

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Poster

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Topic: E.03. Basal Ganglia

Support: NIDA
NINDS

Title: Distinct encoding of reward predictions and outcomes by D1 and D2 neurons of the nucleus accumbens core

Authors: *T. FAUST[1], A. MOHEBI[2], M. DUHNE[3], J. D. BERKE[2];
[1]Neurol., [2]UCSF, San Francisco, CA; [3]Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: The nucleus accumbens (NAc) is a critical component of the brain networks that control motivation to work for rewards. Yet exactly how the NAc operates is unknown. Distinct subsets of NAc projection neurons express distinct dopamine receptors (D1 vs. D2) and are usually considered to boost or diminish motivation respectively. However, recent studies using optogenetic manipulations have cast doubt upon this scheme. One obstacle to understanding NAc is that we have very little data about the firing patterns of identified NAc neurons in behaving animals. To overcome this, we used optogenetic tagging to identify NAc projection cells as rats performed an operant task in which motivation varies with recent reward history. We used AAV5 for Cre-dependent expression of the opsin ChrimsonR, infused into the NAc Core of knock-in D1-Cre or A2a-Cre rats, together with a custom probe combining an optic fiber for illumination and tetrodes for single-unit electrophysiology. We identified n = 502 D1+ cells and n = 265 A2a+ neurons (the adenosine A2a receptor is a more selective marker for D2+ projection cells than D2 itself). As rats initiated a trial by approaching a nosepoke port, D1+ cells ramped up their firing. This increase consistently scaled positively with reward expectation - it was greater when recent prior trials had been rewarded. A2a+ neurons rarely increased firing during this approach behavior, and did not scale their firing (positively or negatively) with recent reward history. During a subsequent leftward vs rightward choice, both D1+ and A2a+ positively scaled with reward history, regardless of the value of the specific choice being made. Finally, at the completion of each trial A2a+ preferentially increased firing in response to a cue signaling reward. This was surprising, as we have shown that this reward cue evokes dopamine release

which might be expected to suppress A2a+ firing via D2 receptors. These new results should be highly valuable for the construction of new models of NAc functions in the pursuit of rewards.

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Poster

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Topic: E.03. Basal Ganglia

Support: NINDS

Title: Zona incerta distributes a broad movement signal that modulates behavior

Authors: S. HORMIGO, J. ZHOU, D. CHABBERT, S. SAJID, N. BUSEL, *M. CASTRO-ALAMANCOS;
Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: The zona incerta is a subthalamic nucleus made up mostly of GABAergic neurons. It has wide-ranging inputs and outputs and is believed to have many integrative functions that link sensory stimuli with motor responses to guide behavior. However, its role is not well established perhaps because few studies have measured the activity of zona incerta neurons in behaving animals under different conditions. To record the activity of zona incerta neurons during exploratory and cue-driven goal-directed behaviors, we used electrophysiology in head-fixed mice moving on a spherical treadmill and fiber photometry in freely moving mice. We found two groups of neurons based on their sensitivity to movement, with a minority of neurons responding to whisker stimuli. Furthermore, zona incerta GABAergic neurons robustly code the occurrence of exploratory and goal-directed movements, but not their direction. To understand the function of these activations, we performed genetically targeted lesions and optogenetic manipulations of zona incerta GABAergic neurons during exploratory and goal-directed behaviors. The results showed that the zona incerta has a role in modulating the movement associated with these behaviors, but this has little impact on overall performance. Zona incerta neurons distribute a broad corollary signal of movement occurrence to their diverse projection sites, which regulates behavior.

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Poster

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Title: Striatal neurons are recruited dynamically into collective representations of self-initiated and learned actions in freely-moving mice

Authors: L. TIROSHI[1], Y. ATAMNA[2], N. GILIN[2], N. BERKOWITZ[2], ***J. A. GOLDBERG**[2]; [1]Neurobio., Northwestern Univ., Evanston, IL; [2]Med. Neurobio., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Striatal spiny projection neurons are hyperpolarized-at-rest (HaR) and driven to action potential threshold by a small number of powerful inputs - an input-output configuration that is detrimental to response reliability. Because the striatum is important for habitual behaviors and goal-directed learning, we conducted microendoscopic imaging in freely-moving mice that express a genetically-encoded calcium indicator sparsely in striatal HaR neurons to evaluate their response reliability during self-initiated movements and operant conditioning. The sparse expression was critical for longitudinal studies of response reliability, and for studying correlations among HaR neurons while minimizing spurious correlations arising from contamination by the background neuropil signal. We found that HaR neurons are recruited dynamically into action representation, with distinct neuronal subsets being engaged in a moment-by-moment fashion. While individual neurons respond with little reliability, the population response remained stable across days. Moreover, we found evidence for the temporal coupling between neuronal subsets during conditioned (but not innate) behaviors.

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Title: Involvement of the mouse posterior striatum in visual perceptual decisions

Authors: *K. K. COVER, K. ELLIOTT, S. M. PREUSS, R. J. KRAUZLIS;
Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: The basal ganglia are associated with perceptual functions as well as action control. Our group has previously demonstrated a role for the basal ganglia direct pathway in biasing visual perceptual decisions in mice based on spatial expectation: optogenetic activation of dorsomedial striatal (DMS) D1-MSNs induced a perceptual bias in favor of valued events expected in the contralateral visual hemifield (Wang et al., 2018; 2020). Here, we investigated the involvement of the posterior striatum (PS) in visual perceptual decisions. We first showed that the direct pathway circuits originating from the PS and DMS are largely anatomically distinct. DMS D1-MSNs innervate the entopeduncular nucleus and substantia nigra pars reticulata whereas PS D1-MSNs route primarily through the substantia nigra pars lateralis. Next, to investigate the role of the PS in visual perceptual decisions, we optogenetically stimulated the direct pathway in mice trained to perform a spatially cued orientation-change detection task. We found that activation of PS D1-MSNs increased the false alarm rate with a significantly larger increase when the cued location was contralateral to the site of stimulation (N = 7 mice; 2-way repeated measures ANOVA: main effects of cued side and light delivery, $p < 0.01$; interaction, $p < 0.05$. In contrast, fluorophore-expressing control mice (N=4) did not show significant changes in false alarm rates as a result of light delivery. Together, these results demonstrate that parallel direct pathway circuits originating from the DMS and PS exhibit a shared role in biasing perceptual choices for expected contralateral events. Future work investigating the output targets of these circuits, such as the superior colliculus, may elucidate the mechanisms by which the basal ganglia influence perceptual decisions.

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NSF Grant CIF-1955981

Title: Sensory modulation of striatal networks during voluntary locomotion

Authors: *S. SRIDHAR[1], E. LOWET[2], H. GRITTON[4], J. FREIRE[3], C. ZHOU[2], F. LIANG[2], X. HAN[2];
[2]Biomed. Engin., [3]Pharmacol. & Exptl. Therapeut., [1]Boston Univ., Boston, MA; [4]Comparative Biosci., Univ. of Illinois, Urbana, IL

Abstract: Sensory modulation of striatal networks during voluntary locomotion Sudiksha Sridhar, Eric Lowet, Howard Gritton, Jennifer Freire, Chengqian Zhou, Florence Liang, Xue Han Boston University, Biomedical Engineering Department, Boston, MA The striatum receives

broad cortical and subcortical inputs and is well positioned to integrate sensory and motor information during behavior. While individual striatal neurons are well known to encode locomotion, particularly movement transitions, it remains unclear how striatal networks integrate sensory and motor information during voluntary movement. We performed calcium imaging from hundreds of individual dorsal striatal neurons simultaneously in mice during voluntary movement, occasionally presented with audio-visual sensory stimulation delivered at either beta (10Hz) or high-gamma (145Hz) frequencies. We found that 10Hz sensory stimulation entrained population striatal dynamics measured via local field potentials (LFPs), whereas 145 Hz stimulation was too fast to entrain striatal circuits. Sensory stimulation at both frequencies increased movement vigor, but only 10 Hz stimulation inhibited onset transitions. Striatal population neuronal activity precedes movement onset by ~150ms and tracked movement stepping during sustained locomotion bouts. While sensory stimulation led to heterogeneous and locomotion-state dependent changes in individual neurons, striatal network was consistently desynchronized regardless of stimulation frequency or locomotion state. Together, these results demonstrate that striatal dynamics track stepping movement during sustained locomotion bouts, in addition to encoding movement transitions. Sensory stimulation could effectively enhance movement vigor, and beta frequency stimulation, but not high-gamma frequency stimulation, effectively entrained striatal circuit dynamics and inhibited movement transitions.Funding: X.H. acknowledges funding from the NIH (1R01NS115797) and NSF (CBET-1848029, CIF-1955981).

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Poster

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Title: Nonlinear neural manifolds underlie naturalistic behaviours

Authors: *C. FORTUNATO, M. SAFAIE, C. GALLEGO-CARRACEDO, G. KAWAKITA, J. GMAZ, J. GALLEGO;
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Abstract: The collective activity of neural populations within a specific brain area can be well captured by a relatively limited set of co-variation patterns, termed 'neural modes'. Studying these neural modes and their activity—the ‘latent dynamics’—is shedding light into the neural

basis of behaviour and cognition. Here we seek to understand how interacting neural populations from various cortical and subcortical regions drive naturalistic behaviour by examining the geometrical properties of their underlying manifolds.
We initially hypothesized that neural manifolds should be intrinsically nonlinear due to the nonlinear responses of single neurons and their complex interactions. For these reasons, we further predicted that two factors would affect manifold nonlinearity: 1) circuit connectivity; and 2) task complexity. We verified these two hypotheses by analysing neural population activity during a variety of motor tasks across monkeys, mice and humans. First, we established that motor cortical manifolds are nonlinear even during stereotypical motor tasks. Then, we showed that the cytoarchitecturally distinct motor cortex and dorsolateral striatum have manifolds with very different degrees of nonlinearity during the same behaviour. Finally, we demonstrated greater manifold nonlinearities in more complex tasks that require a broader set of neural population activity patterns.
These observations on the properties of neural manifolds were primarily from motor-related areas during highly stereotypical laboratory tasks. To expand on these results, we designed a new behavioural paradigm to investigate the complexity of sensorimotor manifolds underlying higher-dimensional 'naturalistic' behaviour in mice. We extended a 3D treadmill setup by integrating linear motors that applied quick, random perturbations on the floating ball, eliciting adaptative behaviour in mice engaged in running. In these experiments, we recorded from various sensorimotor cortical and subcortical regions using Neuropixels probes, and tracked whole-body kinematics using a deep learning-based marker-less pose estimation method. Our analyses focus on two questions. First, whether there is a gradient in manifold nonlinearity and dimensionality as one moves toward higher order regions along both motor and sensory pathways in the brain. Second, whether sensory regions, due to their different role in behaviour, display greater degrees of manifold nonlinearity than motor regions. Collectively, our work explores the need for capturing emergent nonlinear interactions within neural populations, especially as we delve into increasingly complex behavioural paradigms.

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Poster

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Title: The representation of syllable repetition, sentence production and orofacial movement in the subthalamic nucleus

Authors: *Z. JOURAHMAD[1], J. I. BERGER[1], A. H. ROHL[1], C. K. KOVACH[1], F. TABASI[1], K. JOHARI[2], J. D. W. GREENLEE[1,3,4];
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Abstract: Speech production is a complex behavior in humans that involves the interplay of motor and language network. The interaction of various cortical brain regions throughout speech production is broadly studied, however research that investigates the role of subcortical area is more limited. Some evidence suggests that subthalamic nucleus (STN) plays a role in producing speech. Previous studies have shown differential modulation of STN neural firing during speech, but it remains an understudied area. STN is thought to participate in the regulation of motor movement through the classical cortico-subcortical loop receiving inputs from frontal cortex via the hyperdirect pathway. These critical motor circuits regulate and coordinate limb, orofacial and saccadic eye movement. It remains unclear whether speech-associated activity in STN is related to facial movement generically or exhibits specificity for speech production. To investigate this question of speech versus orofacial driven modulation in STN we recorded single- and multi-units from 12 participants (3 female) with Parkinson's disease who were undergoing awake bilateral deep brain stimulation STN electrode implantation surgery. STN unit activity was recorded from Alpha Omega high impedance electrodes. Fifty-eight single- and multi-units clusters were detected and sorted offline using a recently developed automated sorting toolbox by our lab (Kovach et al. Signal Processing 2019 165:357-379). To measure neuronal modulation, we calculated trial-by-trial mean firing rate during baseline and with respect to the specific behaviors throughout separate blocks. The speech task include sentence production ("Buy Bobby a Puppy") and diadochokinetic repetition ("TA/TA/TA") in an interleaved fashion. Non-speech orofacial movement tasks included interleaved trials of jaw opening and closing and tongue protrusion. We observed varied STN neuronal firing response patterns that were time-locked to the initiation of sentence production, syllable repetition and orofacial movement. Some units showed increased firing rates while others exhibited decreased rates. Of particular note, neuronal clusters showing strong speech-related and non-speech orofacial movement-related responses were distinct, with neurons that were responsive to orofacial movement having higher mean baseline firing rate.
Together, these findings suggest that distinct population of neurons in STN are modulated during speech production and orofacial movement. Exploring the involvement of the STN in speech production can contribute to our understanding of the architecture of the speech network.

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Poster

PSTR551. Basal Ganglia: Codes and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR551.19/HH25

Topic: E.03. Basal Ganglia

Support: NIH R01NS118424

Title: Longitudinal Imaging of Basal Ganglia Activity in Juvenile Finches Learning to Sing

Authors: *J. QI[1], M. MARTINEZ[1], J. M. PEARSON[2], R. D. MOONEY[1];
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Abstract: Some complex motor skills, such as human speech and musical performance, are learned by imitation and without external reward or punishment. The neural mechanisms that enable such internally guided forms of imitative learning are poorly understood, partly due to the scarcity of well-documented forms of internally guided learning in animals. Juvenile male zebra finches learn to copy an adult male (tutor) song, a process with many parallels to human learning of speech and music. We sought to better understand how activity in song-specialized region of the basal ganglia (sBG) affects song copying in juvenile finches. We virally expressed an engineered calcium indicator, GCaMP6s, either in the general population of neurons in sBG using a pan-neuronal promoter, CAG, or specifically in sBG spiny neurons (SNs) using a CaMKII promoter. We used a miniature microscope to image singing-related activity of sBG neurons in juvenile male zebra finches as they copied a previously memorized tutor song. We extracted ROIs using MINIPIPE and measured inter-neuronal synchronization as well as other properties. We found that the singing-related activity of SNs was less synchronized than in the general population of sBG neurons. To further investigate these differences, we examined how synchronization between ROIs changed across time by looking at the correlation structure of the neural population. In the general population of sBG neurons, we found that correlation structure across ROIs repeated over the course of each day. However, each song had a nearly unique correlation structure, suggesting a fine-grained relationship between the variability in the song and the neural population activity. These findings are consistent with the idea that variations in sBG ensemble activity enable the juvenile finch to explore vocal “space” as it searches for song variants that better match the tutor song. In ongoing analysis, we are using a variational autoencoder (VAE), an unsupervised machine learning method, to find low-dimensional representations of variability in each song. We are pairing this approach with a linear dynamical system model of the ROI activity to relate each song rendition produced by a juvenile during sensorimotor learning to sBG ensemble activity. These studies are beginning to elucidate the correlation between sBG activity, fast and slow changes in vocal variability, and song copying, thus offering insight into neural mechanisms for complex form of imitative motor learning.

Disclosures: J. Qi: None. **M. Martinez:** None. **J.M. Pearson:** None. **R.D. Mooney:** None.

Poster

PSTR551. Basal Ganglia: Codes and Behavior

Location: WCC Halls A-C

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Program #/Poster #: PSTR551.20/HH26

Topic: E.03. Basal Ganglia

Support: UNAM-DGAPA-PAPIIT: IN200822
Fellowship 857858 from CONACyT-México

Title: The substantia nigra reticulata scales kinematic representations across different behavioral contexts

Authors: *A. BÁEZ-CORDERO, D. ORTEGA-ROMERO, C. PÉREZ-DÍAZ, P. RUEDA-OROZCO;
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Abstract: Movement execution requires coordinated activity between sensory and motor systems and involves several cortical and subcortical regions, including the basal ganglia (BG). While the specific mechanisms by which the BG influence movements remain unclear, previous reports have shown robust kinematic and contextual representations in their input (striatum) and output nuclei (susbtantia nigra pars reticulata, SNr). Hence, an attractive hypothesis is the BG implication in the control and adaptation of movement kinematics to different behavioral contexts. Here, we explore this possibility by using high-density electrophysiological recordings in freely moving rats and analyzing the neural activity of the SNr. Recordings were performed during movement execution in two behavioral protocols with a different range of spatial and temporal content. In the first protocol, animals were required to perform locomotion runs constrained to a spatiotemporal range of meters and a few seconds (around 7 seconds). In the second protocol, the same animals were required to perform forelimb movements constrained to 3 to 10 centimeters in the order of hundreds of milliseconds. We found that the SNr spiking activity in both tasks was linearly correlated with kinematic parameters such as position and velocity. Furthermore, these representations were adjusted to the different spatiotemporal scales of the movement. That is, the minimum and maximum SNr firing rates adjusted proportionally to the minimum and maximum ranges of speed/position on each task. Our data suggest that while position and velocity signals are preserved throughout the BG, they scale in a context-dependent manner, supporting their implication in the abstract representation of movement kinematic control.

Disclosures: A. Báez-Cordero: None. **D. Ortega-Romero:** None. **C. Pérez-Díaz:** None. **P. Rueda-Orozco:** None.

Poster

PSTR552. Neural Decoding and Processing

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Topic: E.05. Brain-Machine Interface

Support: NSF EFRI BRAID 2223822
NIH NIDCD R01DC018446

Title: Evaluating generalization gap in neural decoders for vocal synthesis

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Abstract: Recent advancements in neural network-based decoders have achieved state-of-the-art performance in translating neural activity into synthesized vocalizations. The generalization capabilities of these decoders for synthesis of novel vocal sequences, however, remains uncertain. Evaluation of generalization is critical because errors in predicting novel vocal elements and sequences can severely impede effective communication, given the limited time available to collect data for decoder training. To address this challenge, we propose the vocal unit level generalization gap, measuring the difference between the expectation of training error and holdout generalization error, as an evaluation procedure to assess a model's tendency to memorize the input-output relationship between neural activity and vocal units to make accurate predictions. The concept of generalization gap, initially introduced in computer vision research, quantifies the extent of label memorization by an image classification network. Here, we extend this definition to instances of a vocal unit and quantify the extent to which the model's prediction changes when the vocal unit is included in the training data, serving as an approximation for model memorization of that vocal unit. A lower average generalization gap across all vocal units would indicate the capability of the neural decoder to effectively interpolate and extrapolate, going beyond mere memorization. We evaluated a neural decoder on a zebra finch vocalization dataset containing neural recordings from 143 single-units in the robust nucleus of the arcopallium (RA), the brain region projecting onto motor neurons responsible for effector-muscle control in the vocal apparatus. Our feedforward neural network decoder translates RA activity to song spectral slices at 1ms resolution. We measured the generalization gap at the syllable level (vocal units analogous to phones in human speech). Comparing the non-regularized decoder against the best regularized decoder, there is a 3 times increase in the generalization gap with only a 12% decrease in the training error.These findings match our expectation that effective regularization results in higher performance decoding for novel vocal units while maintaining much of the performance on the non-novel vocal units. Moreover, this measure is extensible to withholding parts of latent acoustic or dynamical features. These findings enhance our ability to evaluate and benchmark neural decoders, particularly as we develop novel decoding architectures motivated by a hypothesis driven design strategy.

Disclosures: J. Huang: None. **P. Tostado:** None. **E.M. Arneodo:** None. **G. Mishne:** None. **T. Gentner:** None. **V. Gilja:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Shareholder in Neuralink, Corp.. F. Consulting Fees (e.g., advisory boards); consults for Paradromics, Inc..

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.02/HH28

Topic: E.05. Brain-Machine Interface

Support: NSF Grant 1849213

Title: Event-related changes in the EEG are associated with graded finger extension: trust but verify?

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Abstract: Brain-computer interfaces (BCIs) are becoming more commonly used in rehabilitation as a means of detecting volitional signals from the brain to assist movement or provide proprioceptive feedback to individuals with disability. However, command signals tend to reflect binary brain states that reflect contrast between movement and rest alone. Here, we seek a correlate in the electroencephalogram (EEG) of different grades of movement associated with finger extension, which is a priority for individuals with hand impairment. In a human study performed with institutional approval and informed consent, able-bodied individuals (n=11, all right-hand dominant) were prompted to extend their fingers in response to visual cues to one of four levels: low, medium, high, or "no-go" (i.e., none). Each session consisted of 12 runs alternating between the left and right hand. Finger movement, extensor muscle, and brain activity were monitored using motion capture, electromyography (EMG), and 32-channel EEG, respectively. Finger movement was measured in terms of the angle of finger extension and distance between the metacarpal joint and fingertips. Event-related desynchronization (ERD) of the sensorimotor EEG was measured in terms of the mu-beta (8-30 Hz) signal power relative to the no-go condition at each scalp location, and ERD strength computed as the vector norm over all EEG channels for each level of extension. The mu-beta ERD strength increased monotonically from low to high finger extension in 5/11 participants on the left hand, a proportion greater than chance ($p<0.05$), and in 4/11 participants on the right hand. This trend reflects a progressive increase in cortical recruitment correlated with extensor activity and indicates the feasibility of deriving a graded volitional signal that represents fine motor control. But though actual finger extension measurements followed the same trend in the mean as the targeted level, the measurements showed significant overlap between targets, which points to limitations in the participants' ability to accurately perform this deceptively simple motor task. Removal of outliers or remapping of target labels based on actual movement may improve the ability to resolve fine control signals in the EEG; these avenues will be explored in future work. We also propose to extend this protocol to include various hand configurations (e.g., two-finger pinch) and actions (e.g., grip force) using a custom-designed sensor glove, meant to provide proprioceptive feedback to the user in movement-related BCI protocols.

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Poster

PSTR552. Neural Decoding and Processing

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Topic: E.05. Brain-Machine Interface

Support: Challengeable Future Defense Technology Research and Development Program through the Agency For Defense Development(ADD) funded by the Defense Acquisition Program Administration(DAPA) in 2023(No.915069201)

Title: Reconstruction of 3D hand trajectories during center-out-and-back task with velocity decoding from electrocorticography signals in humans.

Authors: *M. SHIM[1], Y. YANG[2], J. KIM[2], C. CHUNG[2], S.-P. KIM[1];
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Abstract: Harnessing electrocorticographic (ECoG) signals can offer a compromising solution for neural motor prosthetics to reduce invasiveness compared to using intracortical signals. Many studies have demonstrated successful decoding of limb movements from ECoG data. In particular, continuous kinematic parameters such as hand position and velocity in 2D or 3D space could be predicted from human ECoG using various decoding models, showing a possibility to control upper limb prosthetics in naturalistic environments. Yet, it remains unclear which kinematic parameters should be decoded from ECoG to reconstruct 3D hand trajectories. Also, it would be useful to know the mapping of ECoG grids, which often varies across epileptic patients, affects 3D hand trajectory decoding performance. In this study, we addressed the first question by comparing 3D hand trajectory reconstruction between direct position decoding versus reconstructing position from velocity decoding. As position and velocity are coupled in the conventional center-out task, we analyzed ECoG data in the center-out-and-back task to decouple velocity and position. Also, we addressed the second question by investigating a relation between the properties of ECoG mappings and individual decoding performance. Fourteen epileptic patients participated in the experiment in which they performed the 3D center-out-and-back task with four target locations. In each subject, we used the first 70% of the whole trials as a training set and the last 30% as a testing set. We built two decoders each being trained to decode either 3D hand position or velocity. To test a velocity decoder, we reconstructed position by integrating velocity over time. We evaluated decoding performance using both Pearson's correlation coefficients (CC) and root-mean-square error (RMSE) between predicted and actual hand trajectories. The result showed that the average CC of x-, y-, and z-axis was 0.519, 0,515, 0.454 using the position decoder and 0.602, 0.55, 0.529 using the velocity decoder, respectively. It revealed that decoding velocity from ECoG produced better outcomes than decoding position (signed rank test, $p < 0.05$). Next, we extracted an index of ECoG grid mappings as the number of electrodes located in frontal cortical areas including premotor and primary motor cortex. We found that this index could account for the variance of individual decoding performance ($r2 = 0.407$, $p < 0.05$), showing that when more electrodes were located in

frontal cortical areas, decoding performance tended to increase. Our results may provide additional information to the design of decoding models for 3D hand trajectory reconstruction from human ECoG signals.

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Poster

PSTR552. Neural Decoding and Processing

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Topic: E.05. Brain-Machine Interface

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Title: Decoding 'before the movement' and 'after the movement' signals for Brain machine interface

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Abstract: *Objective:* A brain machine interface (BMI) is designed to convert raw neural signals into motor commands and reproduces the body's movements with a neuroprosthetic device [1]. An invasive study showed that a person with tetraplegia can drink coffee by controlling a robotic arm through neural signals recorded by microelectrode arrays (MEAs) [2]. However, MEAs may have risks of infection and damage to the brain tissue. Moreover, recordings from the MEAs can be unstable for long-term use. Electrocorticography (ECoG) is a beneficial option for BMIs in because it covers wide brain areas with a high signal-to-noise ratio and minimal damage. Thus, ECoG can show important neural signals. Here, we used the ECoG signal to decode human reach-and-grasp movements. The 'before the movement' period, which is the state of before the movement execution, involves the intention and planning of the movement [3]. We wanted to know whether this 'before the movement' signal could be decoded.*Methods:* Five epileptic patients with intracranial electrodes were asked to execute reach-and-grasp movements to random targets. CT and MR images were co-registered using the CURRY software to find the positions of the electrodes. We decoded the 'before the movement' (-1.5 to 0 s from the movement onset) and 'after the movement' (0 to 1.5 s from the movement onset) data by using the Long Short-Term Memory algorithm (LSTM) and conventional multiple-linear regression algorithm (MLR) to compare the decoding performance.*Results:* We successfully decoded the data of five subjects. The 'before the movement' period was also decoded. The decoding

performance of the ‘before the movement’ was similar (Pearson’s $p < 0.05$) to the ‘after the movement’ period. Also, the decoding performance for LSTM algorithm was outperformed compared to the decoding performance of MLR algorithm.*Discussion:* The results suggest that the neural network during ‘before the movement’ has also directional information. Therefore, we suspect that understanding the ‘before the movement’ signal is important in controlling BMI and understanding human neurophysiology during movement.

Disclosures: Y. Yang: None. **J. Kim:** None. **C. Chung:** None.

Poster

PSTR552. Neural Decoding and Processing

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Program #/Poster #: PSTR552.05/II3

Topic: E.05. Brain-Machine Interface

Support: NIH R01EY015545
NIE UG1EY032039
NRSA T32NS105595

Title: Context dependent coordinate-frame transformations in human posterior parietal cortex

Authors: *K. KADLEC[1], J. GAMEZ[2], T. AFLALO[3], M. JAFARI[4], C. GUAN[3], E. ROSARIO[5], A. BARI[4], N. POURATIAN[6], R. A. ANDERSEN[7];
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Abstract: Our ability to interact with objects around us is easily taken for granted, but requires complex neural transformations to convert spatial information from the input of retinal coordinates into the proper motor output. When reaching out to grasp an item, the object position transitions from being coded relative to the position of the eyes to being coded relative to the position of the hand. Traditionally it has been thought that this process, like other parts of motor control, is organized with anatomically separate areas in cortex coding for different effectors. We have the opportunity to evaluate this hypothesis by recording from single neurons in motor areas of human cortex as part of an ongoing brain-machine interface clinical study. We recorded from 96 intracortical electrodes implanted in the left hemisphere of the posterior parietal cortex (PPC) in two subjects and in the left hand knob of the motor cortex (MC) of one subject. We used two versions of the same task to test movements of two effectors. First, the subject positioned their eyes and right hand at varying locations on a monitor. Then a target was shown, followed by a delay period during which the subject maintained gaze and hand position. Finally, after a go cue, the subject performed either an imagined reach (IR) or a saccade to the target depending on the version of the task. We found that in the early phases of both versions of the task when the relevant information is the hand and eye position, the neural population in PPC encoded a vector

from the hand to the eye. When the target became visible, the coding seen in PPC began to differ between the two tasks. In the IR task, the population began to code a vector from the hand to the target in addition to the vector from the hand to the eyes, while in the saccade version of the task, it began to also code a vector from the eyes to the target. Finally, during movement execution, PPC encoded the vector from the hand to the target if an IR was being performed, and a vector from the eyes to the target if the subject saccaded. This population result was also seen at the single unit level, where some individual units showed changes in vector coding through the task. In MC, there was no vector coded during the initial phases of the task without movement. During the go phase, a hand-target vector was coded for the IR task, but no vector is coded for saccades. The results from MC are in line with an effector-organized network, but the flexibility of the vector coding seen in PPC is surprising. As we have been able to study PPC in humans more thoroughly, a higher cognitive movement code has emerged. The exact purpose of this switching of representation is not immediately clear, but is a promising area for future research.

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Poster

PSTR552. Neural Decoding and Processing

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Topic: E.05. Brain-Machine Interface

Support: Hong Kong Research Grants Council (grants N_CUHK456/21, R4022-18F, 14114721, 14119022 to VCKC).

Title: Correlation between neural modes and muscle synergies revealed by optogenetically stimulated muscle and spinal interneuronal population activities

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Abstract: Advances in neurophysiology have enabled simultaneous recording from multiple neurons, leading to new theoretical models of neuronal information processing in which populations of neurons are the primary units of computation, rather than individual neurons. In the context of movement control, it has been shown that motor and premotor cortical activity can be reduced into a smaller set of neural modes controlled by a few independent latent variables that represent the dynamics of the neural modes (Gallego, 2017). An analogous simplifying strategy has previously been proposed for motor control at spinal level. According to this theory, spinal interneurons are organized into functional motor modules, known as muscle synergies, that activate different muscles simultaneously with distinct temporal activation profiles. Electromyographic signals (EMG) can be decomposed into basis vectors and activation coefficients, describing how the synergies combine to produce observed muscle activity. With

our study we aim to investigate the presence of neural modes in the spinal cord and their correlation to the motor output represented as muscle synergies. We optogenetically stimulated excitatory spinal interneurons in the lumbosacral spinal cord of 9 anesthetized Thy1-ChR2-YFP mice while simultaneously recording intramuscular EMG from 6 hindlimb muscles and the neural activity of spinal interneurons. The neural activity was recorded using a 64-channel silicon array, implanted perpendicular to the ipsilateral spinal surface at T11 level, while the laser was advanced in steps of 200 µm, rostrally-caudally, along it. After performing spike sorting on the neural data, we applied spike-triggered averages to all muscle activities. We found that 55% of the units had muscle fields that could be well-matched to the EMG-derived synergies. We then applied principal component analysis to the neurons' firing rates to find the neural modes of the population and calculated the muscle field for each neural mode. We observed that the EMG-derived muscle synergies could be reasonably matched to those estimated from the neural modes. Interestingly, the degrees of matching between the muscle fields and synergies at the population and single-unit levels were consistent. Taken together, our preliminary results confirm that there is a correlation between neural population and muscle synergies in their spatiotemporal activations, suggesting that muscle synergies may be implemented by neural modes rather than individual spinal interneurons.

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Poster

PSTR552. Neural Decoding and Processing

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Topic: E.05. Brain-Machine Interface

Support: JST CREST JPMJCR186
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JSPS KAKENHI 23H03731

Title: Wireless ECoG recording from an unrestrained animal performing a visuomotor task within its home cage

Authors: *T. KAIJU[1], M. INOUE[1], M. HIRATA[2], T. SUZUKI[1];
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Abstract: Introduction: Brain-machine interface (BMI) is a promising technology for supporting individuals with disabilities. In the process of developing and testing new BMI devices, it becomes essential to conduct motor task experiments on non-human primates (NHPs) to interpret their intentions. However, training these primates for behavioral tasks tends to be labor-intensive and often spans several months. This leads to inefficiencies in data collection, which, in turn, slows down the overall device engineering process.

Methods: We have developed a behavioral testing environment that allows NHPs to perform motor tasks within their home cages. Additionally, we have engineered a wireless headstage specifically optimized for ECoG/LFP recording. We demonstrated the utility of this setup by acquiring neural signals from one Japanese macaque while it performed an 8-direction center-out task using a joystick. Recordings were taken from electrodes positioned in the primary somatosensory cortex.
Results: The subject was able to complete over 1000 trials in each daily session. A negative deflection in the broadband signal was observed post the hand movement, modulated by the direction of the movement. The time-frequency analysis showed an increase in signal power from the gamma band to the high gamma band. The direction of movement was decoded with an accuracy of ~50% (compared to a 12.5% chance level).
Discussion: Our system successfully recorded neural activity from an unrestrained animal performing a visuomotor task within its home cage. It enables daily autonomous training of various tasks, with minimal adjustments of task difficulty. The unrestrained conditions might improve animal comfort during the task, possibly explaining the high number of trial attempts observed in a daily session.

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Poster

PSTR552. Neural Decoding and Processing

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Program #/Poster #: PSTR552.08/II6

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS 1R01NS124222-01 (MI)

Title: Reinnervated proprioceptive action potentials recorded by regenerative ultramicro-electrode arrays

Authors: *K. HUSSEIN[1], A. AZAMI[2], S. F. COGAN[2], M. I. ROMERO-ORTEGA[1]; [1]Biomed. Engin., Univ. of Houston, Houston, TX; [2]Bioengineering, The Univ. of Texas at Dallas, Richardson, TX

Abstract: Regenerating axons were observed to be electrically active following amputation injury in rodents implanted with a regenerative multi-electrode array (REMI). However, the specific neuron type was not identified due to the mixed growth regenerative path through the electrodes, and the relatively large size of both the individual needle-like electrodes and the active site's geometric surface area (GSA) in the REMI (i.e., shaft 200 μm outer diameter (OD) and 2000 μm^2 GSA). Here, we fabricated a novel Regenerative Ultramicro-electrode Array (RUMA) with 3 ultramicro-electrode arrays (UMEAs) located in the common, left and right arms (inner diameters (ID): 1.0, 0.6, and 0.6 mm, respectively) of a Y-shape conduit, which was implanted into the rat tibial and peroneal nerves in an end-to-end repair strategy to allow re-

innervation of their natural gastrocnemius and tibialis anterior muscles in the lower limb. The UMEAs are significantly thinner needle-shaped electrodes (8 μm OD) with a 10-fold GSA reduction (200 μm^2) compared to those in the REMI.
Single action potentials from regenerating neurons were recorded from a total of 30 active channels from 14 days after implantation and for 77 days at the writing of this abstract. These single units were recorded using the OmniPlex Data Acquisition System (Plexon Inc) at 40kHz sampling rate and a 300 Hz high-pass filter. Proprioceptive waveforms were isolated by dual thresholding using the Plexon Offline Sorter during passive limb stretching in anesthetized animal and confirmed by active limb stretching during bipedal standing in a freely moving animal. Results show that the signals remained stable from week 8 as indicated by PCA comparison of waveform metrics. In-vivo impedance tests are underway to report changes in electrode integrity. This work further demonstrates that the RUMA provides increased stability for peripheral nerve interfacing and allows for functional identification of individual regenerated proprioceptive neuron types. Acknowledgments: We thank Amairani Ramirez Rendon for experimental assistance, and funding by NIH NINDS 1R01NS124222-01 (MI).

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Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.09/II7

Topic: E.05. Brain-Machine Interface

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Title: A causal test of the flexibility of interactions between cortical areas

Authors: *S. SNYDER[1], E. R. OBY[2], M. A. SMITH[3], S. M. CHASE[3], B. YU[4], A. BATISTA[2]; [1]Ctr. for Neurosci., [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [3]Biomed. Engin., [4]Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Most brain functions rely on interactions between brain areas and these interactions are believed to underlie adaptable sensorimotor behavior. However, the extent to which the activity of distinct populations of neurons can be selectively coordinated with each other is unknown. Here we sought to directly test the flexibility of functional interactions between populations of neurons in distinct brain areas, relative to the flexibility of interactions between different populations of neurons in the same brain area.
The first step in challenging the flexibility of interarea interactions is to identify how they are interacting. We used canonical correlation analysis to identify a pair of dimensions (one in each

area) along which the population activity is maximally correlated. We used multielectrode arrays to simultaneously record from neurons in the dorsal premotor cortex (PMd) and primary motor cortex (M1) of a monkey. After identifying how the two areas are interacting, we used a brain-computer interface (BCI) paradigm to causally test the degree of flexibility between the populations. In our BCI setup, the velocity of a computer cursor was under the direct control of the neural activity in the pair of correlated dimensions. This allowed us to present different targets to the animal. Some targets asked him to reproduce correlated neural activity patterns. Other targets challenged the animal to produce neural activity patterns that were anticorrelated across these dimensions, and thus required the animal to decouple neural activity in the two populations. As a key comparison, we also tested the animal's ability to decorrelate signals shared within a single brain area. We randomly divided the recorded population from a single area into two non overlapping groups and then used the same procedure as described above to identify and challenge interaction modes between the two populations.
We conducted dozens of sessions in which the animal was challenged to decouple activity either between PMd and M1, or within PMd only or M1 only. We found that interactions between PMd and M1 were more flexible than interactions within PMd or M1 alone. This was assessed by faster cursor speeds to the specific targets that challenged the animal to decouple his neural activity. For the across-area experiments, the animal was able to generate population activity over hundreds of trials that were both stronger and more consistently decoupled compared to the activity within either area alone. These results provide causal evidence that the interactions between brain areas can be flexible, perhaps supporting the vast repertoire of existing behaviors or enabling the acquisition of new behaviors.

Disclosures: S. Snyder: None. **E.R. Oby:** None. **M.A. Smith:** None. **S.M. Chase:** None. **B. Yu:** None. **A. Batista:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR552.10/Web Only

Topic: E.05. Brain-Machine Interface

Support: Michael Smith Foundation for Health Research

Title: A generative adversarial framework for mesoscale cortical dynamics decoding, behavior prediction, and mind reading.

Authors: *D. XIAO[1], B. ZHAO[2], T. H. MURPHY[1];
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Abstract: A cardinal objective in systems neuroscience entails elucidating the intricate relationship between neural activity and behavior. Historically, behavioral analysis has

predominantly focused on low-dimensional, task-associated variables such as locomotion velocity or reaction times. However, burgeoning interest in the complex, nonlinear associations between brain function and high-dimensional behavioral data necessitates the development of innovative tools proficient in decoding real-world, brain-related high-dimensional data. In this study, we present MesoGAN, a sophisticated Generative Adversarial Network (GAN) tailored to synthesize authentic behavioral videos derived from the neural decoding of mesoscopic cortical calcium dynamics. Employing wide-field cortical calcium imaging, our model generates synthetic (predicted) behavioral videos. Our results demonstrate that the GAN-based approach can generate realistic fake behavioral videos that closely resemble the actual videos (brain to behavior). The framework can also be used to reconstruct brain activity from behavior video (behavior to brain). The attention maps produced by the GAN further pinpoint critical brain activity features that are highly predictive of specific bodily movements, thereby offering novel insights into the neural activity-behavior relationship. Moreover, we expand our framework to encompass human EEG data, facilitating the prediction of forelimb movement from human cortical activity. This research holds significant implications for fields such as brain-computer interfaces, neuroprosthetics, and personalized medicine. By paving the way for future investigations into brain activity decoding, our study contributes to an enhanced understanding of the human brain and its intricate functions.

Disclosures: **D. Xiao:** None. **B. Zhao:** None. **T.H. Murphy:** None.

Poster

PSTR552. Neural Decoding and Processing

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Program #/Poster #: PSTR552.11/II8

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R00NS101127

Title: Improving BCI accuracy with multiple neural dimensions for a single degree of freedom

Authors: *W. LEE, A. G. ROUSE;
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Abstract: Brain-computer interfaces (BCIs) have emerged as valuable tools for decoding neural activity to help restore motor control and communication. Recent studies raise a focus on the dynamical neural population associating with distinct behaviors, indicating that different encoding models may apply on different behaviors. However, prevailing BCI decoders often fail to account for the unique neural dynamics and state-spaces present across movement types. In this study, we propose a novel state-dependent encoding model that leverages our understanding of these distinct neural dynamics. By capturing and integrating information from multiple neural spaces, our model aims to provide inspiration to improve the accuracy and reliability of neural decoding. It increases the number of neural dimensions used for encoding a given degree-of-

freedom and thus increases the amount information signaled and obtainable precision. We synthesized two neural tuning models to test the hypothesis. For simplicity, the synthetic models are only tuned to velocity. The distinct movement types are identified based on peak velocity of a 1-dimensional reaching movement. The first, classical model includes neurons' firing rates tuned to velocity along a single neural dimension. Our novel model includes neurons that tune to 1-dimensional velocity in a 2-dimensional space. The synthesized firing rates based on a combination of two different encoding models -- one for smaller movements and one for larger -- are then projected into a 2D neural space. The 2D tuning model shows a diverging neural trajectory at the early stage of movement, indicating the planning of movement speed peak is clearly encoded earlier than a standard single dimension encoder and could be predicted by downstream neuromotor processes, or a BCI decoder. Noise was then added to the modeled neural populations for the two encoding models. We then fit decoders as ideal observers of the two encoding models. With the extra dimension to identify magnitude of upcoming movement, we can reconstruct the movement speed profile with higher accuracy. We present these simulation results demonstrating the efficacy of our approach in enhancing decoding performance. This research holds significant implications for moving forward the understanding of brain encoding of distinct reaching movement types and establishing more precise and reliable brain-controlled systems. The proposed state-dependent BCI decoder opens new avenues for investigating the intricate interplay between neural spaces and their impact on decoding accuracy, facilitating the development of more efficient and adaptable BCI systems.

Disclosures: W. Lee: None. **A.G. Rouse:** None.

Poster

PSTR552. Neural Decoding and Processing

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Program #/Poster #: PSTR552.12/II9

Topic: E.05. Brain-Machine Interface

Support: NIH R01 NS125877

Title: Neural population decoding of locomotion on a step-by-step basis from primary motor cortex

Authors: *D. SINGLA, L. YANG, S. C. MASMANIDIS;
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Abstract: Objective: While the primary motor cortex (M1) plays an unquestionably important role in movement control, its role in regulating gait on a step-by-step basis is less clear. Previous studies have shown that a subset of M1 neurons preferentially fire action potentials during specific phases of limb movements during walking. However, it is unknown whether it is possible to precisely decode the phase of individual limbs using population-level dynamics. Here, our objective is to examine whether single-limb gait phase can be decoded from the

population activity of tens of M1 neurons in mice. **Approach:** We performed high-speed (80 Hz) video recordings of self-initiated walking in mice freely behaving in a 60 cm x 60 cm open field. The open field had a transparent floor allowing us to track the position of each limb on a step-by-step basis using open-source pose tracking tools. Simultaneously, we also recorded electrophysiological activity from multiple M1 single-units using chronically implanted silicon probes. We first confirmed that a large fraction of units in M1 encode single-limb gait phase and fire preferentially during specific phases of the gait cycle. To show this, we converted limb position to phase and calculated tuning curves for single-unit firing rate versus gait phase. We then evaluated neural population activity characteristics using support vector machines and decoded the gait phase during walking periods. **Main results:** The average absolute circular distance (AAE) between predicted and actual limb phases was significantly lower than shuffled limb phases. Further, the decoding performance appeared to plateau when the number of units exceeded ~10. Moreover, while the recordings were performed in the right hemisphere, the decoding performance was comparable for all four limbs. **Significance:** This work shows that neural population activity in M1 encodes information about the phasic movement of limbs on a step-by-step basis during normal gait. This may have relevance for understanding the mechanisms of impaired gait in movement disorders and for developing brain machine interfaces (BMIs) that control step-by-step locomotion.

Disclosures: D. Singla: None. **L. Yang:** None. **S.C. Masmanidis:** None.

Poster

PSTR552. Neural Decoding and Processing

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Program #/Poster #: PSTR552.13/II10

Topic: E.05. Brain-Machine Interface

Support: Michael J. Fox Foundation

Title: Using bidirectional deep brain implants in Parkinson’s disease (PD) patients and unsupervised machine learning approaches to study human motor control mechanisms

Authors: *X. XING[1,6], K. PRESBREY[2], K. H. LOUIE[2], M. MORRISON[3], R. BHALERAO[3], P. A. STARR[2,4], R. ABBASI-ASL[5], D. D. WANG[2,4];
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Abstract: The current understanding of human neural dynamics and its projection on motor control is obscure, leading to no good treatments for motor control impairments in PD patients. However, the application of bidirectional deep brain stimulation (DBS) devices in Parkinson's disease (PD) patients has provided a promising approach to investigate this question. In order to decode neural biomarkers associated with motor control, we recorded neural activity from

various brain regions of PD patients while they practiced typing sequences on a custom behavioral apparatus that determines finger kinematics. With the explorative unsupervised machine learning approaches including Hidden Markov Model, we are able to decode online digit and element finger movements from hidden states of neural activities across days and under different treatment conditions.

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Poster

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Title: New insights and methods for automatic and reliable decomposition of the spiking activity of populations of motor neurons from high-density intramuscular electromyography

Authors: *A. GRISON[1], A. CLARKE[1], S. MUCELI[3,2], A. KUNDU[1], J. IBANEZ[4,2], D. FARINA[2];
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Abstract: Recent advances in intramuscular electrode technology and data acquisition systems allow recordings of muscle electrical signals with unprecedented spatial resolutions. The invasive nature of intramuscular EMG (iEMG) recordings enables the measurement of muscle activity in a more selective way than with surface EMG. Modern iEMG electrodes can be microfabricated in arrays with tens of detection sites. The high number of channels represents an impossible workload for manual decomposition of individual motor unit (MU) activity, demanding new automated source separation methods that match the high density of information acquired from increasingly compact detection sites. To explore the applications and limitations of these advanced electrodes, we conducted an extensive study involving intramuscular recordings with high-density electrodes from the major forearm muscles under isometric and

dynamic contractions. Our experimental design accounted for a wide range of muscle activities (e.g., flexion and extension of single and multiple fingers) to provide a comprehensive understanding of how intramuscular electrodes operate under varying circumstances. We developed a novel method of automatic MU decomposition based on a projection-pursuit form of gradient descent independent component analysis, validating the tools by comparison with state-of-the-art techniques (CKC-FastICA) using manually labelled data as a ground truth. In particular, we demonstrated the importance of adapting the nonlinear contrast function to each source, especially when there is a high degree of similarity amongst the MUs. We achieve this by introducing a novel method based on particle swarm optimisation of a family of polynomial contrast functions. The resulting algorithm outperforms current methods, returning 28 units (accuracy=94.75 ± 4.83) from a 16-channel electrode of a Tibialis Anterior muscle contraction at 30%MVC. The same analysis run using the gold standard automatic method returned only 7 MUs. Our result is comparable to the yields of manual decomposition, but runs automatically in a fraction of the time. Our investigation resulted in two significant findings. Firstly, we obtained a comprehensive understanding of the potential of human recordings using thin film electrodes, building a large dataset of iiEMG activity under diverse motor conditions. Secondly, we propose a source separation method that enables detailed automatic analysis of MUs, setting the stage for novel physiological studies and clinical diagnostic methods. Our research marks a significant stride towards accurate and automatic decomposition of high-density iEMG electrodes.

Disclosures: **A. Grison:** None. **A. Clarke:** None. **S. Muceli:** None. **J. Ibanez:** None. **D. Farina:** None.

Poster

PSTR552. Neural Decoding and Processing

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Program #/Poster #: PSTR552.15/II12

Topic: E.05. Brain-Machine Interface

Support: Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro (INAIL)

Title: Mapping neural modulation during a standardized robot-assisted task: a step towards neurorehabilitation benchmarking

Authors: ***F. GARRO**[1,2], E. FENOGLIO[1], I. FORSIUK[1], S. BUCCELLI[1], L. DE MICHIELI[1], M. CHIAPPALONE[2,1], M. SEMPRINI[1];
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Abstract: Robotic neurorehabilitation is a growing area of research driven by evidence suggesting that integrating patients into the control loop of robots promotes motor learning and enhances brain plasticity (Colucci et al. 2022). However, the underlying neural mechanisms are

not yet fully understood, which hinders our knowledge of healthy and pathological human motor control. Besides, there is potential to extract biomarkers that can objectively benchmark robotic technologies and assess the efficacy of rehabilitation therapies (Garro et al. 2021).This study aims to characterize healthy neural correlates during a robot-assisted task and derive standardized measurements to further assess neurorehabilitation interventions.For this, we implemented a protocol to simultaneously collect high-density EEG, surface EMG, and kinematic data during a reaching task from a cohort of 40 healthy right-handed subjects (20 males, 20 females, age 44.5 +/- 15.5 years old) using the FLOAT upper-limb exoskeleton (Buccelli et al. 2022).Preliminary electrode-level analysis of EEG data from a subset of 20 subjects using cluster-based permutation tests based on Monte Carlo method revealed amplitude modulations in event-related potentials (ERP) and event-related synchronization/desynchronization (ERS/ERD) during the motor task ($p<0.05$) when subjects were using the exoskeleton with different assistance levels. Expanding on these initial results, this work examines the entire dataset through source-level analysis, which shows significant modulation over central, frontocentral, and left parietal-occipital areas.Furthermore, a resting-state analysis pre- and post-motor task exploring average power band and brain connectivity analysis (using seed-based techniques) showed significant changes ($p<0.05$) between pre-selected regions of interest.These findings provide evidence that robotic assistance modulates different areas associated with movement planning and execution depending on the level of assistance provided. This modulation offers a non-invasive means to quantify the effects of interacting with robotic technologies and their impact on motor performance. Such characterization can enable patient-tailor rehabilitation by tuning robotic assistance and providing feedback for closed-loop interventions. Furthermore, it can also provide a mean to benchmark and evaluate robotic technologies during their design and development phases, allowing us to compare the effectiveness of different robotic systems, assess their performance, and guide improvements in their design and functionality.

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Poster

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Topic: E.05. Brain-Machine Interface

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Title: Evidence for phonetic and semantic word processing during internal and vocalized speechin single unit recordings of the supramarginal gyrus

Authors: *S. K. WANDELT[1], D. A. BJA°NES[2], K. PEJSA[2], B. LEE[3], C. LIU[3], R. A. ANDERSEN[2];
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Abstract: Speech is a fundamental aspect of human communications, but neurological conditions like amyotrophic lateral sclerosis and cerebral brain lesions can impair the ability to speak, leaving patients without means of expression. Brain-machine interfaces (BMIs) offer a promising approach to restoring communication by capturing neural activity related to speech from the cortex. For patients affected by these speech disorders, a system that can be controlled through internal speech production would be highly desirable. Currently, our understanding of internal speech remains limited due to several factors, including lack of behavioral output, differences in cortical activation pattern compared to vocalized speech, and reduced associated neural signatures.In our previous work, we identified neurons in the supramarginal gyrus (SMG), located in the posterior parietal cortex, that represents internal and vocalized speech. Internal speech selectivity allowed us to develop an online internal speech decoder. In this study, we aimed to investigate the specific aspects of speech production represented in the neural activity of SMG, specifically focusing on phonetic and semantic processes.To that aim, a C5-C6 bilingual tetraplegic participant implanted with Blackrock (Utah) arrays in SMG performed two tasks. The first task involved four English-Spanish word pairs, focusing on varying the phonetic demands while controlling the semantic meaning. The second task involved two groups of homophones (Scent/ Cent/ Sent and Where /Ware /Wear), varying the semantic meaning while controlling the phonetic demands (i.e., pronunciation). Trials were composed of six phases: an inter-trial interval, a written cue to one of the words, a first delay, an internal speech phase, a second delay and a vocalized speech phase.We found both words with the same semantic meaning (English/Spanish pairs) as well as homophones were significantly decoded from the recorded neural data during internal and vocalized speech. To validate the homophones, we employed two tests. First, five individuals listened to the audio produced by the participant and attempted to classify the spoken words. Secondly, recorded audio were classified using a convolutional neural network. In both cases, homophone groups were distinguishable from one another, however within homophone groups, classification was near chance level.These findings suggest the presence of both phonetic and semantic word representation in SMG, indicating that this brain region is involved in active speech processes. Integrating both phonetic and semantic information into speech decoding may result in improved internal speech BMIs.

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Poster

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Title: Fast and accurate decoding of electrocorticograms during hand movements based on dynamic mode decomposition

Authors: *R. FUKUMA[1,2], K. MAJIMA[3], Y. KAWAHARA[4,5], O. YAMASHITA[5,6], Y. SHIRAISHI[1], H. KISHIMA[1], T. YANAGISAWA[1,2];
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Abstract: Dynamic mode decomposition (DMD) approximately decomposes nonlinear spatiotemporal dynamics underlying given time series data as the sum of multiple oscillatory components (modes). Our previous study demonstrated that modes are more informative for classifying electrocorticograms (ECoGs) during different types of hand movements compared to conventional frequency power features (Shiraishi et al. (2020)). The classification method in the study (L2-regularized support vector machine (L2-SVM) with a nonlinear kernel) had a computational complexity of $O(n)$ for testing new samples (n: number of training samples), solely because the characteristic frequencies of the modes differed for each trial, making it difficult to directly compare the modes among the trials. Here, in this study, we propose a new decoding feature that (1) requires small computational complexity for decoding, (2) is mathematically equivalent to the previous method when combined with linear L2-SVM, and (3) can be used with linear L1-regularized SVM (L1-SVM) to achieve higher classification accuracy.
We evaluated our new decoding features on the same ECoG dataset used in our previous study. The dataset consisted of ECoGs, while eleven patients performed three different types of movements once at the given auditory cue. ECoGs of 0-500 ms, according to the cue, were used to decode the movement by using the proposed features and conventional high-γ power features (80-150 Hz). In addition, computational complexity was evaluated by performing the classification multiple times while changing the number of training samples per movement type. With L1-SVM, the classification accuracy using the proposed features was 80.5±7.5% (mean ± 95% confidence interval), which was significantly higher than that of the high-γ power (71.4±8.1%) ($p < 0.001$, paired t test). In addition, the computational complexity to train the decoder and to test a new sample was reduced from $O(n^{2.01})$ to $O(n^{1.24})$ and from $O(n^{0.99})$ to $O(n^{0.05})$, respectively.

Our proposed method is a new promising approach for classifying various spatiotemporal signals, including real-time applications such as brain-machine interfaces.

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Poster

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Title: Differences in spectral contribution of motor- and temporal brain regions in overt and mouthed speech detection

Authors: *A. DE BORMAN[1], I. DAUWE[2], E. CARETTE[2], A. MEURS[2], D. VAN ROOST[3], P. BOON[2], M. M. VAN HULLE[1];
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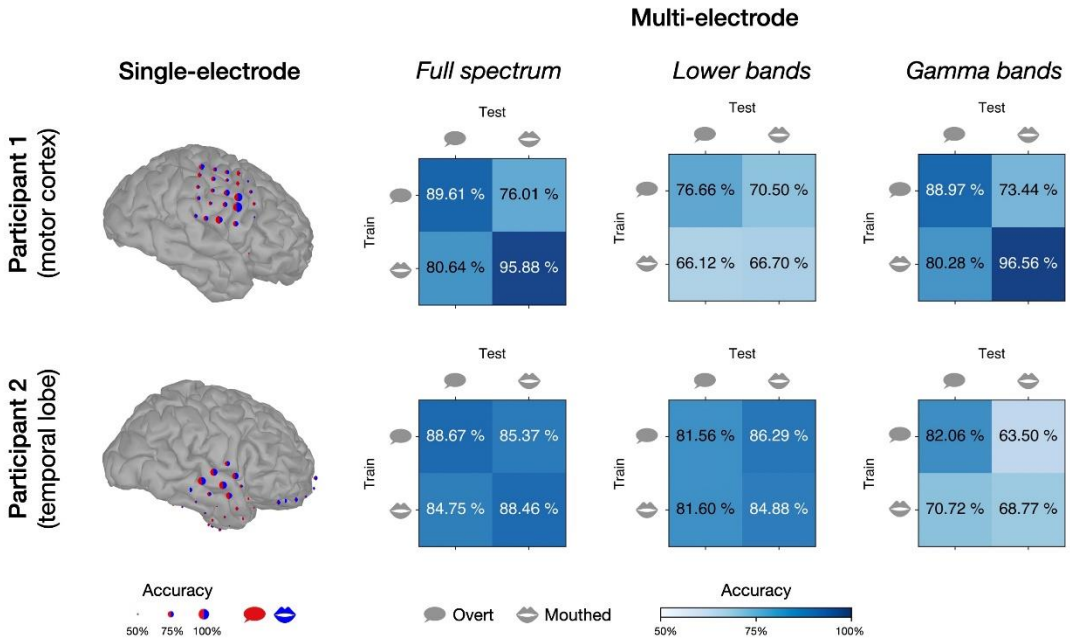
Abstract: In the past decade, many studies investigated performed and perceived speech decoding from neural signals recorded in refractory epilepsy patients with temporarily implanted electrodes. However, to serve the speech-impaired, it is of interest to study speech in the absence of vocalization. In this study, two participants were asked to repeat sentences by vocalizing or not speaking articulations, i.e., overt and mouthed speech. Electrocortigraphy signals were recorded over the motor cortex (participant 1) and the temporal lobe (participant 2). A linear regressor was used to classify neural activity into the presence or absence of speech (binary classification). For both subjects, multiple electrodes yielded a high classification accuracy (left panel). Multi-electrode models of the best electrodes led to higher accuracies compared to single-electrode ones. We also studied the contribution of lower and higher frequencies individually (diagonal elements in the right panel). We observed that, in the motor cortex, the accuracy significantly drops for both speech modes if only lower frequency bands were used contrary to only higher frequency bands. In the temporal lobe, the performance using only gamma bands was particularly low for mouthed speech compared to overt speech. In contrast, the performance using only lower frequency bands remained above 80\%. To assess the relation between speech modes, we applied models trained on one mode to the other mode. When using the full spectrum, a high accuracy can be reached (off-diagonal elements in the right panel). Model transfer is most

successful using high frequencies in the motor cortex and lower frequencies in the temporal lobe. We conclude that, while gamma bands are predominant in the motor cortex, lower frequencies are prominent in the temporal lobe, in particular in the absence of auditory feedback.



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Poster

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Topic: E.05. Brain-Machine Interface

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Title: Two in one: decoding ipsilateral and contralateral movements from one hemisphere using high-density electrocorticography

Authors: ***M. KROMM**[1], F. B. DIJKSTRA[1,2], M. POEL[2], M. A. H. L. RAEMAEKERS[1], N. F. RAMSEY[1], M. P. BRANCO[1];
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Abstract: Implanted Brain-Computer Interfaces (BCIs) aim to facilitate communication for individuals with severe motor impairments. Expanding the range of control signals in implanted BCIs that target one hemisphere could significantly extend their usability. One way to achieve this is by adding ipsilateral movement readout. This study investigates the feasibility of classifying individual finger movements from both the contra- and ipsilateral hand based on brain activity in the sensorimotor cortex. Four participants with intractable epilepsy who were implanted with a high-density (HD) ECoG grid (32, 64, and 128 channels; 3-4 mm inter-electrode spacing) over the hand knob of one hemisphere were included in this study. In a randomized event-related task, the participants performed individual finger movements of the thumb, index, and little finger with the contra- and ipsilateral hand relative to the implanted hemisphere. Spatiotemporal analyses and classification were conducted on the alpha (8-12 Hz), beta (16-30 Hz), and high-frequency (HFB, 60-130 Hz) bands. Preliminary results revealed significant differences between contra- and ipsilateral movements in the high-frequency band. The peak of spectral modulations during ipsilateral movements occurred slightly earlier and showed smaller amplitudes than during contralateral movements in the HFB. The number of significantly activated channels during ipsilateral movements was smaller, primarily present over the primary motor cortex, while contralateral movements evoked more widespread cortical activity, including the somatosensory cortex. For the alpha and beta frequency bands, no significant differences were observed between contra- and ipsilateral hand movements in the spatiotemporal distribution. Linear Discriminant Analysis (LDA) demonstrated successful decoding of both contra- and ipsilateral finger movements and rest trials (7 classes), with performance significantly above chance level for all participants (79.22 ± 6.30% [mean ± SD]; chance level = 14.3%). Although ipsilateral finger movements could be well decoded, they were more likely to be confused with rest trials than the contralateral movements. This research aims to shed light on the potential and challenges of decoding ipsilateral finger movements using high-density ECoG. Preliminary findings suggest that it is possible to discriminate contra- and ipsilateral fingers from rest periods with HD-ECoG grids for all participants. Successful decoding of ipsilateral movements could increase the degrees of freedom of implanted BCIs targeting the hand sensorimotor region, without the need for bilateral electrode placement.

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Poster

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Program #/Poster #: PSTR552.20/II17

Topic: E.05. Brain-Machine Interface

Support: NWO Project Number VI.Veni.194.021
NWO Grant Number 17619

Title: Exploring the spatial distribution of acoustic, articulatory, and semantic speech representations in intracranial EEG

Authors: *M. VERWOERT[1], J. AMIGÓ-VEGA[2], M. C. OTTENHOFF[3], S. GOULIS[3], P. L. KUBBEN[4], C. HERFF[3];
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Abstract: Speech brain-computer interfaces (BCIs) have made remarkable progress in enabling communication for individuals who have lost their ability to speak. Speech can be represented in a variety of ways, these include acoustic, articulatory, and semantic representations. While the acoustic representation captures auditory properties of speech, an articulatory representation estimates the movements and positions of articulatory organs during speech production. The semantic representation, on the other hand, extracts the underlying meaning of spoken words. By considering these multiple representations, we can gain an understanding of the different aspects of speech production and acquire crucial complementary information.
We leveraged the *Single Word Production Dutch* dataset from Verwoert et al. (2022) to explore how these speech representations are spatially represented in the brain. In this dataset, parallel stereo-electroencephalography (sEEG) and audio data were recorded while 10 participants overtly spoke 100 unique words. A total of 1103 electrodes were distributed across the entire brain with a balanced coverage between the hemispheres. To extract neural features, we focused on the broadband gamma signal from each electrode. For the acoustic representation, we captured the acoustic spectrogram of the spoken words. To capture articulatory representations, we utilized a *long short-term memory* neural network as implemented by Gao et al. (2020) to estimate articulatory trajectories from the audio data. Finally, for the semantic representation, we employed a *word2vec* model, trained on the Dutch wikipedia, to create 160-dimensional embeddings for each word to capture its semantic meaning.
By analyzing the spatial distribution of significant electrodes, we aim to identify overlapping regions as well as distinct regions that selectively encode specific aspects of the speech production process. This analysis may reveal a potential hierarchical organization in the neural encoding of speech, as previously demonstrated in a study involving articulated, mouthed, and imagined speech (Soroush et al., 2023). These results may contribute to the development of more efficient and accurate speech BCIs, ultimately enhancing communication capabilities for individuals with speech impairments.

Disclosures: M. Verwoert: None. **J. Amigó-Vega:** None. **M.C. Ottenhoff:** None. **S. Goulis:** None. **P.L. Kubben:** None. **C. Herff:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.21/II18

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01DC019498
DoD Grant W81XWH-21-0538

Title: Quantifying the neuro-anatomical contributions for speech decoding using intracranial recordings

Authors: *S. DURAIVEL[1,4,5], S. RAHIMPOUR[8,4], D. G. SOUTHWELL[6,1,2,7], S. R. SINHA[9], M. VESTAL[4,7], J. VIVENTI[1,4,2,7], G. B. COGAN[5,1,4,7,3];
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Abstract: Neural speech prostheses using intracranial recordings can enable accurate speech restoration for patients with disrupted verbal communication abilities. A crucial requirement for reliable neuroprosthetics is neuro-anatomical targeting using intracranial electrodes, including electrocorticography (ECoG) or stereoelectroenphalography (SEEG), to decode speech. Previous decoding studies have shown that motor speech features (phonemes) can be accurately decoded from the ventral sensorimotor cortex (vSMC). However, neuroimaging studies examining neural speech mechanisms identified activation in multiple neuro-anatomical regions during speech production: vSMC for speech motor articulation, inferior parietal (IPC) and inferior frontal (IFG) regions for phonological coding and syllabification, and middle and superior temporal gyri (MTG & STG) for monitoring self-generated speech. The contributions from these different neuro-anatomical regions for speech decoding have not been systematically characterized. In this study, we sought to quantify the relative strength of decoding from these neuro-anatomical regions using intracranial recordings. We performed cortical recordings from 34 patients using ECoG or SEEG while in the epilepsy pre-operative monitoring unit. Patients performed a non-word delayed repetition task. Our results show a significant increase in high-gamma power (HG: 70 - 150 Hz; $p < 0.05$; one-sided permutation test) across 1429 electrodes spanning a wide range of neuro-anatomical regions of interest (ROI). To quantify the contribution to speech production in an ROI, we developed neural decoding (multivariate) and encoding (univariate) strategies to model speech features associated with HG activations. Our decoding models resulted in above-chance prediction of speech features from each ROI, with varying degrees of decoding strength (All production electrodes: 42%, vSMC: 37%, IPC: 23.4%, IFG: 15%, prefrontal cortex: 25%, MTG & STG: 35%; chance: 11.11%). Since decoding based on ROI can be limited by inadequate sampling, we also developed electrode-specific encoding models to predict HG activations using spoken speech features. Our preliminary findings indicate above-chance prediction from sensorimotor electrodes, with regression weights quantifying the strength of an electrode in relation to its neural-speech features. These results using intracranial recordings indicate distributed representations of speech features from neuroanatomical speech-related ROIs which could influence the design and targeting of cortical electrodes for neural speech prosthetic applications.

Disclosures: S. Duraivel: None. **S. Rahimpour:** None. **D.G. Southwell:** None. **S.R. Sinha:** None. **M. Vestal:** None. **J. Viventi:** None. **G.B. Cogan:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.22/II19

Topic: E.05. Brain-Machine Interface

Support: UH3NS114439

Title: Automated Segmentation and Detection of Silent Speech Attempts

Authors: *Q. RABBANI[1,2], Y. CHEN[3], M. ANGRICK[3], S. LUO[3], S. SHAH[4], D. N. CANDREA[3], K. NATHAN[3], K. WYSE-SOOKOO[3], M. S. FIFER[5], H. HERMANSKY[2], N. E. CRONE[4];
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Abstract: Locked-in Syndrome (LIS) is a neurological disorder, in which cognition remains intact but communication is impaired from paralysis due to a variety of causes, including brainstem strokes and amyotrophic lateral sclerosis. Brain-computer interfaces (BCIs) have the potential for restoring communication in patients with LIS. Training speech BCIs typically relies on microphone recordings to locate ground-truth speech segments, but this is not possible in the absence of audible output. For some patients, silent articulation without phonation may be the only way to train and control speech BCI's. To identify speech segments solely from video data we have developed a method to automatically segment speech-related mouth movements using facial landmarks in a restricted set of words designed specifically for BCI operation. These facial landmark-based labels were meticulously hand-verified and subsequently used to train a neural voice activity detection (nVAD) model to verify their utility for BCI. The facial landmarks served as ground truth for locating silently articulated speech segments from a BCI trial participant. In the laboratory setting, we deployed the trained nVAD model and verified its real-time operation with the participant. Our segmentation algorithm demonstrated robust performance by reliably segmenting speech based on facial landmarks, even when faced with changes in camera distance and physical pose across multiple sessions. The algorithm provided accurate and reliable labels, enabling high-performance nVAD of neural signals during silently articulated speech. This research represents a significant step forward in practical speech segmentation from video and model training for silent speech BCIs.

Disclosures: Q. Rabbani: None. **Y. Chen:** None. **M. Angrick:** None. **S. Luo:** None. **S. Shah:** None. **D.N. Candrea:** None. **K. Nathan:** None. **K. Wyse-Sookoo:** None. **M.S. Fifer:** None. **H. Hermansky:** None. **N.E. Crone:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.23/II20

Topic: E.05. Brain-Machine Interface

Support: NIH R01MH123770
NSF CRCNS IIS2113271

Title: Input-aware dynamical models of neural-behavioral data uncover consistent intrinsic behaviorally relevant neural dynamics across distinct tasks and animals

Authors: *P. VAHIDI[1], O. SANI[2], M. M. SHANECHI[3];
[1]Electrical and Computer Engin., USC, LOS ANGELES, CA; [3]Electrical Engin., [2]USC, Los Angeles, CA

Abstract: Neural dynamics are generated either due to intrinsic dynamics in a brain region or due to input dynamics, for example in the form of sensory stimuli or excitations from upstream regions. Disentangling intrinsic and input dynamics is important for understanding how neural computations give rise to behavior, especially when comparing across tasks with distinct sensory inputs. While measuring all inputs to a brain region is not experimentally feasible, measurements of sensory inputs such as task instructions or partial measurements of neural input are often possible. However, current methods for learning models of neural population dynamics can either account for measured input but not behavior, or vice versa. We develop a new method that simultaneously accounts for measured input, behavior, and neural activity during learning. We show how without this capability, input dynamics or other intrinsic neural dynamics may be mistaken for intrinsic behaviorally relevant neural dynamics. We then use this method to compare the intrinsic behaviorally relevant neural dynamics across distinct tasks by accounting for their different sensory inputs. To do so, we analyze two public datasets in which three non-human primates (NHPs) perform two distinct motor tasks with different task instruction sensory inputs, while spiking activity is recorded from the motor cortical areas. First, using standard neural dynamical modeling with input, we find that the prominent intrinsic neural dynamics are different across tasks and animals. We then use our method to compare the behaviorally relevant subtype of intrinsic neural dynamics. Despite the difference in prominent intrinsic dynamics, our method reveals that the intrinsic behaviorally relevant neural dynamics are remarkably similar across animals and motor tasks. Interestingly, these consistent dynamics are missed without the new method. These results suggest that common behaviorally relevant neural computations may be at play in the motor cortical areas across animals and distinct motor tasks. These common intrinsic and behaviorally relevant dynamics can be unmasked when simultaneously considering input, behavior, and neural activity during learning.

Disclosures: P. Vahidi: None. **O. Sani:** None. **M.M. Shanechi:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.24/II21

Topic: E.05. Brain-Machine Interface

Support: NIH R01MH123770

Title: Cross-session normalization of EEG recordings using sensory tasks

Authors: *O. G. SANI[1], M. M. SHANECHI[2];
[2]Electrical and Computer Engin., [1]USC, Los Angeles, CA

Abstract: Non-invasive EEG recordings can be obtained from healthy participants, which gives them a unique advantage over intracranial recording methods for studying brain functions. However, EEG recording setups are temporary in nature, and basic recording characteristics such as electrode impedance and location may vary significantly across recording sessions. These non-neural cross-session variations can mask real variations in brain signals themselves and thus can confound studies that require multi-session sampling of an individual's brain signals, for example to study the encoding of mood variations in neural signals over days. Here, we address this challenge by devising a new paradigm that enables cross-session normalization of EEG using sensory tasks. In this paradigm, in addition to the primary task being studied, each EEG recording session also includes short sensory tasks that evoke event-related potentials (ERPs) from the low-level sensory processing of the brain. Since low-level sensory processing in the brain is expected to undergo minimal plasticity, changes in low-level sensory ERPs can be largely attributed to changes in the recording setup. Therefore, we propose that EEG recordings can be normalized such that recordings from different sessions yield similar ERPs for low-level sensory tasks. We validate this idea by collecting unparalleled multi-session EEG datasets with 20-40 recordings from each participant each collected on a different day. In each recording, we include a steady-state visually evoked potential (SSVEP) task as our low-level sensory task. We demonstrate that SSVEP responses can be used to learn mappings of the EEG sensor space, which transform EEG in a way that yields matching SSVEP responses from recordings across different sessions. Moreover, EEG sensor space mappings can be learned that can normalize all recording sessions to a comparable sensor space, resulting in similar SSVEPs across all sessions. These results demonstrate the effectiveness of our novel paradigm for cross-session normalization of EEG recordings using sensory tasks. This approach may enable future works to utilize EEG in longitudinal studies of brain functions in individuals over time, in ways that were previously considered impractical with EEG.

Disclosures: O.G. Sani: None. **M.M. Shanechi:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.25/II22

Topic: E.05. Brain-Machine Interface

Support: ARO W911NF-16-1-0368
NIH R01MH123770

Title: Unsupervised learning of behaviorally relevant switching regimes from Poisson spiking activity

Authors: *C. Y. SONG, M. M. SHANECHI;
USC, Los Angeles, CA

Abstract: Switching dynamical system models are powerful tools in probing population spiking dynamics that switch between distinct regimes, e.g., due to changes in task epochs or lapses in engagement. However, in many datasets, regime labels may be absent for example when regime switches happen due to internal state changes or when experiments have no strict trial structure and are naturalistic. Thus, there is a need for accurate unsupervised learning methods for Poisson observations that can capture the statistical profile and fine timescale of spiking activity. While prior learning methods exist, their inference step relies on the Laplace approximation which may fail to capture broader properties of densities and lead to potentially inaccurate model learning. Thus, there is a need for new inference methods that can enable accurate unsupervised model learning. Here, we address this need by developing a novel inference method that uses deterministic sampling on Poisson observations and term it the Poisson Cubature Filter (PCF). We embed this inference method within expectation-maximization learning frameworks to yield high accuracy unsupervised learning methods for both switching and stationary dynamical systems. We validate the method in both extensive numerical simulations and publicly available motor cortical spiking data of a monkey performing a continuous point-to-point reach task. We show how PCF-based learning outperforms Laplace approximation-based learning methods in the accuracy of learned parameters. We also find that PCF-based learning is more data efficient and more reliable in regime identification, which is critical for neural datasets as training samples are often limited. Specifically, PCF-based learning more reliably identified interpretable behavior-related regimes corresponding to changes in direction or magnitude of movement in the motor cortical spiking data completely unsupervised with respect to behavior. This new unsupervised learning method can facilitate investigations of how population dynamics relate to regime changes for example due to higher order cognitive states, task stage, or behavior strategy in naturalistic scenarios.

Disclosures: C.Y. Song: None. **M.M. Shanechi:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.26/II23

Topic: E.05. Brain-Machine Interface

Support: NIH Director's New Innovator Award (DP2), Contract DP2MH126378
ONR Young Investigator Award, N00014-19-1-2128

Title: Describing neural population activity on an entangled geometric manifold

Authors: *D. KIM, H.-L. HSIEH, M. M. SHANECHI;
Electrical Engin., USC, Los Angeles, CA

Abstract: Neural population activity exhibits nonlinear dynamics that may traverse on a low-dimensional latent manifold. Rotational structure in low-dimensional projections of neural population activity has been observed across diverse datasets. Based on this rotational structure, we previously hypothesized that neural population activity may evolve on nonlinear manifolds that contain holes. We thus developed the geometric dynamic modeling (GDM) framework, which is a data-driven method that learns a multi-dimensional nonlinear manifold consisting of a major hole and appending dimensions around it. GDM also learns an intrinsic dynamical system model on top of this multi-dimensional manifold. So far, the GDM learning method disentangles the dynamics along the hole dimension and those along the other appending dimensions to address the challenge of modeling curvatures. Here, we generalized GDM to explicitly model any potential cross-talk between the dynamics along the nonlinear hole and the appending dimensions by combining them into a "tube". We validated this extension through numerical simulations that featured a substantial correlation between the hole and the appending latent dynamics. Our results demonstrate that if cross-talk exists depending on the shape and distortion of the manifold, using this extended approach and modeling the cross-talk can improve the prediction of neural population activity. Overall, this new method provides a valuable tool that can systematically combine and jointly model the geometry and dynamics of neural population activity to provide insight into neural representations.

Disclosures: D. Kim: None. **H. Hsieh:** None. **M.M. Shanechi:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.27/II24

Topic: E.05. Brain-Machine Interface

Support: NIH R01MH123770

Title: Predicting the multiregional human brain dynamics during direct electrical stimulations

Authors: *H. JO[1], O. SANI[1], M. M. SHANECHI[2];
[2]Electrical Engin., [1]USC, Los Angeles, CA

Abstract: Characterizing the dynamic response of human brain networks to time-varying direct electrical stimulation is important for developing more effective deep brain stimulation

treatments. Doing so would allow for personalizing the therapy based on a dose-response model of how an individual's brain networks respond to stimulation. This can lead to model-based closed-loop stimulation systems that vary the stimulation parameters based on real-time feedback from brain signals as well as the dose-response model. However, building model-based stimulation systems remains elusive and hinges on accurate personalized dose-response modeling. Our recent work achieved this modeling in non-human primates for micro-stimulation and LFP recordings. However, dynamical dose-response modeling remains a challenge in the human brain and for recording and stimulation with intracranial EEG (iEEG), which is used in clinical applications. To address this challenge, we designed and applied electrical stimulation patterns with randomly varying amplitude and frequency to elicit and model diverse brain responses to time-varying stimulation. We recorded multiregional iEEG neural activity in epilepsy patients while delivering this stochastically varying electrical stimulation from the iEEG electrodes. We then studied the changes to iEEG spectral powers in several standard frequency bands after the removal of stimulation artifacts. We found that a considerable proportion of the extracted iEEG spectral power features showed a significant predictable response to the amplitude of the ongoing stimulation. This result also held for the frequency of stimulation. Interestingly, the response of some spectral features in a time window with no stimulation that immediately followed the stimulation time window was also predictable from the previously applied amplitude/frequency. Our work demonstrates new experimental design and modeling steps as well as promising results toward building dose-response models for human brain networks in response to electrical stimulation. These models can enable precise neurotechnologies for personalized deep brain stimulation therapy in mental disorders in the future.

Disclosures: H. Jo: None. **O. Sani:** None. **M.M. Shanechi:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.28/II25

Topic: E.05. Brain-Machine Interface

Support: NIH Director's New Innovator Award (DP2), Contract DP2MH126378

Title: Learning the distribution of neural population activity in an intrinsic geometric coordinate system

Authors: *H.-L. HSIEH, M. M. SHANECHI;
Electrical and computer engineering, USC, Los Angeles, CA

Abstract: Extensive evidence in the literature suggests that neural population activity can be described with a low-dimensional manifold. We previously showed that neural population activity can evolve over nonlinear manifolds with holes and developed an algorithm to learn this

manifold if it exists in data. Our results indicated that the intrinsic coordinate for neural activity may differ topologically from the Euclidean coordinate. However, a remaining challenge is to not only quantify the low-dimensional manifold that captures most of the neural variance, but also probabilistically describe the deviations of neural activity from the manifold. Doing so is critical for testing hypotheses about whether the distribution of neural population activity follows the Euclidean coordinate system or a nonlinear geometric coordinate system. To test this hypothesis, new algorithms need to be developed for learning the manifold-based probability distribution from neural population activity, allowing for a fair comparison between Euclidean and geometric probabilistic models based on their log-likelihood. For linear manifolds, probabilistic PCA (PPCA) can probabilistically describe the deviations around hyperplanes, but PPCA is not applicable to nonlinear geometric manifolds. To address this challenge, we developed a novel algorithm called probabilistic geometric dimensionality reduction, which combines our geometric manifold learning method with elements from expectation-maximization (EM) and mean value theorem. Using this new method, we show how we can test whether the intrinsic coordinate that follows the geometric manifold is better suited to describe the distribution of neural population activity compared to the Euclidean coordinate. In summary, this new tool provides a new probabilistic framework for testing hypotheses about the geometry of neural population dynamics and their intrinsic coordinate system.

Disclosures: H. Hsieh: None. **M.M. Shanechi:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.29/II26

Topic: E.05. Brain-Machine Interface

Support: ARO award W911NF-16-1-0368
Army award W911NF1810434
NIH R01MH123770

Title: Prioritized dynamical learning of inter-regional brain dynamics

Authors: *T. JANI[1], B. PESARAN[2], M. M. SHANECHI[1];
[1]USC, Los Angeles, CA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Interactions among multiple brain regions are critical in brain functions. Prior work studying multiregional interactions has often utilized static modeling methods that do not explicitly consider the temporal structure or dynamics of neural data. While recent methods have made progress in incorporating dynamics into models of multiregional interactions, a remaining challenge is that inter-regional dynamics may be mistaken for or masked by within-region dynamics. We show that this challenge can be addressed by prioritizing the learning of inter-regional dynamics over other dynamics, which existing dynamic modeling methods do not

achieve. To do so, we provide a new multi-regional dynamical learning formulation that prioritizes the learning of inter-regional dynamics and separates them out from other dynamics. We show that this approach can address the problem of inter-regional dynamics being masked by the disjoint dynamics within each region. We validate this new approach using both numerical simulations and non-human primate datasets. We first find that this new approach more accurately predicts the inter-regional dynamics when compared to existing static methods and standard dynamic methods without prioritization. We next demonstrate that prioritization is critical for efficient learning of inter-regional dynamics. To show this, we formulate an alternative dynamical modeling approach that numerically optimizes the joint log-likelihood of all dynamics from both regions without prioritization. We find that this joint likelihood optimization approach is significantly less accurate in extracting the inter-regional dynamics for a given number of training samples. Further, to reach the accuracy of the prioritized learning approach, the joint likelihood approach requires significantly more training samples, which highlights the importance of prioritization. Moreover, the prioritized learning approach more accurately finds the dimensionality of inter-regional dynamics. Finally, this prioritized approach applied to experimental recordings of multiregional motor cortical dynamics identifies interaction pathways between bilateral premotor and primary motor cortical areas that are consistent with anatomical projection pathways, which further serves as its validation. Our results show that prioritized dynamical learning can help address the challenge of inter-regional dynamics being masked by, confounded by, or mistaken for within-region dynamics.

Disclosures: T. Jani: None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

PSTR552. Neural Decoding and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR552.30/II27

Topic: E.05. Brain-Machine Interface

Support: NIH Director's New Innovator Award (DP2), DP2MH126378

Title: Multiscale nonlinear dynamical modeling for flexible inference of latent neural population dynamics

Authors: *E. ERTURK, H. ABBASPOURAZAD, M. M. SHANECHI;
Electrical Engin., USC, Los Angeles, CA

Abstract: Behavior is encoded across multiple spatial and temporal scales of neural population activity, from small-scale discrete spiking activity of neurons to large-scale continuous local field potentials (LFP) that measure networks. Joint latent factor modeling of multiscale neural activity while also allowing for flexible inference, whether causally or non-causally, is important for improving real-time brain-machine interfaces (BMI) and for understanding the relationship across neural scales. While there exist recent multiscale dynamical models that also allow for

flexible inference, these models have assumed that dynamics are linear. However, capturing the nonlinearity in neural dynamics can be important in improving the inference of latent factors and behavior. Here, we develop a novel neural network model that can capture nonlinearity in multiscale neural dynamics while also allowing for flexible inference of latent factors. Flexible inference is defined as simultaneously achieving causal and non-causal inference and accounting for missing neural observations, which is critical for modeling multiple timescales. We first validate our model on nonlinear dynamical simulations and show that it can successfully fuse information across multiple scales to improve the latent factor reconstruction and account for missing neural samples. Next, we apply our method to a non-human primate motor cortical dataset consisting of discrete spiking and continuous LFP population activity. We demonstrate that our model improves the decoding of movement compared to models trained on single neural scales, even when neural scales have different timescales. This novel multiscale nonlinear dynamical model can simultaneously admit multiple spatial-temporal scales of data, capture their nonlinear latent structure, and enable flexible inference of latent factors and behavior. By enabling these new functionalities, this multiscale nonlinear model can provide a new tool to study multiscale dynamics and to improve BMI decoding technologies.

Disclosures: **E. Erturk:** None. **H. Abbaspourazad:** None. **M.M. Shanechi:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.01/II28

Topic: E.09. Motor Neurons and Muscle

Support: PAPIIT UNAM IN211720
CONACyT 732830 (DAZL)
CONACyT 628536 (CHB)

Title: The role of obturator internus muscle as a urinary continence mechanism in female rabbit

Authors: *D. ZACAPA[1,2], O. SÁNCHEZ-GARCÍA[2], C. HERNÁNDEZ-BONILLA[3], D. CORONA-QUINTANILLA[2], R. ZEMPOALTECA RAMÍREZ[2], F. CASTELÁN[4,2], M. MARTINEZ-GOMEZ[4,2];
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Abstract: Stress urinary incontinence (SUI) is the most common type of urinary incontinence. This issue is high relevant in an increasingly aging global society. Although it is know that SUI is associated with abnormal function of the sphincter complex and vesicourethral ligament as well as weakening of the muscle-fascial structures of the whole pelvic floor, to understand subjacent mechanism, data is needed on the skeletal pelvic floor muscles that are anatomically

related to the closure function of the urethral, implicated in urinary continence. Aims: To determine the role of obturator internus muscles (OIm) as a urinary continence mechanism in female rabbit. Methods: Virgin female rabbits were used to describe the gross anatomy and innervation of the OIm, to determine the effect of the OIm contraction on urethral pressure, and to evaluate the OIm activity during the induced-micturition. Both electromyogram and cystometrogram activity were simultaneously recorded in urethane-anesthetized rabbits. Bladder function was assessed measuring standard urodynamic variables before and after blocking the OIm activity for approaching its contribution to micturition. The relevance of the OIm activation for micturition was approached applying lidocaine injections. Results: The OIm is a bilateral muscle (length = 2.44 ± 0.05 cm, thickness = 0.11 ± 0.01 cm), it presents a flat structure with a triangular shape (origin width = 1.39 ± 0.07 cm, insertion width = 0.24 ± 0.05 cm). It originates from a tendon, occupying half of the rostral part on the inner surface of the pubic symphysis and is inserted posteriorly through a tendon on the medial surface of the greater trochanter of the femur. It is innervated by a nerve that arises from the anastomosis of branches that emerge from the spinal segments S1, S2 and S3. The OIm showed bursts of tonic activity during the storage phase of micturition that gradually decreased until turning off as the onset of the voiding phase. Electrical stimulation at 50 Hz generates a vaginal pelvic pressure of 24.17 ± 1.04 mm Hg and at 100 Hz this pressure does not change (23.96 ± 1.22 mm Hg). Conclusions: Present anatomical and physiological findings support that OIm have a role of sphincter complex to regulate the continence mechanism of female rabbits.

Disclosures: D. Zacapa: None. **O. Sánchez-García:** None. **C. Hernández-Bonilla:** None. **D. Corona-Quintanilla:** None. **R. Zempoalteca Ramírez:** None. **F. Castelán:** None. **M. Martinez-Gomez:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.02/JJ1

Topic: E.09. Motor Neurons and Muscle

Support: Shirley Ryan Ability Lab
NSF-CMMI1635443

Title: Novel Tattoo Electrode for Long-Term Recording of High-Density Surface Electromyography

Authors: *S. CHANDRA[1], A. BARRY[2], J. LI[3], S. CHEN[3], W. Z. RYMER[4];
[1]Arms and Hands, Shirley Ryan Ability Lab., Chicago, IL; [2]Shirley Ryan Abilitylab, Chicago, IL; [3]Ohio State Univ., Columbus, OH; [4]Shirley Ryan AbilityLab, Chicago, IL

Abstract: High-density surface electromyography (HDsEMG) possesses unique advantages, detection of the neuromuscular junction, estimation of muscle fiber conduction velocity, and

identification of motor units, to name a few. However, long-term HDsEMG recording often faces major technical challenges due to the limitation of the electrode front end. Quality of the skin-electrode interface, skin conformity of the electrode, and skin breathability are some of the significant factors that affect signal quality, particularly for long-term recording of more than a few hours.
Here, we demonstrate the long-term HDsEMG recording performance of a 3cm×4cm, 64 channel (8×8) tattoo electrode, faithful recording of the tattoo was demonstrated in our earlier works. We recorded HDsEMG from the medial gastrocnemius muscle response during the isometric plantar flexion on day 1 and a follow-up session on day 2. The subject was instructed to perform a maximum voluntary contraction (MVC) trial followed by two trials of 50% of MVC trials on day 1, and two trials of 50%MVC were also performed on day two. The contraction on day 2 was force matched to the performance of day 1, the posture of the subject was similar on both days. The subject performed their usual daily activity while the tattoo electrode stayed on the muscle the whole time. Before placing the electrode the skin surface was cleaned with standard procedure. During the experiment, the tattoo connectors were attached to the amplifier(TMSi Refa 128).
The signal was acquired in a monopolar mode, the reference electrode placed on the skin surface of the medial malleolus. We have obtained signals from more than 95% of the channels across the grid. The baseline recording during the resting state between day-1 and 2 was found to be varying within 7±12% across the channels. The steady state to baseline ratio of signals was found to be 20±4 across all the channels on day 1, and 19±7% on day 2. The frequency response of the sEMG signal was calculated, spectral power distribution shape was estimated between the day 1 and day 2 recordings from each channel, and the similarity of the shape was preserved. The single differential values were calculated row-wise and propagation of the MUAP was observed in both sessions. The motor units were also identified through the signal decomposition algorithm. We obtained 10±2 matched MUs from those two days of recording.
The dry tattoo electrodes ensure superior quality HDsEMG signals for long-term recording, offer flexibility of layout design, and a secure way of analyzing muscle activity. It will potentially facilitate long-term HDsEMG recording in neurorehabilitation, emg based prostheses, and other areas of neuroscience.

Disclosures: **S. Chandra:** None. **A. Barry:** None. **J. Li:** None. **S. Chen:** None. **W.Z. Rymer:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.03/JJ2

Topic: E.09. Motor Neurons and Muscle

Title: Two classes of action-stabilizing synergies revealed in a study of unintentional force drifts

Authors: *S. DE[1], J. RICOTTA[1], A. BENAMATI[2], M. LATASH[1];
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Abstract: We explored two types of action-stabilizing synergies during unintentional force drifts observed in the absence of visual feedback during finger pressing tasks. Task-level synergies were quantified in the space of finger modes, precursors of finger forces. Intra-muscle synergies were quantified in the space of motor unit groups (MU-modes) within the extrinsic flexor (FDS) and extensor (EDC) muscles. We hypothesized that force drifts would be associated with disappearance of force-stabilizing synergies in the space of finger modes, but not in the space of MU-modes. Young, healthy subjects (n = 12) performed accurate total force (F_{TOT}) production tasks at 25% of maximal voluntary force level while pressing on individual force sensors with the fingertips. In each episode, visual feedback on F_{TOT} was provided for the first 5 s, and the subjects were instructed to keep F_{TOT} unchanged for 15 s more. Surface electromyography was used to identify motor unit action potential using the Delsys Galileo system. Principal component analysis was used to identify two MU-modes using MUs within FDS, EDC, and across both muscles. The framework of the uncontrolled manifold hypothesis was used to quantify an index (ΔV_Z) of F_{TOT}-stabilizing synergies. All subjects showed force drift to lower magnitudes; on average, the force drop was 17.82 ± 0.35 percent of the initial level. Force-stabilizing synergies in the space of finger modes stabilized F_{TOT} when visual feedback was available (ΔV_Z = 1.41 ± 0.02), and these synergies disappeared after the force drift (ΔV_Z = -0.12 ± 0.01; $p < 0.01$). In contrast, there were no changes in the indices of F_{TOT}-stabilizing MU-mode-based synergies for all three analyses, within FDS (ΔV_Z = 2.49 ± 0.04), within EDC (ΔV_Z = 1.81 ± 0.04), and across FDS+EDC (ΔV_Z = -0.60 ± 0.06). Our results suggest that there are two basic types of performance-stabilizing synergies, task-related synergies organized at the level of subcortical circuitry involving the basal ganglia and cerebellum, and MU-mode-based synergies organized at the spinal level. The results support the interpretation of unintentional force drift as consequences of drifts in referent coordinates for the effector.
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Disclosures: S. De: None. **J. Ricotta:** None. **A. Benamati:** None. **M. Latash:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.04/JJ3

Topic: E.09. Motor Neurons and Muscle

Support: Imperial President's PhD Scholarship
Meta Platform Technologies, LLC

Title: Towards the identification of behavioral and neural constraints on motor control

Authors: *C. F. GIBBS, S. AVRILLON, A. KUNDU, N. M. TORRES CÓNSUL, D. FARINA, J. A. GALLEGO;
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Abstract: The repertoire of human movement is made possible by control of the elementary actuators of our neuromuscular system - the 'motor units'. A motor unit comprises an alpha motor neuron and the muscle fibers it innervates. Motor unit behavior is thought to be constrained by 1) common synaptic inputs that prevent individual motor units from being selectively recruited, and 2) biophysical constraints, primarily Henneman's size principle. These constraints fit well with traditional views on motor control based on basic modules that simplify the control problem (e.g., muscle synergies), but are at odds with recent work hinting at more flexible motor unit control. Accordingly, the field is yet to identify the underlying principles of motor unit control.

An intriguing middle ground consistent with experimental observations would be control of subpopulations of motor units—'motor unit modules'—partitioned via reception of common synaptic input. Their anatomical definition would then be unrestricted, spanning parts of a muscle, or even multiple muscles. To explore the existence of these motor unit modules and gain insight into the neural constraints on motor unit control, here we present an experimental paradigm and initial results, aiming to delineate the organizational principles of synaptic input within muscles.

We focus on muscles partitioned by compartments given earlier reports on differences in motor unit correlation across compartments, as a mechanism in part to perform individual digit movement. Of particular interest however is the human Flexor Carpi Ulnaris, a superficial forearm flexor with two distinct compartments. Importantly, unlike muscles of prior studies, each compartment is not associated with a unique function; yet, they work together in achieving wrist flexion and adduction. With intramuscular EMG, we record the activity of populations of motor units in each compartment. We also detect motor unit activity of synergistic and antagonist muscles through decomposition of high-density surface EMG recordings. We aim to compare motor unit correlation metrics across both muscle compartments and muscles for several contraction types and arm configurations, to decouple the influences of task-related and neural constraints on motor control. This will help us to establish whether motor unit modules exist at the most coarse, compartmental level within muscle, and whether they remain stable across behaviors. Overall, our study will aid in defining what is the smallest functional unit of the motor system, shedding light into a fundamental question in systems neuroscience and informing the design of future `muscle-computer interfaces.

Disclosures: C.F. Gibbs: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Meta Platform Technologies, LLC. **S. Avrillon:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Meta Platform Technologies, LLC. **A. Kundu:** None. **N.M. Torres Cónsul:** A. Employment/Salary (full or part-time):; Meta Platform Technologies, LLC. **D. Farina:** 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.; Meta Platform Technologies, LLC.. F. Consulting Fees (e.g., advisory boards); Meta Platform Technologies, LLC. **J.A. Gallego:** 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.; Meta Platform Technologies, LLC..

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.05/JJ4

Topic: E.09. Motor Neurons and Muscle

Support: Shake It Off, Inc., West Chester, PA

Title: Initial Motor Unit Discharge Behavior in Motor Segmentation in Parkinson's Disease

Authors: *R. DANIELS[1], C. A. KNIGHT[2];
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Abstract: In healthy adults, high rates of force development (RFD) are achieved by rapid initial motor unit (MU) firing. Healthy older adults have lower initial firing rates (FR) than young and the distribution of the first three interfiring intervals (IFIs) shifts to lower frequencies, resulting in lower RFD. MU FR during rapid contractions have not been studied in people with Parkinson's disease (PwPD). However, surface electromyography (EMG) measures indicate that the initial amplitude and duration of neuromuscular excitation is stunted compared to healthy adults, and sometimes interrupted by silent periods that result in disrupted RFD and irregular force-time curves (motor segmentation). Motor segmentation is a phenomenon specific to PwPD that relates to clinical status. The aim of this study was to compare initial MU firing characteristics in rapid force pulses with and without motor segmentation in PwPD. Six PwPD on their usual medications (aged 63.0 ± 8.7 years, 4.1 ± 0.3 years since diagnosis, levodopa equivalent dose=1005.0 ± 1053.5 mg, Hoehn and Yahr stage 1-3) performed rapid isometric finger abduction pulses at 20-60% of their maximal voluntary contraction force (MVC) while MU action potentials were measured from a needle electrode. Using custom MU sorting algorithms, a total of 16 MUs were discriminated. Within each subject, three force pulses with segmentation were identified as well as three amplitude-matched (±5% MVC) pulses without segmentation. Dependent variables were calculated for each MU and force pulse and averaged across segmented and non-segmented pulses. Variables included the number of force segments, peak RFD, IFIs, and peak FR. Paired t-tests assessed differences in dependent variables between segmented and non-segmented force pulses with alpha set to 0.05. Cohen's *d* was used to evaluate effect sizes. There was a significant difference in the number of force segments (3.0 ± 1.2 vs. 1.0 ± 0 force segments, t=6.75, p<0.001), though peak RFD (309.2 ± 152.0 vs. 358.1 ± 152.5 %MVC/s, t=0.9, p=0.60, *d*=0.32), IFI 1 (58.6 ± 32.2 vs. 63.4 ± 42.1 ms, t=0.40, p=0.35, *d*=0.13), IFI 2 (50.7 ± 23.4 vs. 47.3 ± 21.0 ms, t=1.54, p=0.08, *d*=0.15), IFI 3 (60.6 ± 28.9 vs. 43.7 ± 16.4 ms, t=1.54, p=0.08, *d*=0.72), and peak FR (39.4 ± 18.8 vs. 47.5 ± 30.6 pulses per second, t=1.60, p=0.065, *d*=0.32) were not significantly different. Though small to large effects

were found in RFD, IFI 3 and peak FR, the insignificant results partially reflect high variability in MU behavior in this sample of PwPD which resulted in impaired rapid force performance.

Disclosures: R. Daniels: None. **C.A. Knight:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.06/JJ5

Topic: E.09. Motor Neurons and Muscle

Support: European Research Council Consolidator Grant INcEPTION (contract no. 101045605).

Title: Central and peripheral mechanisms modulating alpha band oscillations for enhanced precise force generation: experimental evidence from human motor control

Authors: H. V. CABRAL[1], *F. NEGRO[2];
[1]Clin. and Exptl. Sci., Universita' degli Studi di Brescia, Brescia, Italy; [2]Dept. of Clin. and Exptl. Sci., Brescia, Italy

Abstract: Previous studies have demonstrated a precise correspondence between muscle force output and the low-frequency components of the synaptic inputs broadly shared across the alpha motor neuron pool. In voluntary tasks, these shared inputs consist of control components that determine the command for optimal force generation, and common noise oscillations such as alpha band oscillations or physiological tremor, which reduce task precision. Thus, achieving precise force generation requires minimizing these shared noise components to maximize the control components in the force output. In this study, we conducted two sets of experiments to investigate central and peripheral mechanisms that may influence alpha band oscillations in the neural drive to the muscle to enhance precise force generation. The first experiment involved acquiring and decomposing high-density surface electromyography (HDsEMG) from the first dorsal interosseous (FDI; 13 participants) and tibialis anterior (TA; 11 participants) during the short-term acquisition of a new force-matching skill. Participants performed 15 trials of an isometric, challenging force-matching task. We selected the trials with the highest and lowest errors between the force and target (pre- and post-skill acquisition). We then quantified the ratio (post/pre) of the area under the curve of motor units' z-coherence within delta (1-5 Hz), alpha (5-15 Hz) and beta (15-35 Hz) bands. We found that improvements in force-matching were accompanied by significant reductions of ~22% and ~13% in the area under the curve within the alpha band for the TA and FDI muscles, respectively. No changes were observed in the delta or beta bands. In the second experiment, 12 participants performed dorsiflexion isometric contractions for two TA muscle length conditions: shortened length (ankle at 90^{o}) and optimal length for force production (110^{o}). HDsEMG acquired from TA were decomposed into motor unit spike trains. The averages of motor units' z-coherence within delta, alpha and beta bands

were calculated and compared between TA muscle lengths. We found a significant reduction of ~14% in the alpha z-coherence values when the muscle length changed from shortened to optimal length. No such reduction was observed in the delta or beta bands. Our results provide experimental evidence that acquiring a force-matching skill and force production at optimal muscle length are associated with reductions in physiological tremor. These findings suggest that central (experiment 1) and peripheral (experiment 2) mechanisms may modify the characteristics of the shared noise inputs to alpha motoneurons and ultimately achieve optimal force generation.

Disclosures: H.V. Cabral: None. **F. Negro:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.07/JJ6

Topic: E.09. Motor Neurons and Muscle

Support: NSERC Discovery Grant

Title: Influence of system impedance on limb displacements following mechanical perturbation in control and co-contracted muscular states

Authors: *D. P. ARMSTRONG[1], K. J. DELUZIO[2], S. H. SCOTT[1];
[2]Mechanical and Materials Engin., [1]Queen's Univ., Kingston, ON, Canada

Abstract: Co-contraction is thought to provide an instantaneous response to unexpected disturbances to the body by increasing muscle stiffness. Contrary to this belief changes in muscle activity level led to negligible differences in initial limb motion (0-50 ms) after postural perturbations, arguing against zero-lag muscle stiffness as a mechanism for control [1]. However, inertia plays a key role influencing limb displacement 0-50 ms after perturbation, limiting our ability to quantify the effects of increased muscle impedance (both stiffness and damping) when co-contracting. To test the potential for muscle impedance to contribute to instantaneous control we compared limb displacements after perturbation where system 1) stiffness, 2) damping and 3) inertia were experimentally increased while in both co-contracted and relaxed muscle states. Three participants (1 ♀, 2 ♂; stature = 1.77 ± 0.16 m; body mass = 75.0 ± 9.6 kg) were recruited to complete the study. A Kinarm Exoskeleton Robot platform was used to apply a 5 Nm torque to the elbow joint after the participant held their fingertip at a controlled position for a random time of 2-4 seconds. Five trials were completed in each combination of system property (control, high stiffness, high damping, or high inertia), muscle activation (relaxed or co-contracted) and perturbation direction (flexor or extensor torque) conditions. Statistical parametric mapping two-way repeated measures ANOVA procedures were used to quantify differences in absolute elbow displacement angle (collapsed across perturbation direction) from 0-50 ms post-perturbation across system property and muscle activation conditions. Elbow displacements significantly differed from 9-50 ms as a function of system

property, with no significant muscle activation state or interaction effects. System property effects were driven by absolute elbow angular displacement 50 ms post-perturbation being significantly greater in control (6.15 ± 0.64º) and high inertia (5.94 ± 1.04º) compared to high stiffness (5.27 ± 0.53º) and high damping (2.82 ± 0.24º) conditions. While system impedance was shown to reduce limb displacement following perturbation, increases in muscle impedance while co-contracting did not similarly do so. This suggests that zero-lag muscle impedance is unlikely to be the primary mechanism in which muscular co-contraction aids in limb control. Rather it supports the need to further investigate contributions from neural mechanisms active beyond 50 ms post-perturbation contributing to limb control when co-contracting.
[1] Crevecoeur, F., & Scott, S. H. (2014). *PloS computational biology*, *10*(10), e1003869.

Disclosures: D.P. Armstrong: None. **K.J. Deluzio:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder and CSO of Kinarm.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.08/JJ7

Topic: E.09. Motor Neurons and Muscle

Title: Comparison of intramuscular and surface EMG of gluteus maximus during TMS: a case study

Authors: *M. MUNK[1], C. POWERS[2], B. E. FISHER[3];
[1]Biokinesiology and Physical Therapy, USC, Alhambra, CA; [2]Biokinesiology and Physical Therapy, [3]USC, Los Angeles, CA

Abstract: Background: Gluteus maximus (GM) weakness is associated with lower extremity (LE) pain and injury. Transcranial magnetic stimulation (TMS) measures brain changes related to muscle strength and impairment. However, TMS requires an electromyographic (EMG) signal. Adipose tissue, which is thicker over the GM than other muscles and varies significantly between individuals, can attenuate surface (s)EMG signals. Intramuscular EMG (iEMG) is not subject to attenuation caused by adipose tissue. Corticomotor excitability as measured by motor evoked potentials (MEP) as well as short interval intracortical inhibition (SICI), the degree to which inhibitory receptors in the motor cortex influence motor output, are two important TMS measures of the brain's influence on the GM. As such, the method which delivers the most robust EMG signal would be optimal for any TMS measurement. The purpose of this case series was to compare MEP amplitude and SICI of the GM using both sEMG and iEMG. **Case Description:** Subjects were a 27 y/o female and a 29 y/o male with no history of LE injury. sEMG electrodes were placed 30mm apart over the midpoint between the greater trochanter and sacrum. iEMG electrodes were inserted 3cm deep between sEMG electrodes and placement was confirmed with electrical stimulation. The GM motor hotspot and active motor threshold (aMT) were determined

before measuring MEP amplitude at 150% aMT. SICI, which uses 2 pulses in quick succession to assess the influence of inhibitory neural circuits on the GM, was measured at 120% aMT. **Outcomes:** At 150% aMT, iEMG MEP amplitudes were 311% (sd 118%) of sEMG MEP amplitudes. SICI is reported as a ratio of inhibited MEP amplitudes over uninhibited amplitudes. iEMG SICI was 69.4% (sd 2.16%), while sEMG SICI was 58.2% (sd 4.05%). **Discussion:** As expected, iEMG MEP amplitudes were greater than sEMG, and the difference was greatest in the female subject whose adipose tissue was visibly thicker at the recording site. However, iEMG and sEMG SICI, which were expected to be of equal magnitude, differed significantly. Difference in inhibition may be due to iEMG recording from a small number of small and easily excitable motor units, which were less affected by intracortical inhibition than larger motor units in the motor unit population recorded with sEMG. Also, the GM is composed of two regions with distinct activity patterns. iEMG may record activity in one region of the GM whereas sEMG records activity from the whole muscle. Measurement of changes in MEP amplitude would be unaffected by adipose tissue thickness using iEMG and thus preferable to sEMG. However, iEMG measurement of inhibitory mechanisms may be too focal to characterize the entire GM.

Disclosures: M. Munk: None. **C. Powers:** None. **B.E. Fisher:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.09/JJ8

Topic: E.09. Motor Neurons and Muscle

Support: NIH F31 NS124347
NIH MPI R01 NS104194
NSF CRCNS 1515140
NIH U01 EB021921

Title: Adding stochastic phase states and associated stochastic dynamic operators to a modular framework captures observations of modular motor physiology and behavior in the spinal frog

Authors: *T. S. SMITH[1], M. ABOLFATH-BEYGI[2], T. D. SANGER[3], S. GISZTER[1]; [1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Dept. of Biomed. Engin., USC, Los Angeles, CA; [3]UCI, CHOC, Redondo Beach, CA

Abstract: How the spinal cord translates various motor tasks into muscle activations is uncertain. In the motor modularity model of movement, dynamic ‘building blocks’ are combined to compactly construct most motor commands. We define a motor ‘module’ as a neural element evoking stereotyped motor activity, extracted from kinetic or biological features (e.g., synergist muscle EMG). In the spinal frog, modularity can be studied using the hindlimb-to-hindlimb wiping reflex as a behavior composed of adjusted phases and amplitudes of three motor modules. The spinal capacity to execute this motor activity can be challenged by varied perturbations,

including intraspinal microstimulation (ISMS). During wiping, the spinal cord must control when modules are recruited and how modules coordinate the motor pools. Previously, we adapted the Sanger stochastic dynamic operator (SDO) framework as a state-dependent spike-triggered average to better describe spike-correlated effects of spinal interneurons and single motor units on EMG in real data. Spike-triggered SDOs generated for single units across EMG channels can capture fixed patterns of muscle coactivations, consistent with synergy. Interneuron classes within the two-layer Rybak central pattern generator (CPG) model yield distinctive SDOs. SDO matrix 'motifs' qualitatively describe state-state relationships yet confer certain properties when used synthetically. Here, we describe a simple CPG-like network which can phenomenologically recapitulate probabilistic modular motor behavior in the spinal frog wiping reflex. Network state variables, including phase and amplitude, and their interactions (or lack thereof) are mediated by SDOs. Under the SDO framework, perturbations to the system may be readily incorporated as linear modifications to differential probability matrices, and network interactions probed, to test system connectivity or constraints. Furthermore, experimentally-estimated spike-triggered SDOs can be incorporated into the SDO-CPG, and the network readily scaled to encapsulate more complicated behavior. We will use this model in real data to explore if modules recruited during the wiping reflex require a coordinating rhythm generator or interacting phase-controls to maintain phase or amplitude when challenged by ISMS. This model extends the capability of the freely-available SDO Analysis Toolkit code package we have released. We anticipate this framework is useful for modeling black-box portions of CPG systems, where the biological circuit composition of the system is still uncertain, or synthetic systems where stochastic but stable limit cycle-driven properties are desired.

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Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.10/JJ9

Topic: E.09. Motor Neurons and Muscle

Title: Exploring The Neuromechanics of Object Manipulation in Physical and Virtual Environments

Authors: *D. L. DESPRADEL[1], N. POLLARD[2], D. J. WEBER[3];
[1]Mechanical Engin., [2]Robotics Inst. and the Computer Sci. Dept., [3]Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: This study focuses on analyzing the neuromechanics of user-object interaction in virtual environments (VEs) within the context of virtual reality (VR) technology. VR aims to replicate real-world (RW) sensory experiences, making the understanding of sensory cues on behavior and neuromechanics crucial for optimizing VR applications. By adopting a user-centric

approach, we investigate the direct interaction between physical and neurophysiological processes involved in object manipulation to enhance our understanding of the neuromechanics underlying user experiences in VEs.
We examine how sensory cues (visual and haptic) influence the neuromechanics of VR users and their alignment with RW experiences. We hypothesized that incorporating these cues during interactions with virtual objects will improve finger stability enhancing motor control during grasping. To test this, we analyzed the effects of sensory cues on muscle activation and hand kinematics, including maximum grip aperture (MGA), time to MGA (T-MGA), percent overshoot (PO), grasp variability (GV), and speed profiles. The study involved nine able-bodied individuals performing a pick-and-place task with physical objects and corresponding virtual renderings. Hand and finger movements were tracked using a motion-tracking system, while muscle activity was recorded using wireless EMG sensors on the forearm and upper arm.
In comparing user kinematics and EMG between the VE and RW under different feedback conditions (Visual, Vibrotactile, and No feedback), we observed significant neuromechanical differences. In the no feedback condition, VE exhibited higher values for MGA, PO, and GV compared to RW, while T-MGA and speed profiles during transport were longer in VE due to visual and motor uncertainty and lack of physical contact. However, in the vibrotactile and visual feedback conditions, participants achieved improved finger stability, as indicated by reduced uncertainty during grasping and transport (reflected in MGA). Muscle activity analysis revealed distinct patterns, with higher extensor muscle amplitudes in VE during the no feedback condition and increased flexor muscle amplitudes in the vibrotactile and visual feedback conditions. These findings shed light on the complex interplay of sensory perception, motor control, and cognitive processing in VR, highlighting its potential applications in cognitive support, neurorehabilitation, and human-computer interaction.

Disclosures: D.L. Despradel: None. **N. Pollard:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power Inc, IOTA Biosciences Inc, Neuronoff Inc, Neuroone Inc, Reach Neuro Inc.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.11/JJ10

Topic: E.09. Motor Neurons and Muscle

Title: Corticospinal excitability and interconnectivity of the limbs during arm cycling

Authors: *F. FARAHMANDFARADDONBEH[1], E. LOCKYER[1], C. COMPTON[1], K. RAIT[1], J. A. OGOLO[1], K. POWER[1,2];
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Abstract: Corticospinal excitability and interconnectivity of the limbs during arm cycling

Fattaneh Farahmand[1], Evan Lockyer[1], Christopher Compton[1], Katlyn Rait[1], Jirho A. Ogolo[1], and Kevin Power[1, 2]1Human Neurophysiology Laboratory, School of Human Kinetics and Recreation, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; [2]Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Appropriate coordination between upper and lower limbs is an important characteristic of quadrupedal and human locomotion and part of this coordination involves cortical and spinal control. This communication between the cortex and spinal cord suggests that neural coupling between the upper and lower limbs occurs during rhythmic movement. The purpose of this study was to investigate the effect of 1) arm cycling on corticospinal excitability to the resting vastus lateralis, 2) leg cycling on corticospinal excitability to the resting biceps brachii and 3) whether potential effects were intensity dependent. Twelve healthy participants (3 females and 9 males 28.8 ± 3.7 years of age, height 17.5 ±5.6, and weight 80±7.9, one left hand dominant) with no known neurological impairments participated in our study. Corticospinal excitability was assessed using transcranial magnetic stimulation (TMS) of the motor cortex. Motor-evoked potentials (MEPs) elicited by TMS were recorded at two power outputs 25 and 50% peak power output (PPO) from resting biceps brachii and vastus lateralis during leg and arm cycling, respectively. Our results demonstrated that biceps brachii (BB) MEP amplitudes were significantly increased at 25 and 50% PPO during leg cycling compared to rest (0.24 and 0.37mV vs 0.10mV; P=0.03 and P=0.005, respectively), but there was no significant difference in BB MEP amplitudes between 25 and 50% PPO (p=0.13). In addition, MEP amplitudes significantly increased to the vastus lateralis (VL) at 25 and 50% PPO compared to rest (0.3 and 0.5mV vs 0.11 mV; P=0.02 and P=0.004, respectively), as well as, increased at 25 compared to the 50% PPO during arm cycling (0.3mV vs 0.5mV; P=0.03). Our data demonstrate that MEP amplitudes increased in the resting BB and VL during leg and arm cycling, respectively, an effect that was intensity dependent.

Disclosures: F. Farahmandfaraddonbeh: None. **E. Lockyer:** None. **C. Compton:** None. **K. Rait:** None. **J. A. Ogolo:** None. **K. Power:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.12/JJ11

Topic: E.09. Motor Neurons and Muscle

Title: Neurofencing: Evaluating the benefits of warmup in competitive fencing through EMG, EEG and EKG.

Authors: *S. NAIR[1], M. DOERSTLING[2], D. F. PUTRINO[2], J. WOOD[2], A. SAWYER[2], B. W. HAMILTON[2], M. ZAKHARY[3];
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Med. Advisor, USA Fencing, Rehabil. & Human Performance, Icahn Sch. of Medicine, Mount Sinai, New York, NY

Abstract: Fencing coaches often emphasize the importance of warmup for their students prior to a bout of fencing. However, the performance benefits of a warmup remain understudied. In these experiments we analyzed activity in the brain, heart, and muscles. Our hypothesis suggested that the brain would shift from a relaxed to an active state, the heart from rest to increased energy, and muscle movement would become faster after warmup. Multiple competitive fencers, ages 10-15, participated in the Mount Sinai IRB-approved study protocol (STUDY 22-01661) over the course of two days. One day included a 15-minute warmup of predetermined exercises, and the other day excluded the warmup. All fencers performed ten lunges towards a fixed target 6ft away each day. Delsys Trigno sensors were placed on various muscle groups to collect electromyogram (EMG) and inertial measurement unit (IMU) data. Two-minute recordings of electrocardiogram (EKG) and electroencephalogram (EEG) were conducted at baseline and post-lunge timepoints on both days and post warm-up on one day. EEG data were collected via Neuroelectrics Enbio to assess band power across eight channels. Particular attention was given to the C3 and C4 channels which are associated with the motor cortex. EKG data were gathered using Polar H10 and HRVElite. Lunge videos were analyzed with a machine learning model for human pose estimation (HPE). A 9.4% faster touch speed was observed in participants on the day a 15-minute warmup was performed ($p=0.01$*). There was significant reduction in the magnitude of the EMG spike in the forearm ($p<0.01$**) and bicep ($p=0.02$*) muscle groups, indicating improved coordination between the nervous system and muscles. Better coordination between the nervous system and muscles yields controlled, precise movements, potentially reducing the need for large, forceful contractions, seen as larger EMG spikes. Analysis of EKG data showed a significantly higher minimum heart rate post-lunge with warmup compared to the non-warmup day ($p<0.01$**). Additionally, a significant reduction in (HRV PNN50) proportion of time that the interval between heart beats is greater than 50ms ($p=0.03$*) was seen. Preliminary EEG analysis of C3 and C4 channels demonstrated a significantly higher delta wave band power post-lunge with warm-up compared to post-lunge without warmup ($p<0.01$**), indicating an impact on the amplitude and power of EEG component bands. These findings support the objective value of warmup to young fencers and encourage future application of the study's methodology and data analysis framework on fencing as well as other athletic sports.

Disclosures: S. Nair: None. **M. Doerstling:** None. **D.F. Putrino:** None. **J. Wood:** None. **A. Sawyer:** None. **B.W. Hamilton:** None. **M. Zakhary:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.13/JJ12

Topic: E.09. Motor Neurons and Muscle

Support: NIDILRR-90RE5013

Title: A method of quantifying the MAS test for evaluating spasticity treatment in chronic stroke survivors

Authors: H. XU[1], Z. RYMER[2], ***N. SURESH**[3];
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Abstract: Background: The Modified Ashworth Scale (MAS) is a gold standard for the clinician to evaluate spasticity in chronic stroke survivors. While a single MAS score is assigned to assess the severity of spasticity, inter as well as intra rater reliability is often questioned. Quantification of the clinical MAS test might help to assess more precisely the effects of interventions as well to help elucidate underlying neurological and biomechanical origins of the resistance to stretch. **Methods:** Two chronic stroke survivors who were identified by their physicians as eligible for botulinum toxin(BT) injections for the treatment of spasticity in their biceps brachii were tested. Each participant was tested before the BT injection and every two weeks after until weeks 12 and 14 post injection. At each session, the participant had a digital goniometer, EMG electrodes and accelerometer attached to their joint/muscles in order to record the speed, and angle of joint rotation and record any EMG activity or reflex response to stretch. A research therapist performed the MAS test in each session, 36 assessments were done on both affected side and contralateral side at three different randomized speeds. Catch angle, EMG onset angle and EMG summation during the range of motion (ROM) were calculated and compared over sessions. **Results:** The computed catch angle had negative correlations with stretch velocity. EMG activities decreased significantly at week 2 after the initial injection of BTX-A for both subjects, which matched with the MAS scores, but the catch angle increased for subject A and decreased for subject B. While catch angle and EMG activities showed decreasing and rebounding trend, they varied over sessions for both subjects, but clinical MAS scores did not reflect the underlying details. **Conclusions:** Via wearable sensors, quantitative measurements in addition to MAS scores can help differentiate the effect of treatment. By applying the method in the clinical assessments, therapists might make better decision for further intervention.

Disclosures: H. Xu: None. **Z. Rymer:** None. **N. Suresh:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.14/JJ13

Topic: E.09. Motor Neurons and Muscle

Title: Force-stabilizing intra-muscle synergies in tibialis anterior and their changes with fatigue

Authors: *M. LATASH[1,3], J. M. RICOTTA[2], S. D. DE[2], M. NARDON[3], A. BENAMATI[3];
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Abstract: We applied the recently introduced concept of intra-muscle synergies in spaces of motor units (MUs) to quantify indices of such synergies in the tibialis anterior during accurate

ankle dorsiflexion force production tasks and their changes with fatigue. We hypothesized that MUs would be organized into robust groups (MU-modes), which would co-vary across trials to stabilize force magnitude, and the indices of such synergies would drop under fatigue. Healthy, young subjects (n = 14; 7 females) performed accurate cyclical force production tasks in isometric conditions. Surface electromyography using the Delsys Galileo system was used to identify action potentials of individual MUs. Principal component analysis was used to define MU-modes. The framework of the uncontrolled manifold (UCM) hypothesis was used to analyze inter-cycle variance and compute the synergy index, ΔV_Z. The tests were repeated after a fatiguing exercise and a non-fatiguing exercise (control). Across subjects, fatigue led, on average, to a 43% drop in maximal force and fewer identified MUs per subject (29.6±2.1 vs. 32.4±2.1). The first two MU-modes accounted for 81.2±0.1% of variance across conditions. The synergy index was positive across tests confirming the existence of force-stabilizing synergies. This index was unaffected by the control exercise but dropped significantly under fatigue (1.76±0.75 vs. 1.49±0.40, $p < 0.01$). This was primarily due to an increase in the amount of variance orthogonal to the UCM. These findings are in contrast to earlier studies of multi-effector synergies demonstrating increased synergy index under fatigue. We interpret the results as reflections of a drop in the gain of spinal reflex loops under fatigue. The findings confirm an earlier hypothesis on spinal nature of intra-muscle synergies.

Disclosures: M. Latash: None. **J.M. Ricotta:** None. **S.D. De:** None. **M. Nardon:** None. **A. Benamati:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.15/JJ14

Topic: E.09. Motor Neurons and Muscle

Support: Coulter-Drexel Translational Program
PA Dept. Health CURE

Title: Emg recordings in human and rats with braided multi-electrode intramuscular probes

Authors: *T. KIM[1], T. SMITH[1], A. BORISYUK[1], B. BINDER-MARKEY[2], S. GISZTER[3]; [1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Physical Therapy and Rehabil. Sci., Drexel Univ., Philadelphia, PA; [3]Drexel Univ. Col. of Medicine, Neurobio., Philadelphia, PA

Abstract: At SfN 2022, we introduced braided multi-electrode intramuscular EMG probes for single motor unit recordings, with 6 pairs of 12 ultrafine microwires tubularly braided over Natus 25G monopolar needle electrodes (used for electrodiagnostic testing in the clinic). Our probe has 12 channels on 12 microwires + the needle tip. Recording sites on each microwire are made by laser ablation. With these novel EMG probes, we demonstrated high yields of single motor units

successfully recorded in frogs and rats. We are now working on human tests with these probes under a Drexel IRB. For the human tests, we have also developed additional outer coatings and tip beveling techniques to assist insertion, USB-C based EIBs (Electrode Interface Board) for the multi-channel, small form factor probes, cable & adapters and a channel switching system to be used with conventional clinical single channel recording systems. We demonstrate and discuss benefits of multi-channel capability of intramuscular EMG probes by comparing recordings with clinical single channel systems to recordings with multi-channel parallel recording systems and advanced signal processing across multiple channels in human subjects compared to our animal tests. A second variation of the braided multi-electrode intramuscular probes developed is flexible and releasable. The braided microwires of the releasable version are released from the needle after being inserted into muscle in order for the braid to have very high compliance, this mechanical flexibility not causing any discomfort. We believe that this releasable version will allow single unit EMG recording during patients' regular movements and provide semi-chronic EMG monitoring options to observe changes in muscle activities after injuries and therapeutic options, with potential local intramuscular electrical stimulation. We demonstrate this releasable braid design, the releasing mechanism and recording results from the releasable braid probes tested in freely moving frogs and rats.

Disclosures: **T. Kim:** None. **T. Smith:** None. **A. Borisyuk:** None. **B. Binder-Markey:** None. **S. Giszter:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.16/JJ15

Topic: E.09. Motor Neurons and Muscle

Support: NIH UG3NS12313501A1

Title: Decoding hand gestures using HDEMG and Graph Neural Networks

Authors: *P. YADAV[1], D. J. WEBER[2];
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Abstract: Humans use their hands to perform a variety of intricate tasks for activities of daily living (ADLs), and they are the primary means of interaction with the environment. The loss of hand function greatly diminishes a person's ability to independently perform ADLs. The restoration of hand function through prosthesis that can perform basic functions like grasping, pointing, and picking, would assist people with ADLs and assume some functional independence. Such dexterous prosthetic hands require intuitive, multichannel control interfaces, which can be achieved using high-density electromyography (HDEMG) . The translation of myoelectric signal from the hand, to a functional control for the prosthesis requires low-latency decoding of the signals. Effective decoding would require understanding the patterns in the EMG

signals associated with each of the intended gestures and their subsequent classification. A machine learning (ML) based framework would be an ideal solution for such a decoding problem. ML methods can learn the aforementioned patterns in EMG signals and allow for rapid inference which can enable the desired low-latency decoding of EMG signals for controlling a prosthesis. However, the ML based decoding of EMG signals suffers from issues with generalization to differences in recording setup, orientation of electrodes and other typicalities, making it difficult to design subject-and-session agnostic methods. Some of these constraints, such as orientation and differences in electrode montages, can be addressed by utilizing permutation invariant ML models, such as the Graph Neural Networks (GNNs). To explore the efficacy of GNNs at gesture decoding from EMG signals, we have developed a message passing GNN based on the graph convolution neural network for able-bodied people. We model the HDEMG array as a partially connected graph, with electrodes as the nodes and distance between electrodes as edges. We collect HDEMG signals from the forearm flexor and extensor muscles associated with specific tasks as our training data. The GNN model trained on this data achieved an overall classification accuracy of 82.7% (n=13 classes). The model had highest accuracy for classifying index finger pointing, grasp, and wrist movements. Additionally, the low dimension visualization of the representations learnt by the model showed that it had learnt to distinctly cluster the various hand gestures. This work presents promising evidence for the utility of GNNs as a more general ML solution of gesture decoding and its subsequent application to prosthetic control.

Disclosures: **P. Yadav:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power Inc, Iota Biosciences, Inc, Reach Neuro, Inc, Neuronoff Inc, Neuroone Inc.. F. Consulting Fees (e.g., advisory boards); Neuronoff Inc, Neuroone Inc..

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.17/JJ16

Topic: E.09. Motor Neurons and Muscle

Support: AHA Award - 20PRE35211085
National Institute on Disability Independent Living and Rehabilitation Research

Title: Alterations in MU Firing Behavior post stroke may be accessible by mechanomyogram

Authors: ***F. SHI**[1,2], J. SON[3], W. Z. RYMER[1,2];
[1]Northwestern Univ., Chicago, IL; [2]Shirley Ryan AbilityLab, Chicago, IL; [3]Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ

Abstract: Purpose: During isometric contraction, the muscle force output fluctuates about a constant value. The steadiness of this output can be quantified by the amplitude of force fluctuation (hereafter called F_{SD}). This fluctuation can also be accessible by measuring surface vibrations using mechanomyogram (MMG) [1]. These measurements may link to motor unit (MU) behavior, namely, motor unit recruitment and rate modulation may affect the degree of force fusion and thus the force variability. This study aims at exploring the associations of the MU firing behavior with the F_{SD} and MMG post stroke. **Methods:** Four chronic stroke survivors were seated an upright position with the wrist immobilized in a fixture, which is attached with a 6-DOF load cell. A Trigno Galileo sensor and a 3-axis accelerometer was attached on each head of biceps brachii, which was used to decompose MU firings and to assess the surface vibration perpendicular to the skin, respectively. Data were collected at different forces from both sides. The maximum voluntary isometric contraction (MVIC) of the paretic side was used to match the absolute force for both sides. Dataset from the head with higher MU yields was used for further analyses. F_{SD} and RMS MMG were evaluated between sides at each force. The mean firing rate of MUs across all the contraction levels and subjects binned at different stages of recruitment threshold were compared between sides. A spearman correlation was performed to evaluate the associations of changes in MU firing rate with F_{SD} and RMS MMG. **Results and Discussion:** The magnitude of F_{SD} and RMS MMG was greater and the mean firing rate was lower at each matched force on the paretic side (Figure 1). There was a positive correlation of the change in mean MU firing rate with increased F_{SD} (r = 0.42, p = 0.27) and RMS MMG (r = 0.82, *p = 0.01). These preliminary results suggest that the altered MU firing rate post stroke may impact the steadiness of motor output, and RMS MMG may better reflect the altered MU firing rate compared to F_{SD} due to load sharing. **Ref:** [1] Yoshitake et al., 2008.

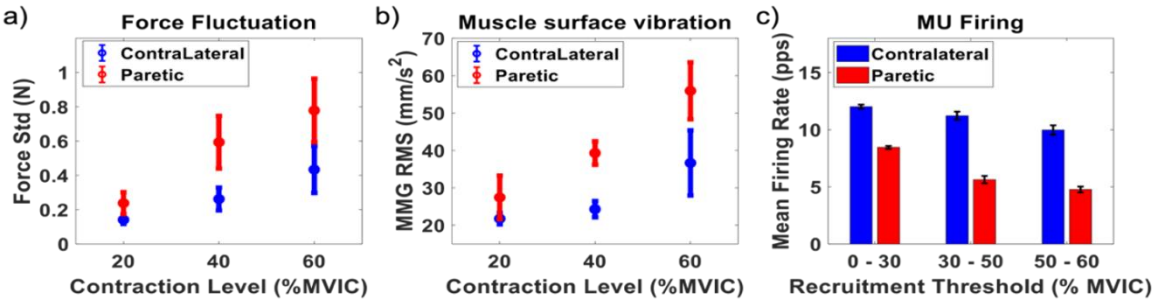

Figure 1. (a) Comparison of F_{SD} between sides across different contraction levels, (b) Comparison of RMS MMG between sides across different contraction levels, (c) Mean Firing Rate within each recruitment threshold bin between sides. Error bars represent standard errors

Disclosures: F. Shi: None. **J. Son:** None. **W.Z. Rymer:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.18/JJ17

Topic: E.09. Motor Neurons and Muscle

Support: N_CUHK456/21
R4022-18F
14114721
14119022

Title: Representation of Muscle Synergies in the Ensemble Activity of Motor Units

Authors: *M. BAI[1], Y. GENG[2], G. LI[2], V. C. CHEUNG[1];
[1]Sch. of Biomed. Sciences, The Gerald Choa Neurosci. Inst., The Chinese Univ. of Hong Kong, Hong Kong, China; [2]Shenzhen Inst. of Advanced Technology, Chinese Acad. of Sci., Shenzhen, China

Abstract: Muscle synergies are known as basic modules of motor control implemented by the nervous system to coordinate multi-muscle activities. Putative neural representations of muscle synergies have been previously identified in motor cortical neurons and spinal premotor interneurons, yet whether the ensemble activity of the motor units reflect the structures of muscle synergies, which are often defined and identified at the whole-muscle level, remains unconfirmed. We hypothesize that whole-muscle muscle synergies derived from traditional surface electromyographic recordings (tr-EMG) are represented in the activities of the motor units of the same muscles. Neurologically intact human adults (N=12) were recruited and instructed to perform 6 isometric finger pinch tasks, each at 3 force levels (thus totaling 6x3=18 tasks) with 3 repetitions (thus 12x3=36 trials). During task performance, tr-EMGs were recorded from 13 superficial forearm muscles while high-density EMGs (hd-EMGs; 8x8 grid) were simultaneously recorded from either the anterior or posterior forearm surface. Muscle synergies were extracted from tr-EMGs using non-negative matrix factorization while activities of motor units were decomposed from hd-EMGs using blind source separation. To build a relationship between motor-unit activities and muscle synergies, we applied representational similarity analysis, which involves first calculating the pairwise between-task similarity for the motor-unit firing rates and the muscle-synergy temporal coefficients, respectively, and then correlating the similarities from the motor units with their counterparts from muscle synergies. We found that in 33 out of 36 trials, the motor-unit-derived and synergy-derived between-task similarities were significantly correlated ($p<0.05$). Thus, across tasks, the synergies' temporal coefficients and the motor-unit activities carry similar information, thus indirectly suggesting that multi-muscle motor units are coordinated in a way that is consistent with the structures of muscle synergies.

Disclosures: **M. Bai:** None. **Y. Geng:** None. **G. Li:** None. **V.C. Cheung:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.19/JJ18

Topic: E.09. Motor Neurons and Muscle

Title: Serotonergic neurons inbrainstem nucleus MRAN regulate sustainedspinal motor output

Authors: *R. DE SA[1], M. SKROBOT[2], E. GARULLI[3], N. WENGER[4];
[1]Charité Berlin, Berlin, Germany; [2]Charite, Berlin, Germany; [3]Charite Universtätsmedizin Berlin, Berlin, Germany; [4]Wenger Nikolaus, Wenger Nikolaus, Berlin, Germany

Abstract: The reticular formation (RF) is a key brainstem center for the control of movement. Yet, the functional roles of several genetically identified cell populations with the RF remain unclear. Here, we addressed the contribution of serotonergic neurons within the magnocellular reticular nucleus (MRAN) to motor activity. For this, we first identified the location of serotonergic neurons in the MRAN using viral tracing tools. Next, we delivered hm3d for pharmacogenetic gain-of-function experiments. Activation of serotonergic neurons induced prolonged periods of limb hyper-extension in both upper and lower extremities. These kinematic changes were reflected by increased muscle tonus measured in baseline EMG activity. Together, our results highlight a role of brainstem serotonergic neurons in regulating descending command signals for sustained activation of spinal motor output.

Disclosures: R. De sa: None. **M. Skrobot:** None. **E. Garulli:** None. **N. Wenger:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.20/JJ19

Topic: E.09. Motor Neurons and Muscle

Title: Ultra-flexible Nanoelectronic Thread Enabled High-density, Chronic Tracking of Neural Ensemble Dynamics During Reach Learning in the Cortex of Freely Moving Mice

Authors: *Y. Y. SUN[1,2], R. YIN[2,1], P. ZOLOTAVIN[2,1], L. LUAN[1,2], C. XIE[2,1];
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Abstract: Motor learning, the consolidation of automatic reactions to memory by long-term practicing, has been theorized to occur in three stages. Initially, when a new stimulus shows up, the brain processes occur on an intentional level. As guidance and practice continue, the mice progressively fine-tune their behavior, making subtle adjustments to improve their skills through the modulation of the neural population activities and the establishment of monosynaptic connectivity. Eventually, with repetitive practice, the motor performance becomes automatic, requiring minimal cognitive processing. However, the precise evolution of neural ensemble dynamics and the concurrent changes in cortical representations throughout these stages remain largely unexplored. One key challenge is the lack of tools that can simultaneously meet the multi-level needs of resolving the fast dynamics underlying each event and longitudinally tracking these activities at the single neuron resolution over a large population. In this study, we

implant large-scale, high-density ultra-flexible nanoelectronic threads (NETs) in the motor cortex of mice to track the neural representation during the single-pellet reach and grasp task covering the whole learning stages. Over a two-week training period, the success rate (N=4) of the single-pellet reach and grasp task increased from 20% to 60%, demonstrating motor skill learning and the intricate coordination of neural ensemble involved in motor control. To reveal the functional tuning and its evolution during motor learning, we tracked hundreds of neurons, with their activities reliably aligned with their paw trajectory. We report the firing rate changes throughout the reach kinematics and its variation over time in the training course. Furthermore, we examine the cell-type specific plasticity by conducting a putative cell-type-specific analysis using their electrophysiological signatures. Having recorded a large number of neurons also allows for identification of existing and newly formed connections across the learning process. These on-going efforts showcase the capability of large-scale, stable recording in the study of neural plasticity underlying learning.

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Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.21/JJ20

Topic: E.09. Motor Neurons and Muscle

Support: NIH R01NS109237
Novo Nordisk Foundation
Simons Foundation
U24 NS126936

Title: Myomatrix arrays for recording muscle activity

Authors: *A. L. JACOB[1], M. ZIA[3], A. SOTIRESCU[1], S. O'CONNELL[1,4], A. PACK[1], K. A. THOMAS[2,4], M. WILLIAMS[1,4], B. CHUNG[1], C. P. ELEMANS[6], M. BAKIR[5], S. J. SOBER[1]; [1]Biol., [2]Emory Univ., Atlanta, GA; [3]Electrical Engin., Georgia Instutite of Technol., Altanta, GA; [4]Biomed. Engin., [5]Electrical Engin., Georgia Instutite of Technol., Atlanta, GA; [6]Biol., Univ. of Southern Denmark, Odense M, Denmark

Abstract: Production of complex animal behaviors, such as vocalization, locomotion, and reaching, is one of the primary functions of the nervous system. These actions result from the precise electrical activity (“spikes”) generated by motor neurons that innervate individual muscle

fibers. Fine wire EMG, the standard method used to record muscle activity, typically cannot resolve the spiking activity of single motor units (composed of one motor neuron and the muscle fibers it innervates) in freely moving subjects, limiting our understanding of how motor circuits generate behavior. Our team has developed a novel high-density, multielectrode system ("Myomatrix arrays"; Chung et al. 2023) that is highly customizable, capable of being used acutely and chronically, and can be used across a wide variety of muscles within multiple model systems ranging from insects, birds, mice, rats, monkeys, and humans. To highlight the use of our arrays, we present data from our novel electrode system recording precise spiking patterns in the vocal muscles of the awake, behaving rats producing ultrasonic vocalizations - a means of social communication used by rats to signal affective states. Our methods, therefore, facilitate observations of the neuronal control of muscle activity in unprecedented detail, providing an important tool to advance our understanding of motor control in both research and clinical settings.

Disclosures: **A.L. Jacob:** None. **M. Zia:** None. **A. Sotirescu:** None. **S. O'Connell:** None. **A. Pack:** None. **K.A. Thomas:** None. **M. Williams:** None. **B. Chung:** None. **C.P. Elemans:** None. **M. Bakir:** None. **S.J. Sober:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.22/JJ21

Topic: E.09. Motor Neurons and Muscle

Support: Simons Foundation
NIH U24 NS126936
NIH R01NS109237
NSF 1937971

Title: Motor unit coordination within and across muscles during locomotion in mice

Authors: *K. THOMAS[1,2], R. GIBBS[1], H. MARQUES[3], M. R. CAREY[3], S. SOBER[1,2];
[1]Emory Univ., Atlanta, GA; [2]Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA;
[3]Champalimaud Fndn., Champalimaud Ctr. For the Unknown, Lisboa, Portugal

Abstract: A fundamental goal of neuroscience is to understand how the nervous system flexibly changes muscle activation patterns to drive movement. Locomotion specifically requires rapid changes in speed, which is potentially driven by fundamental changes in the coordination of motor neurons innervating muscles. Using novel electrodes we developed (Myomatrix arrays), we recorded motor units, which consist of a single motor neuron and the muscle fibers it innervates, from forelimb muscles in freely locomoting mice. Combining these high-resolution recordings with quantitative behavioral analysis revealed how the nervous system reshapes motor unit activity across variations in walking speed in mice. Individual motor units were

reliably active during particular phases of the stride cycle, with times of peak firing varying both within and across muscles. Burst timing and spike count varied dramatically in relation to key stride landmarks across speeds. Ensemble analysis of simultaneously recorded units demonstrated complex patterns of co-timing across motor units, including epochs of highly ordered recruitment and highly variable de-recruitment apparent across motor units within the same and different muscles. The variation in these patterns across speeds depended on the muscle, likely reflecting the biomechanically defined role of a given muscle during locomotion. Overall, connecting the kinematics behind locomotion with the neural inputs to muscles revealed how variability in spike patterns are used to generate flexible behaviors.

Disclosures: K. Thomas: None. **R. Gibbs:** None. **H. Marques:** None. **M.R. Carey:** None. **S. Sober:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

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Program #/Poster #: PSTR553.23/JJ22

Topic: E.09. Motor Neurons and Muscle

Support: Simons Foundation Grant 8310000132
NIH U24NS126936
NIH R01NS109237

Title: Neuromuscular Control During A Bimanual Precision Force Production Task

Authors: *M. J. WILLIAMS[1], A. P. KEIM[2], E. AZIM[3], S. J. SOBER[1];
[1]Biol., Emory Univ., Atlanta, GA; [2]Univ. of California San Diego, La Jolla, CA; [3]MNL-E, Salk Inst., La Jolla, CA

Abstract: To interact with the outside world, the brain must flexibly control and coordinate muscle groups to create complex movements. However, we cannot fully understand how the nervous system achieves the goal of complex motor behavior without observing the precise spiking patterns at the output of the motor system, the motor unit. To better understand how the central nervous system coordinates muscles to produce smooth behavior, we must pair these electrophysiological recordings with similarly precise measures of behavioral output. We trained mice in a bimanual force plate task wherein they must generate a specific combination of isometric forelimb forces to receive a water reward. The exact combination of forces can be rapidly updated by the experimenter or automatically updated based on the subject's success rate. This allowed us to experimentally control not just the absolute force level, but also the covariance between forces, which will enable continued studies of behavioral redundancy. We then paired this novel behavioral measure with precise measures of muscle activity. Previous studies of motor unit spiking patterns have enabled a richer understanding of motor control and learning, but they have been limited by the small number of recorded units and a short recording

duration. We have developed custom electrode arrays that enable the simultaneous, chronic recording of multiple individual motor units to study muscle coordination during a goal-oriented isometric force task. These high-density microelectrode arrays were implanted in several muscles of each forelimb: the deltoids, trapezius, and the triceps muscles. Simultaneous recordings of both output forelimb force and single motor unit spiking enable the study of muscle coordination both within and across limbs at the level of single motor units. The long-term goal of this study is to determine how descending signals from the brain are translated by motor units into complex behaviors. Future experiments will employ cortical and subcortical perturbations to probe the role of descending control during bimanual coordination.

Disclosures: **M.J. Williams:** None. **A.P. Keim:** None. **E. Azim:** None. **S.J. Sober:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.24/JJ23

Topic: E.09. Motor Neurons and Muscle

Support: NIH Intramural Research ZIA CL090099
NSF award 1646204
NIH award u01EB027601
Department of Defense award W81XWH-16-1-0722

Title: Ultrasound imaging as a tool to track joint kinematics across persons with varying muscle sizes

Authors: *S. PATWARDHAN[1], J. SCHOFIELD[2], W. M. JOINER[3], T. BULEA[4], S. SIKDAR[5]; [1]Rehabil. Med. Dept., Natl. Inst. of Hlth. Clin. Ctr., Bethesda, MD; [2]UC Davis, Davis, CA; [3]Dept. of Neurobiology, Physiol. and Behavior, Univ. of California, Davis, Davis, CA; [4]Clin. Center/ Rehabil. Med. Dept., NIH, Bethesda, MD; [5]Bioengineering, George Mason Univ., Fairfax, VA

Abstract: Sonomyography measures muscle deformation with ultrasound. The extracted signals can be used to proportionally control a device, such as the position of an end-effector. Although point-to-point reaching movements in healthy individuals are known to follow a minimum jerk trajectory, it is unknown if muscle activation follows a similar control policy. To test this, we performed an experiment (10 subjects) in which seven virtual targets (five trials each) were presented on a screen. Subjects were asked to acquire these virtual targets by flexing/extending their flexor digitorum superficialis and flexor digitorum muscles, that in turn drove a virtual cursor left/right based on the extent of flexion/extension measured by sonomyography. To assess potential feasibility in individuals with neurological disorders that result in reduced muscle volume, we examined how differences in muscle size affect the control relationships and the ability of ultrasound to capture them. Muscle thickness was computed from B-mode ultrasound

images with 4 cm depth captured with the transducer placed perpendicular to the volar aspect of the forearm. Combined flexor digitorum muscle thickness was defined as the distance between the superficial adipose tissue-muscle interface and the muscle-bone interface. The velocity profiles derived from imaging muscle activation during target acquisition followed a minimum jerk trajectory indicating sonomyographic control was comparable to a point-to-point reaching task. Average muscle thickness across subjects was 3.35 ± 0.44 cm. Despite this range, target acquisition results showed very low average standard deviation in position traces across trials (4.4%). These results demonstrate that motor planning based on muscle activation follows the same movement properties of multi-joint limb movement, suggesting a common control policy despite the execution involving different levels of the motor system. Furthermore, the current results show that sonomyography can track muscle kinematics to reveal the common minimum jerk trajectory control policy in real-time across a group with varying muscle thickness. Collectively these findings support the use of sonomyograpy to characterize muscle contraction trajectories in individuals with upper neuromotor pathology, such as stroke or cerebral palsy, which could reveal whether their isolated muscle contractions follow similar control policies as healthy individuals despite degradation of multi-joint movements. Ultimately, such studies may lead to improved rehabilitation device design and development of objective assessment metrics to track rehabilitation via sonomyography.

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Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.25/JJ24

Topic: E.09. Motor Neurons and Muscle

Title: Synergistic and individual digit movements are controlled by task-specific modulation of motor neuron recruitment and firing rate

Authors: *M. OßWALD, D. SOUZA DE OLIVEIRA, A. CAKICI, D. BRAUN, A. DEL VECCHIO;
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Abstract: Voluntary hand movements require precise neural control of spinal motor neurons. The mechanisms of motor unit recruitment and rate coding during dynamic synergistic hand and individual digit movements are not fully understood. We developed a framework for synchronized markerless acquisition of 3D hand kinematics and motor unit (MU) spike times from high-density surface EMG (HD-sEMG) signals. We recorded 320 HD-sEMG channels from the extrinsic hand muscles. Twelve human participants (10 m, 2 f) performed 13 dynamic hand and digit tasks, covering >20 degrees of freedom of the hand. Tasks consisted of 45 seconds of periodic single digit flexion/extension at fast (1.5 Hz) and slow (0.5 Hz) movement

speeds of all five digits and 3 mechanically synergistic grasping tasks (0.5 Hz, 2-digit precision pinch, 3-digit precision pinch, 5-digit power grasp). MUs were identified and tracked across tasks through matching of motor unit action potential shapes. We computed the coefficient of variation (CoV) of the smoothed MU discharge rate with a high CoV corresponding to strong task modulation of MU firing activity. We found two clusters of MUs based on CoV. The discharge rate of one group of MUs was highly correlated to the kinematics of the digits, with a CoV of >0.5. These MUs were considered as "prime mover MUs" of the respective movement task. The second group showed little to no modulation of MU discharge rate with CoV values of <0.5, considered as "postural MUs". Of the 554 MUs found across all tasks and subjects (7.8 ± 1.8 MUs per task and subject), 182 were identified across a minimum of two tasks (tracked MUs), while 372 were only identified in a single task (unique MUs). In single digit tasks, unique MUs had a mean CoV of 0.62 with 52.1% showing prime moving behaviour (CoV >0.5). Tracked MUs show a mean CoV of 0.36 and 24.5% prime mover MUs, exhibiting strong task modulation in multiple tasks. However, inspection of these MUs firing activities and corresponding 3D kinematics reveals prime moving behaviour for only one distinct digit. Prime moving activity of a MU in multiple digit tasks could be attributed to enslaving (kinematic coupling) of two digits or counter activation by the subject to actively prevent such enslaving. In synergistic grasping tasks, we identified only 24.4% of MUs as also active during the respective involved single digit tasks, with the remaining 75.6% being unique MUs of the grasping tasks. We draw two main conclusions. (1) Single digit movements are controlled by distinct pools of prime mover MUs, with no prime mover MUs shared between digits. (2) Synergistic grasping movements are controlled by more than just the sum of the MUs controlling the involved single digits.

Disclosures: M. Oßwald: None. **D. Souza de Oliveira:** None. **A. Cakici:** None. **D. Braun:** None. **A. Del Vecchio:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.26/JJ25

Topic: E.06. Posture and Gait

Support: NSF NeuroNex: Communication, Coordination, and Control in Neuromechanical Systems (C3NS)

Title: Exploring stance phase hindlimb dynamics in rats through randomized perturbations

Authors: *Z. WANG[1], S. TRAN[1], G. SERRANCOLI[3], M. C. TRESCH[2];
[1]Neurosci., [2]Biomed. Engin., Northwestern Univ., Chicago, IL; [3]Mechanical Engin., Univ. Politècnica de Catalunya, Barcelona, Spain

Abstract: Joint dynamics, i.e., the relationship between joint torque/force and joint movements are critical for understanding the neuromuscular mechanisms underlying motor control during locomotion. In this study, we developed a technique to investigate rat hindlimb dynamics, which induces high acceleration (up to 20000 mm/s^2) perturbations using a linear motor with servo-loop control with position accuracy of ± 1 μm. With force/torque recording from six degree-of-freedom force transducer and precise position feedback, we were able to simultaneously obtain both the force in all directions and limb motion during high-speed excitations using pseudorandom binary sequence (PRBS). We used 2 mm oscillations with randomized pulse widths to perturb the rat hindlimb because it caused much less disturbances to the hindlimb configuration compared to 5mm and 10mm oscillations. We chose PRBS based on its excitation of limb dynamics across the frequency spectrum and because it required fewer trial repetitions compared to other perturbation methods. The system dynamical properties were then characterized by a quasi-linear model between force outputs and position inputs (elasticity) and its first and second derivatives (viscosity and inertia, respectively). To apply this technique, we characterize the rat hindlimb dynamics across a range of configurations including those corresponding to stance phases during locomotion. The generated parametric impedance/passive equations relating force with elasticity, viscosity and inertia in different configurations were compared with traditional in-vitro/vivo measurements to evaluate the model validity. These measurements provide information that can be used to investigate the mechanical properties of animal motor systems and their influence on motor control strategies.

Disclosures: Z. Wang: None. **S. Tran:** None. **G. Serrancoli:** None. **M.C. Tresch:** None.

Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.27/KK1

Topic: E.06. Posture and Gait

Support: NSF DGE 0718128
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The Dr. Benno Nigg Chair in Biomechanics
Canada Research Chairs Program

Title: A simple optimal control policy may predict human walking speed on multistep uneven terrain

Authors: *O. DARICI, A. D. KUO;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Uneven terrain causes fluctuations in walking speed. Some fluctuations follow the height change of each step, but humans might also actively adjust their speed in anticipation of

upcoming terrain. Simultaneity makes these two effects difficult to separate, but speed fluctuations over a complex ground profile could potentially be deconstructed into a fluctuation waveform for a single (1-) perturbation alone. The 1-perturbation waveform should then be able to predict speed fluctuations for other ground profiles. We tested this by measuring human speed (N = 11) walking over a 17 steps complex uneven terrain and deconstructing the 1-perturbation waveform. This waveform exhibited anticipatory adjustments several steps in advance and predicted different uneven terrain speed profiles showing humans make systematic and anticipatory speed adjustments for each upcoming perturbation, even when they occur in succession. Uneven steps cause a momentary loss or gain of walking speed. Any speed changes observable prior to the terrain signify active (visual) anticipation by the human. We developed a simple optimization model suggesting that walking speed dynamics may be approximately linear and superimposable. Superposition means that the speed fluctuations for a single uneven step may be deconstructed from a complex profile. Superposition also suggests that movement planning over complex terrain could be a simple matter of repeatedly applying the 1-perturbation locally, one step at a time, rather than requiring global planning for all steps at once. Superposition facilitates the linear operations of deconvolution and convolution. Deconvolution is a form of linear regression, applied here between a complex ground height profile and corresponding speed fluctuation data (the measured speed on the terrain), to deconstruct the 1-perturbation waveform, or kernel. The kernel describes speed fluctuations in the steps before and after a single small perturbation. It may also be convolved with an arbitrary ground profile to predict new speed fluctuations, and therefore test for generalizability. We tested whether the kernel included an anticipatory speed adjustment, as well as its ability to predict fluctuations for an independent terrain profile. The 1-perturbation kernel was found to reproduce the training data (R = 0.82) and 5 independent and different terrain profiles (R = 0.6, 0.77, 0.79, 0.82, 0.85) reasonably well. The strategy for a single perturbation applies to many in succession, suggesting a simple and general way of control on uneven terrain which could be interpreted as an optimal, inverse internal model of walking within the central nervous system.

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Poster

PSTR553. Motor Unit Recordings, Kinematics, and EMG

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR553.28/KK2

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant R21NS111310
DE-CTR Grant P20GM103446

Title: Subthreshold tms for cortical inhibition of the long-latency response in the forearm

Authors: *C. A. HELM, S. A. AMOFAH, F. SERGI;
Biomed. Engin., Univ. of Delaware, Newark, DE

Abstract: The reticulospinal tract (RST) plays an important role in motor control and is specifically involved in long-latency responses (LLR). Our lab has recently developed StretchfMRI, a technique that uses fMRI and an MRI-compatible robot to quantify LLR-related activity in the brainstem where the RST originates. However, StretchfMRI cannot decouple the contributions of the RST and of the corticospinal tract (CST), both involved in LLRs. Thus, whether the RST has a causal role in generating LLRs is unknown. Our goal is to combine TMS with fMRI to establish the causal role of the RST in LLRs. Inhibitory TMS delivered to the primary motor cortex can be used to decouple the contribution of the CST and RST, but the TMS parameters that result in signficant cortical inhibition during the LLR are unknown. Therefore, we conducted a study to determine the TMS parameters that result in cortical inhibition, enabling the development of a protocol that combines TMS with StretchfMRI to quantify the causal role of the RST on LLRs. We combined surface electromyography (EMG), a wrist perturbation robot, and subthreshold TMS to determine the effect of TMS timing and intensity on the LLR amplitude (LLRa) in the forearm. We divided participants into two groups (N=12 each), each exposed to a different TMS intensity (90% and 95% of their active motor threshold). We tested five different TMS modes, including three latency conditions (motor potential evoked 0, 20, and 50 ms before perturbation onset), perturbation only, and background only mode. We performed a linear mixed model to determine the effect of TMS timing and intensity on the LLRa. We found a significant effect of TMS mode on the LLRa ($p<0.0001$). However, no significant effect was found for TMS intensity or the interaction between TMS intensity and mode. From post-hoc analysis, two levels of TMS latency were significantly different compared to the control (20 ms: (-36.1%), $p=0.0231$; 50 ms: (-56.9%), $p<0.0001$). Our group is currently replicating this experimental protocol while using fMRI to measure BOLD signal during robot-evoked LLRs in presence and absence of TMS. We have currently piloted data collection on three participants and established feasibility of this simultaneous TMS/fMRI protocol. We plan to report extensive data on this component of the study for the conference. In conclusion, we have shown the possibility of using TMS to partially inhibit the cortical contribution to LLRs of forearm muscles. Our future work using simultaneous TMS and fMRI will help elucidate the causal role of secondary motor pathways such as the RST in producing LLRs.

Disclosures: C.A. Helm: None. **S.A. Amofah:** None. **F. Sergi:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.01/KK3

Topic: F.01. Neuroethology

Support: NSF IOS 2154203

Title: Using a customizable recorder of animal kinesis (CRoAK) to measure female mating decisions in gray treefrogs

Authors: *A. ALAYOUBI[1], M. A. BEE[2], K. L. HOKE[1];
[1]Biol., Colorado State Univ., Fort Collins, CO; [2]Ecology, Evolution, and Behavior, Univ. of Minnesota, St. Paul, MN

Abstract: A fundamental question in neuroscience is how the activity of the nervous system can respond to the environment and produce behavior. However, determining the instance a behavior is initiated can be difficult and variable with visual observation alone, and may require multiple high-speed cameras or other instruments such as electromyograms, both of which can be costly and inaccessible. Recently, the use of an accelerometer and gyroscope has been developed to measure response latencies to sounds with a temporal resolution on the millisecond timescale with a customizable recorder of animal kinesis (CRoAK). Here, we tested the efficacy of using a CRoAK to quantify movement directionality and subject localization with open-source programing and data analysis. To do this, we tested gravid female Cope's gray treefrogs (Hyla chrysoscelis) by playing male calls on a multi-speaker array in an anechoic chamber and matched CRoAK readings with video. Females responded robustly to speakers playing a conspecific male call with the direction of their movements, location in the apparatus, and several kinetic characteristics. Future studies plan to pair the CRoAK with chronic, single-unit, extracellular recordings to understand how neuronal activity in the auditory midbrain relates to behavioral output.

Disclosures: A. Alayoubi: None. **M.A. Bee:** None. **K.L. Hoke:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.02/KK4

Topic: F.01. Neuroethology

Support: NIH Grant DC020279

Title: The onset of independent behavior: Continuous individual tracking of gerbil family members

Authors: M. DIEZ CASTRO[1], C. C. MITELUT[1], R. E. PETERSON[1], M. GONÇALVES[1], T. D. PEREIRA[2], *D. H. SANES[1];
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Abstract: Behavioral interactions within the nuclear family occur over a prolonged period, and may play a pivotal role in the emergence of normal social and cognitive skills. However, behavioral studies are typically limited by observation periods of minutes to hours. We addressed this limitation with continuous video recordings of gerbil families over a two-week undisturbed period, and used a machine learning framework for multi-animal pose-tracking to investigate the emergence of independent behaviors in gerbil pups. Video recordings (Blackfly S, 24 fps) of 3

separate gerbil families (2 parents, 4 pups) were obtained from hearing onset to weaning (P15-29). Families were housed in an enlarged home cage (57 x 36 cm; 12 hr light/dark cycle) in an isolated room. Using unique fur shave patterns, individual animal identification and social behavior was quantified with a deep-learning based framework (SLEAP; Pereira et al., 2022). We found that families maintained a single nest site in which they huddled for the majority of each day (P15-29: All animals in nest, 13.52 ± 0.35 hours/day; Unoccupied nest, 0.45 ± 0.10 hours/day). Furthermore, pups transitioned to an independent, exploratory state beginning at ~P20. At P15, pups spent only 0.8 ± 0.2 hours/day foraging outside the nest. However, by P24 they foraged 4.1 ± 0.2 hours/day, mirroring their parents that foraged 5.1 ± 0.2 hours/day. Pup transition to foraging was associated with independent acquisition of food and water. At P15, pups allocated a mere 0.05 ± 0.01 hours/day to the food hopper region. By P24, this increased to 0.8 ± 0.02 hours/day, and to 1.4 ± 0.1 hours/day by P29. Parental behaviors compensated for pup independence. For example, maternal feeding declined from 1.3 hours/day when the pups were P15 to 0.5 hours/day by P29. The developmental trajectory of water acquisition was consistent with feeding, but was ~3x shorter. Finally, correlated social behavior transitioned: At P15, ~90% of the adults' nest exits followed pup egress within 10s, but by P24 following behavior was equivalent for pups and parents. Taken together, continuous video recordings coupled with a deep-learning approach enabled us to quantify intricate social and environmental interactions and explore an early stage of family development, the emergence of pup independence.

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Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.03/KK5

Topic: F.01. Neuroethology

Support: R01DC020279
R01DC018802

Title: Deep sound source localization for the study of social vocal interactions in rodents

Authors: *R. E. PETERSON[1], A. TANELUS[1], A. CHOUDHRI[3], A. PRASAD[4], M. KIRCHGESSNER[2], J. MAGLAND[5], J. SOULES[5], R. C. FROEMKE[2], D. M. SCHNEIDER[1], D. H. SANES[1], A. H. WILLIAMS[1];
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Abstract: Identifying the emitter of a vocalization among a group of animals is a persistent challenge to studying naturalistic social behavior. Invasive surgical procedures - such as affixing

custom-built miniature sensors to each animal in a group - are often needed to obtain ground truth and temporally precise measurements of whether an individual animal is vocalizing. In addition to being labor intensive and species specific, these surgeries are often not tractable in very small or young animals and may alter or restrict the natural behavioral repertoire of an animal. Thus, there is considerable interest in developing non-invasive sound source localization and vocal call attribution methods that work off-the-shelf in typical laboratory settings. Here, we demonstrate that deep learning frameworks for sound source localization display favorable performance as compared to existing state-of-the-art methods, work very well in reverberant environments, and produce calibrated measures of uncertainty. Given the lack of existing data to train and evaluate such models, we acquired a diverse and publicly available benchmark dataset consisting of ground truth microphone array data from known sound sources.

Disclosures: **R.E. Peterson:** None. **A. Tanelus:** None. **A. Choudhri:** None. **A. Prasad:** None. **M. Kirchgessner:** None. **J. Magland:** None. **J. Soules:** None. **R.C. Froemke:** None. **D.M. Schneider:** None. **D.H. Sanes:** None. **A.H. Williams:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.04/KK6

Topic: F.01. Neuroethology

Support: NIH Grant DP2-DC016163
The New York Stem Cell Foundation NYSCF-R-NI40)

Title: Effects of deafening on vocal production learning in the Egyptian fruit-bat

Authors: *J. E. ELIE[1], S. E. MUROY[2], D. GENZEL[1], L. BEYER[4], D. L. SWIDERSKI[4], Y. RAPHAEL[4], M. M. YARTSEV[1,3];
[1]Bioengineering, [2]Mol. and Cell. Biol., [3]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA; [4]Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract: Humans and some species have evolved the ability to use auditory input, including auditory feedback of their own vocal production, to modify innate vocalizations, or even create new vocal signals. This ability corresponds to various forms of vocal production learning or plasticity. Deafening is a powerful tool for both identifying whether a species is capable of any form of vocal plasticity or learning as well as for deciphering which aspects of their vocal behavior is learned rather than innate. Bats are a clade of mammals that are presumed to be vocal learners, yet the necessity of auditory feedback for the development of the species typical vocal repertoire has never been tested through deafening experiments. Here, we are reporting how the lack of auditory input impacts the Egyptian fruit-bat vocalizations. Leveraging wireless audio recordings of freely interacting adults that followed a deafening treatment at birth, we show that the repertoire of Egyptian fruit-bats consists of both innate and learned vocalizations. The

learned vocalizations were not lacking in the repertoire of deaf bats, but rather did not show the typical spectro-temporal patterns found in hearing animals. Furthermore, while deafening was affecting both sexes' vocal behaviors, males showed a deficit for a group of vocalizations that was unique to them, and females for a group of vocalizations that was shared by both sexes. These results open new avenues for studies of the mammalian neural circuits that enable sexually dimorphic forms of vocal learning.

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Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.05/KK7

Topic: F.01. Neuroethology

Support: R01 NS113071

Title: Vocal production and territoriality in the singing mouse

Authors: *Y. FUJISHIMA, M. A. LONG;
Neurosci. Institute, NYU Sch. of Med., New York, NY

Abstract: Alston's singing mouse (*Scotinomys teguina*) provides an excellent opportunity to study how the brain generates and modifies complex social behavior. Their vocalization involves the production of a series of structured human-audible trills, together with their ability to "turn-take" with a conspecific in a temporally precise manner. However, the ethological relevance of this behavior is poorly understood. To address this issue, we tracked the vocal behavior of singing mice in the laboratory across different social contexts. Our first step was to replicate our initial findings of counter-singing in remotely caged pairs of male singing mice. We found that animals in this configuration robustly engaged in turn-taking, and the frequency of song occurrence was correlated within a pair. When housed individually in a large (3' x 4') terrarium equipped with 10 hiding shelters, the resident males showed distinct daily patterns of physical exploration and singing, often exposed on top of shelters. When an intruder was introduced to the terrarium, however, the mice rarely engaged in turn-taking. Instead, the resident often produced exposed advertisement calls, while the intruder occasionally sang under a protective shelter. The lack of turn-taking within the terrarium led us to hypothesize that the turn-taking behavior is exclusively used for longer distance communication. To test this hypothesis, we played back a set of recorded songs while changing the distance of the speaker from the mouse. We found that singing mice were most responsive when songs were played from moderate distances. We therefore suggest that the singing mice use turn-taking to communicate with a remote conspecific, possibly to announce one's presence and territory.

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Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.06/KK8

Topic: F.01. Neuroethology

Support: R01-DC018691

Title: Using ultrasound-capable miniature microphones to identify mouse USVs

Authors: *E. N. WAIDMANN, V. H. Y. YANG, E. D. JARVIS;
Lab. of Neurogenetics of Language, The Rockefeller Univ., New York, NY

Abstract: Vocal communication is a major component of animal social behavior. Vocalizations can be learned or innate, and can convey a variety of social signals, including territorial limits, the presence of predators, or courtship intent. Mouse ultrasonic vocalizations (USVs) are a promising model in which to study mammalian vocal production circuits. While mouse USVs are innate, mice still show complex vocal behavior, including the production of structured song composed of multiple syllable types, and the ability to modify their vocal rate and syllable repertoire based on the specific social condition. Though in courtship interactions male mice produce the majority of the emitted USVs, both male and female mice are capable of and emit these vocalizations. In order to study the underlying circuitry and behavioral motifs of vocal production in freely behaving pairs of mice, it is necessary to identify the individual responsible for each syllable, and to segment the specific vocalizations produced by each vocalizing individual. Previous methods to identify the source of an individual USV have used high-density microphone arrays and triangulation methods, which involve the use of multiple costly microphones and require implementation of more complex computational methods.Using inexpensive ultrasound-sensitive MEMS microphones, we developed custom circuit boards that can be fitted to individual mice and connect to a variety of existing USV recording systems. We found that these miniature microphones reliably detected mouse USVs, and that a high percentage of vocalizations could be attributed to a specific animal in a vocalizing pair based on the relative amplitude differences alone. This simple readout method avoids the implementation of complicated triangulation methods, and can readily segment male vocalizations with high confidence from a vocalizing pair. By pairing this method with simultaneous video recording, we also were able to study and describe the broader courtship behavioral landscape, in which USV production is one component. These results offer a promising, low-cost, and simple method that researchers can implement to study the courtship interactions between pairs of vocalizing mice.

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Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.07/KK9

Topic: F.01. Neuroethology

Support: NIH Grant 1RF1NS132046-01
Searle Scholars Program

Title: Context-dependent vocal flexibility in singing mice

Authors: *C. E. HARPOLE, X. ZHENG, M. B. DAVIS, A. BANERJEE;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Whether to laugh at a joke or to engage in a lively debate, we flexibly modify our vocalizations based upon social contexts. Such adaptive behavior requires real-time adjustments of motor outputs in response to rapidly changing sensory inputs. To understand the underlying neural mechanisms, we investigate vocal communication in a highly vocal cricetid rodent - the short-tailed singing mouse (*Scotinomys tequina*). We developed a behavioral assay in which two individuals interacted across a perforated divider with a large repertoire of vocalizations. Using custom analysis based on note shape similarity and relative loudness from signals recorded by two microphones on either side of the enclosure, we were able to assign a vast majority (~90%) of vocalizations to individual animals. This allowed us to discover that during social interactions, singing mouse vocalizations are organized into two distinct vocal modes - their namesake songs and the less-characterized calls. Songs were comprised of a series of increasingly longer notes that progress predictably over multiple seconds. In contrast, calls were much quieter, higher pitched, and display much lower supra-syllabic stereotypy than songs. The usage between these vocal modes depended upon social context: calls were only produced when within a few body lengths of a conspecific, while songs were produced over a wider range of inter-animal distances. Interestingly, individual animals could switch between these two vocal modes in a fast and flexible manner, adjusting vocal usage based on sensory cues from the social partner. Our behavioral description of calls produced by singing mice resemble the social affiliative ultrasonic vocalizations (USVs) of laboratory mice and rats. To probe neural mechanisms underlying vocal production, we optogenetically activated excitatory neurons in the caudolateral periaqueductal gray (PAG), which evoked species-typical calls. This result is consistent with reports from analogous experiments in other rodents. Based on our analyses of acoustic properties, vocal usage, production mechanism, and neural control, we posit that singing mouse calls are akin to USVs representing an ancestral behavioral feature shared across many rodents, while the songs represent a more recent evolutionary behavioral novelty. Our investigation of vocal communication across multiple species provides a framework to understand the function and evolution of neural circuits underlying vocal communication in mammals.

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Poster

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Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.08/KK10

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: The acoustic characteristics of ultrasonic vocalizations emitted by individual mice change throughout development

Authors: *C. J. COLLINS[1,2], A. Y. MA[1,2], J. D. TURK[1,3], J. P. NEUNUEBEL[1,2,3];
[1]Psychological and Brain Sci., [2]Interdisciplinary Neurosci. Grad. Program, [3]Bioinformatics Data Sci., Univ. of Delaware, Newark, DE

Abstract: Throughout an animal's life, vocal communication plays an integral role in modulating behavior (Froemke et al., 2021). For example, mice emit ultrasonic vocalizations (USVs) during distress as pups (Liu, 2009) and while engaging in courtship behaviors as adults (Neunuebel et al., 2015). While adult and pup USVs have been compared between mice (Grimsley et al., 2011), it is unclear how an individual's vocal emission and acoustic characteristics change over time. We recorded isolation vocalizations of individual male mice (n = 22) across postnatal (PN) days 2 through 63. In total, 18,599 vocalizations were recorded during 1,364 three-minute sessions. From PN days 2 through 15, an individual mouse pup emitted an average of 38 vocalizations per day. However, from PN days 16 through 35, an individual mouse emitted an average of fewer than two vocalizations per day. Subsequently, as the mice became young adults, from PN days 36 through 63, an individual mouse emitted an average of 13 vocalizations per day. To compare the acoustic features of an individual across developmental time points, we grouped the vocalizations an individual emitted between PN day 2 and 15 (pup) or PN day 36 and 63 (young adult). Across individuals, pup vocalizations had significantly lower peak frequencies (Wilcoxon rank-sum test, $p < 0.01$) and low frequencies ($p < 0.001$) than vocalizations emitted during young adulthood. The inter-vocal interval, or time between vocalizations, was significantly shorter as a pup compared to those emitted during young adulthood ($p < 0.0001$). Vocalization durations and the range in frequency, or bandwidth, were significantly longer as a pup compared to those emitted during young adulthood (duration, $p < 0.0001$; bandwidth, $p < 0.001$). Likewise, vocalizations emitted as pups had a higher intensity than those emitted as adults ($p < 0.0001$). Using acoustic features as predictors, we trained a support vector machine classifier to accurately identify (74%) whether a vocalization was emitted as a pup or an adult. We also trained support vector machine classifiers to predict the day of vocal emission. The highest accuracy (65%) occurred when predicting the last day of pup vocalizations with over 150 signals and the first day of young adulthood. When predicting pup vocal emission for pairs of consecutive days, 7 of 9 classifiers were significantly above chance accuracy (50%). Only 5 of 28 classifiers could accurately differentiate the day adult vocalizations were emitted. Our results suggest that the acoustic characteristics of vocalizations produced by individual mice change throughout development.

Disclosures: C.J. Collins: None. **A.Y. Ma:** None. **J.D. Turk:** None. **J.P. Neunuebel:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.09/KK12

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Examining vocal communication and mating preferences in mice during courtship

Authors: *D. PANDE[1,2], M. R. WARREN[1,4], R. S. CLEIN[1], J. P. NEUNUEBEL[1,2,3]; [1]Psychological and Brain Sci., [2]Interdisciplinary Neurosci. Grad. Program, [3]Bioinformatics Data Sci., Univ. of Delaware, Newark, DE; [4]Biol., Emory Univ., Atlanta, GA

Abstract: Animals modify their courtship behaviors by observing or mimicking the behaviors of others (Freeberg, 2000). Female mice prefer males that have been exposed to the odor of other females, suggesting that the presence of female scent cues influences mate choice (Kavaliers et al., 2006). During courtship, both male and female mice communicate using ultrasonic vocalizations (USVs) ranging from 35 to 110 kHz (Neunuebel et al., 2015). While olfactory cues play a role in mate choice (Ferkin, 2018), how social and vocal signals influence mate preference in naturalistic conditions is less clear. To address this question, we recorded adult mice (13-21 weeks old; B6.CAST-Cdh23Ahl+/Kjn) interacting in mixed-sex groups (n = 11; two males and two females per group, arbitrarily assigned first and second) (Sangiamo et al., 2020). Using a supervised machine learning approach (Kabra et al., 2013), we extracted courtship chases (number of chases: 92.1 ± 43.4). To measure preferences, we calculated a preference index (PI) for each male by dividing the difference between the number of times the male chased the two females by the total. The PI values ranged from -0.35 to 0.29, with positive values indicating a preference for the first female. We discovered a significant positive correlation (Pearson's $r = 0.62$, $p < 0.05$) between the preference indices of two males, suggesting that both males preferred the same female. Next, we used a sound source localization system (Warren et al., 2018) to estimate the vocalization rate (VR) of each mouse during chases in which the animal participated. The VR was calculated by dividing the number of USVs each animal emitted in the chase by the duration of the chase. We then calculated a VR index (VRI) for each animal by dividing the difference between the mean VR by the total. For males, the VRI compared their mean VR while chasing the two females and ranged from -0.18 to 0.43, with positive VRI indicating that the male had a higher mean VR when chasing the first female. For females, the VRI values ranged from -0.91 to 0.93 with positive values indicating a higher mean VR when chased by the first male. We found a significant positive correlation between the VRI of females (Pearson's $r = 0.91$, $p < 0.0001$) but not males (Pearson's $r = -0.41$, $p = 0.2$), indicating that females tended to exhibit a higher mean VR when being chased by the same male. We also observed a significant positive correlation (Pearson's $r = 0.97$, $p < 0.0001$) between the VRI of a male and a female engaged in a chase, suggesting a consistent pattern in male and female VR

during chases. Our results suggest that males show similar preferences during chases, and a relationship may exist between vocalizations and preferences.

Disclosures: **D. Pande:** None. **M.R. Warren:** None. **R.S. Clein:** None. **J.P. Neunuebel:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.10/KK13

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Sex and Behavioral Differences in Bioacoustic Directionality of Mouse Ultrasonic Vocalizations

Authors: *A. Y. MA[1,2], C. D. ESCOBAR-AMADO[3], M. R. WARREN[4,6], J. P. NEUNUEBEL[4,5];
[1]Psychological and Brain Sci., [2]Interdisciplinary Neurosci. Grad. Program, [3]Electrical and Computer Engin., [4]Univ. of Delaware, [5]Bioinformatics Data Sci., Univ. of Delaware, Newark, DE; [6]Biol., Emory Univ., Atlanta, GA

Abstract: In vocally communicating animals, bioacoustic directionality defines the focus of the signal and could reflect functional relevance (Yorzinski and Patricelli, 2010). Across the animal kingdom, bioacoustic characteristics differ between males and females (Miller et al, 2004). In mice, vocalization characteristics differ between sex (Warren 2018), but whether sex differences in directionality exists is unclear. To explore potential sex differences and their relationship to behavior, we used a microphone array system to compare male and female ultrasonic vocalizations (USVs). The system included a camera for tracking behavior and eight microphones for localizing and assigning USVs to individual mice recorded in two (male-female dyads) or four (two males and two females) mouse social contexts. We portrayed the overall sound radiation pattern of USVs emitted by each mouse (8 males and 7 females in the two-mouse context; 22 males and 22 females in the four-mouse context) using sound level data at microphones and the microphone locations relative to the vocalizer. Directionality of USV was evaluated with a directional index (DI) bounded between -1 and 1, representing a highly focused beam posterior and anterior to the vocalizer. In the two-mouse context, male and female DIs had medians of 0.020 (IQR = 0.012) and 0.0027 (IQR = 0.0089). In the four-mouse context, the median DIs were 0.017 (IQR = 0.0088) and 0.0060 (IQR = 0.014) for the males and females. In both contexts, male USVs had higher DIs than female USVs (Wilcoxon rank-sum test, all $p < 0.05$). Chasing and investigating were then annotated using a supervised machine-learning approach (Kabra et al., 2013). We next examined the directionality of mouse USVs emitted as mice in distinct behavioral roles (i.e., chasing vs. chased and investigating vs. investigated) by comparing the DI of USVs emitted when the mouse was in a behavioral role with 1000 DIs

calculated from shuffled data (i.e., the locations of sound level values are randomized). We observed that when a male was chasing a female, USVs were more directional than chance (permutation test, $p < 0.001$), while USVs of the chased female were less directional ($p < 0.05$). When a male was chasing another male, the USVs of the chasing male were more directional ($p < 0.001$), while USVs of the chased male did not differ from chance ($p = 0.18$). We also obtained comparable results during investigations (male investigating a male or a female, both $p < 0.001$; investigated female, $p = 0.17$; investigated male, $p = 0.061$). Our results demonstrate that male mice produced more directional USVs than females, and behavioral roles might explain the differences.

Disclosures: A.Y. Ma: None. **C.D. Escobar-Amado:** None. **M.R. Warren:** None. **J.P. Neunuebel:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.11/KK14

Topic: F.01. Neuroethology

Support: Cornell Center for Social Sciences Research Grant

Title: Developmental time course and neural mechanisms of mouse social USVs

Authors: *N. PRANIC[1], K. A. TSCHIDA[2];
[2]Cornell Univ., [1]Cornell Univ., Ithaca, NY

Abstract: Innate vocalizations of many altricial mammals exhibit a pronounced developmental transition during early adolescence. As young animals gain the ability to survive without parental care, they stop producing vocalizations that elicit parental care and begin producing vocalizations during interactions with social partners and social cues (social vocalizations). Although this transition in vocal behavior is widespread across the animal kingdom, the neural mechanisms underlying the emergence of social vocalizations remain poorly described. In the current study, we first performed longitudinal recordings from age-matched and sex-matched pairs of laboratory mice (*Mus musculus*) to pinpoint the age range over which young mice stop producing isolation ultrasonic vocalizations (isolation USVs; which elicit parental care) and begin producing social USVs that are coupled to social investigation of conspecifics. We find that mice began producing social USVs earlier than previously described, approximately 1-2 days following weaning (postnatal days (P) 22-23). Given the role of the preoptic hypothalamus in regulating social USV production in adult mice, we next used Fos mapping to explore the role of the POA in regulating the production of social USVs in adolescent mice, as well as isolation USVs in mouse pups. We find that a subset of POA neurons upregulate Fos in adolescent mice (P25-P30) the produced social USVs, and we are currently exploring whether the numbers of Fos-positive POA neurons are well related to social USV rates in both adolescent females and

males, as well as whether these neurons express molecular markers that characterize USV-related POA neurons in adult mice (i.e., VGAT and Esr1). In P10 pups, we find that POA Fos expression is strongly correlated with rates of isolation USVs. Notably, these Fos-positive POA neurons appear to be localized more medially than the POA neurons that regulate adult social USV production, suggesting that different subsets of POA neurons may regulate the production of isolation USVs and social USVs. These results will inform future experiments in which we will manipulate the activity of molecularly-defined subsets of POA neurons at different times during development, to test the hypothesis that a developmental switch in the POA neurons that regulate vocal production contributes to the developmental emergence of social vocalizations in mice.

Disclosures: N. pranic: None. **K.A. Tschida:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.12/KK15

Topic: F.01. Neuroethology

Support: NIH Grant 1RF1NS132046-01
Searle Scholars Program

Title: The role of the midbrain periaqueductal gray in controlling vocal flexibility in singing mice

Authors: *X. ZHENG, C. E. HARPOLE, M. B. DAVIS, A. BANERJEE;
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Abstract: Vocal production is an important component of animals' behavioral repertoire. Many mammalian species can produce multiple types of vocalizations in different environmental and social contexts. The flexible selection and control of vocal production requires the coordination of multiple hierarchically organized neural circuits spanning the brain. However, circuit-level understanding of how these neural pathways mediate context-specific vocal behaviors remains less understood. To address this issue, we are investigating neural circuits for vocal flexibility in a rodent species from the cloud forests of Central America - the singing mice (*S. teguina*). Using a novel behavioral assay, wherein two singing mice interact across a divided cage, we first showed that singing mice possess a large vocal repertoire. We found that vocalizations are organized into two distinct vocal modes - calls and songs. Songs are comprised of a series of progressively longer notes that evolve predictably over 6-10 seconds. In contrast, calls are much shorter (~100 ms), quieter, and display much lower supra-syllabic stereotypy. Individual animals can switch between these two vocal modes in a fast and flexible manner. To understand the neural circuits underlying this behavior, we focused on the midbrain periaqueductal gray (PAG) based on prior studies in multiple mammalian species. We tested whether the PAG is necessary

to produce both vocal modes by bilateral expression of tetanus toxin light chain (TeLC). In all animals tested, we found that synaptic blocking of PAG_{CAMKII} neurons resulted in the mice becoming progressively mute over a few days, unable to produce neither songs nor calls. We then used optogenetics to test whether activation of the PAG is sufficient to elicit either vocal mode. Unilateral optogenetic activation of ChR2-expressing PAG_{CAMKII} neurons was sufficient to elicit calls throughout the stimulation period (1 - 4 s). Moreover, brief (0.1 s) activation of the same population during song caused a pause in the song progression, consistent with inhibitory interactions between the two vocal modes. In summary, our behavioral paradigm allowed us to decipher the flexible production of two vocal modes in the singing mice. Through perturbation studies, we found the PAG plays an important role in the production of both vocal modes. In ongoing experiments, we plan to determine whether the two modes are controlled by shared or distinct neurons. More broadly, our behavioral and neural experiments highlight that the singing mice model system is ideally suited to provide insights into the brain-wide mechanisms of context-specific vocal communication in mammals.

Disclosures: **X. Zheng:** None. **C.E. Harpole:** None. **M.B. Davis:** None. **A. Banerjee:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.13/KK16

Topic: F.01. Neuroethology

Title: Female mice do not produce USVs during courtship interactions with muted males

Authors: *C. A. MALONE, J. W. SOKOL, K. TSCHIDA;
Cornell Univ., Ithaca, NY

Abstract: Vocalizations produced by males and females during courtship interactions may influence mate choice and reproductive success. Older studies in mice (*Mus musculus*) attributed the production of courtship ultrasonic vocalizations (USVs) entirely to males, but more recent studies using microphone arrays to localize and assign USVs to individual mice support the idea females also produce USVs during courtship interactions (~15% of total USVs). However, such localization methods can be computationally intensive, and the contribution of female USV production to courtship interactions remains understudied. As an alternative approach to study female courtship USVs, we used a combination of viral tools and activity-dependent labeling to ablate male midbrain neurons essential for USV production, generating male mice that are 'muted' for USV production but still court females at normal levels. By measuring the vocal behavior of females as they interact with these muted males, we tested the hypothesis that female courtship USVs act as a signal for sexual receptivity. Female mice were recorded during interactions with muted males, and vaginal cytology was used to measure female estrous state in each trial. We predicted that sexually receptive females would produce higher rates of USVs during interactions with males than non-receptive females. Surprisingly, our data indicate that

females produce few or no USVs during interactions with muted males, regardless of estrous state. In ongoing work, we are conducting similar recordings of muted males and intact females in combination with playback of male USVs, to test the idea that female USV production is contingent on male USV production.

Disclosures: C.A. Malone: None. **J.W. Sokol:** None. **K. Tschida:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.14/KK17

Topic: F.01. Neuroethology

Support: Whitehall Foundation Grant

Title: Mapping the effects of midbrain activation on vocal production in mice

Authors: *D. ZHENG, K. TSCHIDA;
Psychology, Cornell Univ., Ithaca, NY

Abstract: The midbrain periaqueductal grey (PAG) is a functionally heterogeneous brain region important to a variety of survival-related behaviors, including reproductive behaviors, defensive behaviors, and autonomic function. Recent work in mice characterized a specialized population of neurons in the caudolateral PAG that gate the production of ultrasonic vocalizations (USVs) that mice produce during social interactions (i.e., PAG-USV neurons). Whether other subregions of the PAG regulate USV production or the production of other acoustic categories of vocalization remains unknown. To address this question, we tested the effects on vocal and non-vocal behavior of artificial activation of lateral PAG neurons throughout the rostral-caudal extent of the PAG. The PAG of male and female mice was injected unilaterally with an AAV driving expression of Channelrhodopsin and then implanted with a fiber optic. Two weeks later, we measured the effects of optogenetic activation on the behavior of subject mice, tested alone as well as during social interactions. Our findings will further our understanding of how the midbrain is organized to regulate the context-dependent control of vocalization.

Disclosures: D. Zheng: None. **K. Tschida:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.15/KK18

Topic: F.01. Neuroethology

Title: Midbrain circuits for context-dependent control of vocalization in mice

Authors: *P. ZIOBRO, E. WOO, K. TSCHIDA;
Cornell Univ., Ithaca, NY

Abstract: To communicate effectively, animals produce different acoustic categories of vocalizations in different behavioral contexts that in turn, serve different communicative functions. Despite the importance of vocalization to social behavior, the neural circuits that allow animals to produce different acoustic categories of vocalizations remain unknown. We addressed this question in mice, which produce ultrasonic vocalizations (USVs) during social interactions and lower-frequency broadband vocalizations (i.e., squeaks) in painful or aversive contexts. The midbrain periaqueductal gray (PAG) plays an obligatory role in vocal production, and a recent study identified a specialized population of PAG neurons in the mouse whose activity is both necessary and sufficient for the production of USVs (PAG-USV neurons). In the current study, we ask whether PAG-USV neurons are uniquely important for USV production or alternatively, contribute to the production of multiple acoustic categories of vocalizations. We employed an activity-dependent labeling strategy (TRAP2) to ablate PAG-USV neurons in both female and male mice, and we then tested the effects of this manipulation on USV production during social interactions and on the production of squeaks in response to mild footshocks. Consistent with prior work, ablation of PAG-USV neurons in both females and males abolishes USV production without affecting non-vocal social behaviors. In contrast, the production of squeaks is not affected by the ablation of PAG-USV neurons. These findings support the idea that distinct populations of PAG neurons control the production of different acoustic categories of vocalizations and set the stage for identifying additional vocalization-related PAG neurons, as well as exploring how these different PAG populations act on hindbrain premotor circuits to control the acoustic features of vocalizations.

Disclosures: P. Ziobro: None. **E. Woo:** None. **K. Tschida:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.16/KK19

Topic: F.01. Neuroethology

Support: NIH Grant DC020279

Title: Adolescent maturation of vocalization processing in auditory cortex of freely moving gerbils

Authors: *E. IN 'T ZANDT, D. SANES;
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Abstract: Throughout development, we are exposed to a range of natural sounds, including vocalizations, that gain meaning through experience. In fact, neural remodeling remains sensitive to the environment throughout adolescence, a time during which many behavioral skills remain immature. An abundance of behavioral work shows that the natural acoustic environment, including speech sounds, has a long-term impact on perceptual skills later in life. However, at the neural level, developmental remodeling of auditory cortex (AC) has largely considered how early juvenile exposure to non-natural stimuli (i.e. tones, white noise) can permanently modify sound coding properties. It thus remains unknown whether vocalization encoding continues to mature through adolescence following early experience with the full vocalization repertoire. Here, we investigate the development of AC responses to vocalization sequences in awake, freely-moving adolescent and sexually mature Mongolian gerbils (*Meriones unguiculatus*). Gerbils are a highly social rodent species with a rich vocal repertoire and a prolonged adolescent period. We used chronically-implanted high-density silicon probes to wirelessly record single neuron responses in the same animals across weeks of adolescence or adulthood. Initial analyses have focused on AC neuron firing rate, trial-to-trial variance, and dynamic range of response to vocalization sequences. Cross-sectional comparisons of adult and adolescent responses suggest that the percent of the AC single neurons that are modulated by vocalizations increases through late adolescence. Similarly, our preliminary analyses suggest that the AC neuron dynamic range also increases during this interval. These results suggest that AC responses to natural calls do continue to mature long after the canonical early critical period has closed. Our current analyses are focusing on whether specific vocalizations demonstrate unique developmental trajectories, and how neural responses to specific syllables in the vocalization sequence develop. The results of these experiments will provide insight into how responses to natural, behaviorally-relevant sounds mature in the cortex.

Disclosures: E. in 't Zandt: None. **D. Sanes:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.17/KK20

Topic: F.01. Neuroethology

Support: NIH F32 DC018721-01A1
NIH R01 DC013826-07

Title: Evidence of a vocal motor corollary discharge signal in the mouse auditory cortex

Authors: *T. HARMON[1], S. MADLON-KAY[2], J. M. PEARSON[3], R. D. MOONEY[1]; [2]Neurobio., [3]Biostatistics and Bioinformatics, [1]Duke Univ., Durham, NC

Abstract: An influential idea is that corollary discharge from vocal motor signals suppresses auditory cortical responses to auditory feedback, ultimately helping the brain to distinguish self-

generated vocal sounds from other sounds. Support for this idea stems mostly from studies in humans and monkeys, and whether it is a general principle of mammalian auditory cortical function remains unclear. Male mice vocalize extensively during courtship, but mouse courtship is a complex behavior that also involves both vocal and non-vocal movements, odor cues from the female, and heightened arousal, any or all of which could potentially modulate auditory cortical activity. We developed a protocol for studying courtship interactions between female and head-fixed male mice to systematically search for evidence of vocal motor modulation in the mouse auditory cortex. We found that the male's ultrasonic vocalizations (USVs), as well as its arousal and locomotion, increased when he interacted with a female. We used two photon calcium imaging in the head-fixed male to monitor the activity of auditory cortical neurons during vocalizations and playback of the same vocal bouts. We found neurons that responded strongly to playback stimuli but only weakly responded during vocalization, consistent with a suppressive corollary discharge mechanism. Comparing vocal and non-vocal courtship interactions allowed us to control for effects of arousal, odor, and locomotion, revealing a specific influence of vocalization on a subset of auditory cortical neurons. The activity of many of these vocalization-modulated neurons positively scaled to the amplitude of the vocal bout, consistent with the influence of vocal feedback, and many responded prior to vocal onset, pointing to a motor influence. To further isolate and characterize the influence of a vocal motor signal from vocal feedback, we imaged from the auditory cortex of congenitally deaf (TMC1-/-) male mice. Notably, vocal modulation was inverted in deafness, with the majority of neurons showing negative modulation which scaled to the amplitude of the vocal bout. Taken together, these results show that the auditory cortex of the hearing mouse integrates vocal motor signals and vocal feedback to suppress responses to self-generated vocalizations. Therefore, vocal corollary discharge mechanisms are likely to be a general feature of the mammalian auditory cortex.

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Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.18/LL1

Topic: F.01. Neuroethology

Support: NIH Fellowship F32MH125562
NIH Grant R01NS113071

Title: Integrating social and energetic cues that promote advertisement vocalization

Authors: *J. TRIPP[1], K. RAGHURAMAN[2], R. V. BHALLA[2], S. M. PHELPS[2];
[2]Integrative Biol., [1]Univ. of Texas at Austin, Austin, TX

Abstract: The motivation to engage in social behaviors is influenced by environmental factors, such as time of day, season, or social context, as well as by internal factors, such as an individual's reproductive, hormonal, or energetic state. Understanding where and how internal and external cues that regulate social behaviors are integrated is a key step in understanding the neural control of social motivation. Conspicuous display vocalizations are excellent behaviors to study in this context because vocal behavior is natural, social, and influenced by both external context and internal state. We investigated how the brain integrates the exteroceptive and interoceptive cues that influence social communication using Alston's singing mice (*Scotinomys teguina*), small rodents native to Central America that are named for their distinctive trill vocalizations. Prior research has established that singing mouse song effort (e.g., singing rate, song length) is modulated by both social and hormonal factors. Specifically, singing mice increase song effort in response to playback of conspecific songs while circulating levels of leptin, a hormonal cue of high energetic stores, are strongly associated with high song effort. In this study, we sought to identify where in the brain cues of social context and perceived energetic state that regulate song effort are integrated. First, we manipulated social context using playbacks of conspecific songs and manipulated perceived energetic state using injection of leptin. We found that male singing mice sang significantly more when played conspecific songs compared to control tones, and that this effect was enhanced by leptin injection compared to vehicle. Next, we used the immediate early gene c-Fos to identify regions in the brain with increased activity in response to leptin injection and song playback. For these analyses we focused on regions known to play important roles in social behavior or energy balance and that are a part of the singing mouse vocal circuit, including the medial preoptic area, arcuate nucleus of the hypothalamus, paraventricular hypothalamus, and midbrain periaqueductal gray. Our results provide a better understanding of how the brain integrates diverse cues that influence social communication behavior.

Disclosures: **J. Tripp:** None. **K. Raghuraman:** None. **R.V. Bhalla:** None. **S.M. Phelps:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.19/LL2

Topic: F.01. Neuroethology

Support: MH117778
NS107466

Title: Brainstem control of larynx and vocal-respiratory coordination

Authors: *J. PARK[1,2], S. CHOI[1], J. TAKATOH[1], S. ZHAO[3], A. HARRAHILL[1], B.-X. HAN[3], F. WANG[1];
[1]MIT, Cambridge, MA; [2]Biomed. Engin., [3]Neurobio., Duke Univ., Durham, NC

Abstract: Vocalization and respiration are closely related behaviors, and their neural circuits are also heavily intermingled in the brainstem. Therefore, it was difficult to dissect precise neural mechanisms of vocal production and vocal-respiratory coordination. The Retroambiguus Nucleus (RAm) in the brainstem regulates vocal pattern generations and their coordination with breathing, but the details are still unclear. Here, we identified a vocalization-specific laryngeal premotor population in the RAm using an activity-dependent labeling approach in adult mice. Strong Fos activity was found in neurons in the RAm after vocalization (RAm^{VOC} here after), and therefore we tagged those neurons with a Fos-based tagging technique. Monosynaptic tracing of laryngeal motoneurons and molecular identification of the RAm^{VOC} neurons confirmed that RAm^{VOC} neurons are excitatory laryngeal premotor neurons. Inhibition of the RAm^{VOC} neurons by expressing tetanus light chains abolished vocalization in mice, including ultrasonic vocalizations (USVs) and audible stress-response squeaks. Optogenetic stimulation of the RAm^{VOC} neurons induced vocal cord closure and sufficiently evoked USVs without any behavioral contexts. Interestingly, the opto-induced USVs were coordinated with on-going respirations: 1) the duration of USV syllables and post-inspiratory phases were highly correlated, and 2) the opto-induced post-inspiratory phases and vocal cord closures were overridden by inspiration needs during prolonged opto-stimulation. RAm^{VOC}-neurons receive inhibitory inputs from the preBötzinger complex. Ablating inhibitory synapses in RAm^{VOC}-neurons compromised this inspiration overriding of laryngeal adduction, resulting in de-coupling of vocalization and respiration. Our study revealed the hitherto unknown circuits for vocal pattern generation and vocal-respiratory coupling.

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Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.20/LL3

Topic: F.01. Neuroethology

Support: NSF IOS 1934386

Title: Large-scale neural activity during calling and breathing in frogs

Authors: A. N. MALOY[1], N. BUSH[2], J. M. RAMIREZ[3], ***A. YAMAGUCHI**[4];
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Abstract: Understanding the neural basis of behavior is a fundamental goal in neuroscience. An isolated brain preparations of African clawed frogs which produce fictive vocalization and breathing *in vitro* provides a powerful model which allows us to study the dynamic neural activity to address this question. Historically, the identification of the neurons mediating

behavior involved obtaining electrophysiological recordings from one or a few neurons in behaving animals or fictive preparations. Neurons displaying activity corresponding to or causing the behavioral output were considered to code for behavior, while neurons with unrelated activity were disregarded. However, recent studies employing large-scale neural recordings have revealed the presence of neural mechanisms that code for behavior that exists only at the level of neuronal populations. These studies emphasize the importance of understanding these two types of neuronal coding: one at the single or few-cell and the other at the neuronal population levels. Here we performed large-scale electrophysiological recordings using Neuropixels from isolated brains of frogs during fictive calling and breathing. Simultaneous recordings were obtained from the brainstem, enabling the identification of dozens to hundreds of units. At the single-neuron level, we identified distinct but overlapping populations of single units during breathing and calling. These units exhibited spiking activity that coincides with different vocal and breathing phases and were sequentially recruited throughout the evolution of the call and the inhalation. We further characterized the spatial location of the recorded units with respect to each other through 3D histological reconstruction to identify their anatomical extent. To analyze the population-level coding, we applied dimensionality reduction (PCA) to the spiking data and projected these neural activities into a low-dimensional neural manifold. We found a distinct evolution through the PCA space, where fast and slow trills, two distinct vocal phases of a male call, occupied separable regions within the neural manifold. Moreover, as the fast trill progresses over time, the activity of the neural population sequentially evolved through a plane in the low-dimensional space, while the slow trill remained restricted to a single vector orthogonal to the fast trill. These results indicate that the population activity motifs and the time-evolution underlying these two vocal phases are distinct. These results indicate that calling and breathing behavior are represented both at the single-cell and neural population levels within the frog brainstem.

Disclosures: **A.N. Maloy:** None. **N. Bush:** None. **J.M. Ramirez:** None. **A. Yamaguchi:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR554.21/LL4

Topic: F.01. Neuroethology

Support: NIH grant: 1RF1NS132046-01
Searle Scholars Program
NSF GRFP

Title: Using comparative connectomics to understand the evolution of neural circuits underlying vocalizations in rodents

Authors: *E. C. ISKO, M. B. DAVIS, H. ZHAN, A. M. ZADOR, A. BANERJEE;
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Abstract: A fundamental goal of neuroscience is to understand how neural circuits evolve to enable novel behavioral phenotypes. Since behaviors do not fossilize, our strategy is to identify neural circuit modifications - both structural and functional - among closely-related species with large behavioral divergences. While most rodents, including lab mice (*M. musculus*), produce short, ultrasonic vocalizations (USVs), the singing mice (*S. teguina*) produce not only USVs but also human-audible, stereotyped songs that are many seconds long. We hypothesize that singing, a behavioral novelty in this species, arises from modifications in ancestral vocal circuits. Here, using comparative connectomics, we probe the brain-wide projection pattern differences of two cortical brain areas implicated in mammalian vocalizations: the anterior cingulate area (ACA) and the orofacial motor cortex (OMC). To test whether there are qualitative (e.g., absent or novel) differences in projection patterns, we used viral tracing of neurons from OMC and ACA. We identified bulk projection targets using serial two-photon tomography (STPT). We found that OMC and ACA neurons in both species project to identical downstream brain areas including the contralateral cortex, striatum, thalamus, superior colliculus, PAG, and others (N=2-3 mice for OMC and ACA). Having found no bulk differences between species, we next used MAPseq, a high-throughput barcoding technique, to characterize projection patterns at single-cell resolution. Compared to lab mice, we found that a larger proportion of OMC neurons in the singing mice project to the midbrain periaqueductal grey (PAG) and a temporal cortical area (singing mouse: n= 5114 neurons, 7 animals; lab mouse: n=71704 neurons, 5 animals). No other target region (total of 11) showed significant species-specific differences. This increased projection strength from OMC was driven by neurons with direct projections to temporal cortex showing no/few collaterals. Crucially, identical analyses of the ACA neurons did not show any significant differences in projections strengths between species. In summary, we found evidence for expansion of existing vocal motor circuits in the singing mice compared to lab mice, which may explain their species-typical vocal behaviors. Ongoing experiments will determine the functional roles of these projections using neural circuit perturbations such as chemogenetics and optogenetics. Our study highlights the utility of the singing mouse model system for studying the evolution of neural circuit mechanisms underlying vocal behaviors in mammals.

Disclosures: E.C. Isko: None. **M.B. Davis:** None. **H. Zhan:** None. **A.M. Zador:** None. **A. Banerjee:** None.

Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.22/LL5

Topic: F.01. Neuroethology

Support: Marie Sklodowska-Curie Action 101018877
NIH R01 DC012087

Title: Anterior cingulate cortex (ACC) encodes conversations in marmoset monkeys

Authors: *A. LEFEVRE[1,2], V. PAL SINGH[1], J. LI[1], J.-R. DUHAMEL[2], C. T. MILLER[1];
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Abstract: Acoustic communication is of critical importance for primates. It is involved in every type of social interactions and individuals thus need to adapt their vocalizations to different social contexts. However, research has mostly focused on vocalizations production and perception separately, uncovering distinct networks of brain regions for these functions. Here we investigated the neural basis of conversation, i.e., production and perception of vocalizations consistently emitted by two interacting subjects. Using microwire brush arrays we wirelessly recorded neurons from the anterior cingulate cortex (ACC), a region involved in vocalization production but also receiving inputs from auditory areas, in marmoset monkeys as animals engaged in their natural vocal behaviors inside the colony room. We simultaneously recorded the calls (8 distinct types) from a pair of bonded individuals using wearable microphones and videotaped them. We recorded isolated single units from dorsal ACC (area 24) and quantified the relationship between different properties of neural activity and marmoset vocal behavior, including during conversational exchanges. Analyses revealed that 60 percent of ACC neurons displayed activity related to vocalization production and/or perception. Most neurons (35%) displayed a significant increase in firing rate up to 3 seconds before call production. More critically, a subset of the population (15%) exhibited responses in response to hearing calls and even anticipatory activity before call perception, suggesting that ACC neurons may predict the occurrence of vocalizations from the partner during conversations. Interestingly, activity was modulated by the type of call, irrespective of the sound intensity. Finally, we found that some ACC neurons responded to both vocal production and vocal perception. These results indicate that in primates, ACC is a key region for natural and dynamic acoustic communication, which could adapt vocalization production depending on the social context and the type of calls emitted by a partner.

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Poster

PSTR554. Vocal/Social Communication—Non-Avian

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Program #/Poster #: PSTR554.23/LL6

Topic: F.01. Neuroethology

Support: NIH Grant R01 DC012087

Title: Allocentric representations of auditory space in marmoset hippocampus

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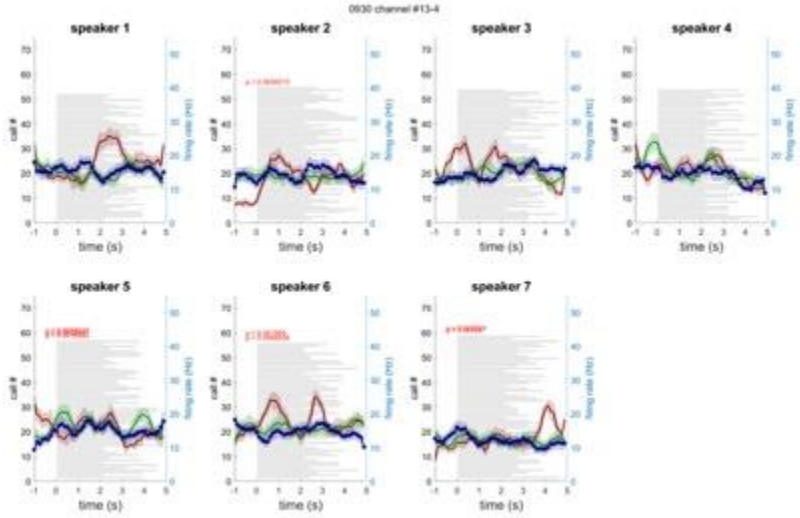
Abstract: The capacity to form internal representations of space is fundamental to all animals. While a large body of literature shows that neurons in the hippocampus of mammalian species encode an individual's self-position while locomoting through space (i.e. place cells), species that rely on distal active sensing are also able to encode space without the need to physically enter a scene. Primates, for example, use both vision and audition to encode spatial scenes. But while previous experiments have shown primate hippocampal neurons encode visual space (e.g. spatial view cells), no previous studies have shown whether similar mechanisms exist to encode auditory space in this substrate. Here we sought to address this question by recording single-neuron activity in the hippocampus of freely-moving marmosets (*Callithrix jacchus*) while subjects participated in a multi-speaker, naturalistic communication network paradigm. Preliminary analyses from two monkeys revealed a subpopulation of neurons in marmoset hippocampus (31%) was spatially selective; that is, they exhibited significantly higher call-evoked activity for calls coming from a single speaker location or adjacent speaker locations. Analyses indicated that the subject's relative location and head orientation at the time a stimulus was broadcast had no effect on neural activity, indicating that the selectivity of auditory space neurons was allocentric. We next found that 39% of these neurons were selective for the interaction of an individual caller and their spatial location. Further analyses showed that both single-unit and population-level linear models can accurately decode the speaker location of call events suggesting a broader ensemble code for representing allocentric space may be present in primate hippocampus. These findings are the first evidence that neurons in primate hippocampus encode allocentric locations in space and are selective for an individual caller's position in an acoustic scene and have significant implications for conceptions of the neural mechanisms underlying the cocktail party problem.



Disclosures: **B. Prendergast:** None. **J. Li:** None. **C. Miller:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR555.01/LL7

Topic: F.03. Stress and the Brain

Support: NIH Grant DK132566
iThrive Grant KL2TR003016/ULTR003015

Title: Lateral hypothalamic proenkephalin neurons drive threat induced overeating associated with a negative emotional state

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Abstract: Psychological stressors, like the nearby presence of a predator, can be strong enough to induce physiological/hormonal alterations, leading to appetite changes. The increased propensity toward eating palatable foods particularly appears after threatening events, yet little is known about how threats can alter feeding-related hypothalamic circuits. Here, we found that proenkephalin (Penk)-expressing lateral hypothalamic (LHPenk) neurons of mice exposed to predator scent stimulus (PSS) show sensitized responses to high-fat diet (HFD) eating, whereas silencing of the same neurons normalizes PSS-induced HFD overconsumption associated with a negative emotional state. PSS triggers an increase in serum corticosterone, which enhances the reactivity of glucocorticoid receptor (GR)-containing LHPenk neurons to HFD. Pharmacological inhibition of GR in the LH normalizes the heightened reactivity of LHPenk neurons to HFD and suppresses emotional overeating after PSS. We have thus identified the LHPenk neurons as a critical component in the threat-induced neuronal adaptations that lead to emotional overconsumption.

Disclosures: Y. Bae: None. **I. You:** None. **S. Shin:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR555.02/LL8

Topic: F.03. Stress and the Brain

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KAKENHI 19H04907, 19H05212, 21H02580 by Japan Society for Promotion of Science
Hirose research grant
Takeda research grant
Yamada research grant

Title: Behavioral characterization of mice with forebrain deletion of m^{6}A reader YTHDF3 reared in enriched environment

Authors: *J. CONTRERAS[1], S. HOU[2,3], M. SUKEGAWA[2,3], F. ZHANG[4,5], X. LI[4,5,6], T. YOSHIHARA[2], T. ENDO[7], A. J. HANNAN[8], D. WANG[1,2,3];
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Abstract: This study investigates the impact of 7 weeks of enriched environment housing (EE) and the forebrain deletion of YTHDF3 protein on the behavior of adult male C57BL/6 mice. YTHDF3 is a reader protein that specifically recognizes and binds to N^6-methyl-adenosine (m^{6}A), the most abundant modification on messenger RNAs in the mammalian brain. YTHDF3 protein expression was found to be localized to dendrites and axons of neurons, suggestive of its potential role in regulating synaptically localized m^{6}A-mRNAs. Its knockdown in dissociated cultures of rodent hippocampal neurons induced abnormal spine phenotypes. However, the physiological and behavioral consequences of its knockout in living animals remain poorly understood. Hence, this study aimed to characterize the physiology and behavior of mice lacking YTHDF3 in mature excitatory neurons in the cortex and hippocampus, brain regions that are found to be impacted by EE. Our results elucidated the putative role of YTHDF3 in mediating gene-environment interactions manifested in physiological changes and stress-coping responses in mice. Specifically, YTHDF3 cKO mice had apparently stronger grip strength and less time immobile during forced swimming in EE mice, and decreased social interaction in mice from standard housing (ST). YTHDF3-cKO also interacted with EE to drive a more passive coping response in the tail suspension test (TST), evident in their increased time immobile. This behavior was not observed in cKO mice reared in ST, suggesting that EE was required for the manifestation of the genotype effect. In contrast, EE-induced changes in body weight, rectal temperature, acoustic startle response, motor functions, exploration and anxiety-like behaviors were not affected by the absence of YTHDF3. These results demonstrate that the absence of YTHDF3 in mature excitatory neurons in the forebrain impacts mice behaviors in a context-dependent manner and is affected by the housing environment, providing a novel molecular link between epitranscriptomic regulation and housing effects on the behaviors of adult mice.

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Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.03/LL9

Topic: F.03. Stress and the Brain

Title: The effects of social stimuli and deprivation on dopaminergic pathways and neuronal activity in the monodelphis domestic

Authors: *C. M. BOTELLO[1], E. I. ALANIZ[1], J. L. ESCOBEDO[2], J. L. VANDEBERG[3], M. GIL[4];
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Abstract: Elucidating the neural pathways that regulate behaviors in animal models is important for understanding the possible neural interactions that underlie complex behaviors in the human condition. The Monodelphis domestica is an animal model that has lacked the same kind of research as other types of animal models. This study serves as an exploratory effort into the interactions between social factors and neural networks using the M. domestica as a model for both social deprivation and social stimuli. In a preliminary experiment, immunohistochemistry techniques were utilized to characterize the expression of Tyrosine Hydroxylase (TH), a marker for dopamine neurons in both the ventral tegmental area (VTA) and the nucleus accumbens (NAc) of the M. domestica using a social deprivation paradigm. Socially isolated opossums (3 female and 2 males) were separated from other animals whereas animals in the social group (2 females and 1 male) were housed with two or three same-sex partners. It was found that social isolation led to an increase in the detection of TH-expressing neurons in the VTA. The social isolation group averaged 11.67 TH-expressing neurons per 2500 µm2 whereas the social group averaged 7.4 TH-expressing neurons per 2500 µm2. Given that social deprivation was studied, the next step was to see the effect that social stimulus presentation would have on the same neural pathway. In another experiment, animals in the experimental group were exposed to stimulus animal of the same sex for 10 minutes. The animals in the experimental group were then sacrificed and perfused 30 minutes after the presentation of the social stimulus. Animals in the control group were not exposed to another animal prior to sacrifice and perfusion. Tissue from the M. domestica were processed and immunohistochemistry was used to characterize the difference in expression of c-Fos in the VTA and NAc of the M. domestica. C-fos is an immediate early gene whose expression is used to characterize neuronal activity. We are currently conducting image analysis and quantifying cells and fibers in the target areas of both isolated and group-housed animals. An independent samples t-test will be used to compare the number of c-Fos-expressing neurons between the socially stimulated and the control groups. This study could serve as the first to look at the effect of social deprivation and social stimulus presentation on the key reward pathway in the brain of M. domestica.

Disclosures: C.M. Botello: None. **E.I. Alaniz:** None. **J.L. Escobedo:** None. **J.L. VandeBerg:** None. **M. Gil:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR555.04/LL10

Topic: F.03. Stress and the Brain

Support: NIH Grant AA023288
NIH Grant AA010761
Charleston Alcohol Research Center

Title: The Dorsal Peduncular Cortex as an understudied stress target in the prefrontal cortex

Authors: *K. O'HARA[1], K. L. LINDQUIST[2], S. BERTO[3], J. RINKER[3], P. J. MULHOLLAND[4];
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Abstract: The neurobiological adaptations underlying the transition from a normal adaptive response to stressors to a pathological maladaptive response are poorly understood. In humans, this change can result in conditions including post-traumatic stress disorder (PTSD) and other anxiety disorders. The role of the medial prefrontal cortex (mPFC; i.e., ACC - anterior cingulate, PL - prelimbic, and IL - infralimbic cortices) in aspects of behavioral responses to stress (e.g., stages of fear memory/consolidation or cognition) are well defined. However, the most ventral aspect of the mPFC, the dorsal peduncular (DP) cortex has been widely ignored and is often subsumed into the IL cortex. Here we examined the role of the DP cortex and its response after stress incubation in the mouse single prolonged stress (SPS) model, which includes a two-hour restraint stress, 10-minute forced swim stress, 15-min predator odor exposure, followed lastly by slow loss of consciousness by isoflurane exposure. In this initial examination, only male mice were used as accommodations to the protocol are necessary for female mice. We first used a viral-genetic strategy in Fos-TRAP2 transgenic mice with a Cre-dependent fluorescent reporter protein to label cells in the DP activated by SPS. SPS significantly increased cFos positive neurons in the DP cortex ($p < 0.05$), and immunolabeling identified activated neurons as CamKII-positive pyramidal cells. Next, we utilized bulk RNASeq to investigate the widespread genetic changes after stress incubation, finding over 1000 differentially expressed genes. Gene ontology enrichment analysis identified candidate neuronal subtype (i.e. deep layer pyramidal cells) and biological processes (i.e., potassium channel mediated changes) enriched 7 days after SPS. Then, using patch-clamp electrophysiology, we identified a significant increase in resting membrane potential and evoked action potential of deep layer DP pyramidal neurons after SPS. Our preliminary behavioral data indicate that complex choice behaviors and escape strategies may be altered in a subset of SPS-treated mice, demonstrating the SPS-treated mice make riskier decisions. In conclusion, the DP cortex represents a new and promising region for preclinical study of stress-related neuropsychiatric disorders.

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Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.05/LL11

Topic: F.03. Stress and the Brain

Support: DA011289 (JAK)
Knight Brain Resilience Catalyst Grant KCG-113 (JAK)
Stanford Dean's Fellowship (VMD)
Stanford NeuroChoice (JAK)

Title: Kappa opioid control of a GABAergic stress-sensitive circuit involved in reinstatement

Authors: *V. MARTINEZ DAMONTE[1], L. BAILEY[2], T. E. BROWN[2], J. A. KAUER[1]; [1]Stanford Univ., Stanford, CA; [2]Washington State Univ. Grad. IPN, Washington State Univ. Grad. IPN, Pullman, WA

Abstract: GABAergic synapses control the excitability of dopamine neurons in the ventral tegmental area (VTA). Plasticity at these synapses is a target of drugs of abuse and acute stress. We previously reported that a single acute exposure to cold-water swim stress can block a form of potentiation of inhibitory postsynaptic currents in onto VTA dopamine cells (LTP_{GABA}) via a persistent activation of kappa opioid receptors (kORs). Importantly, this stressor also induces reinstatement of cocaine seeking in rodents. LTP_{GABA} can be rescued by norBNI, a long-lasting kOR inverse agonist, either before stress or well after stress, indicating that kOR activation is sufficient for stress to block LTP_{GABA}. We hypothesize that loss of LTP_{GABA} contributes to stress-triggered reinstatement of drug seeking. We used electrophysiology in brain slices from genetically modified mice and intracranial viral delivery to identify/manipulate the kOR and dynorphin circuit elements responsible for the block of LTP_{GABA}, and we performed behavioral experiments to study the involvement of this circuit in reinstatement.Selectively deleting KORs from postsynaptic dopamine neurons does not prevent stress-induced loss of LTP_{GABA} (stress: 89.5 ± 18.5 % of baseline IPSC amplitude, n=10 p=0.065 Wilcoxon test; stress+norBNI: 152.1 ± 35.7 % of baseline IPSC amplitude, n=10 p=0.013 Wilcoxon test) suggesting that instead the relevant kORs are on presynaptic GABAergic terminals. The VTA receives GABAergic inputs from several different brain regions. We found that lateral hypothalamus (LH)-to-VTA synapses do not display LTP_{GABA} (92.6 ± 17.5 % of baseline IPSC amplitude, n=9 p=0.51 paired t test), whereas synapses from nucleus accumbens (NAc) do (119.5 ± 15.3 % of baseline IPSC amplitude, n=20 p=0.03 Wilcoxon test). Next, we selectively recruited NAc GABAergic terminals in which we had deleted kORs and found that this removal prevents stress-induced block of LTP_{GABA} (135.5 ± 22.5 % of baseline IPSC amplitude, n=7 p=0.031 Wilcoxon test).We have begun to identify putative dynorphin-containing brain nuclei that might be responsible for the dynorphin release during acute stress that results in KORs activation. Using chemogenetics in pdynCre mice, we triggered dynorphin release from two major dynorphinergic inputs to the VTA, the LH and the NAc and tested for the presence of LTP_{GABA}.Finally, we tested whether intra-VTA delivery of the KOR agonist U50,488 mimics reinstatement of cocaine in a conditioned place preference assay.

Together, these findings position KOR specifically in GABAergic projections to the VTA as a target for therapeutic intervention for treatment of stress-triggered relapse.

Disclosures: V. Martinez Damonte: None. **L. Bailey:** None. **T.E. Brown:** None. **J.A. Kauer:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.06/LL12

Topic: F.03. Stress and the Brain

Support: NIH Grant MH116429

Title: Loss of Dopamine D1 Receptors in Nkx2.1+ Neurons Alters Mood-Related Behavior and Frontal Cortical Gene Expression

Authors: *N. DELVA[1], D. L. GRAHAM[2], B. NOBLE[1], G. D. STANWOOD[2];
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Abstract: Dopamine D1 receptors are expressed early in forebrain development in a variety of neuronal subtypes including future cerebral cortical inhibitory interneurons. These interneurons are specified in part by the transcription factor Nkx2.1, and these GABAergic neurons appear to regulate mood-related behaviors and circuits. We used Cre-loxP-induced recombination to delete D1 receptors from Nkx2.1-specified progenitor cells and characterized the cKO mice behaviorally and cellularly. Nkx2.1-*Drd1*-cKO mice developed normally and had normal phenotypes in a variety of behavioral assays measuring motor activity, anxiety, and different types of learning and memory. However, Nkx2.1-*Drd1*-cKO mice demonstrated reduced immobility in the forced swim test (-22%, $p<0.01$ vs. controls) and reduced latency to consumption in an assay of novelty-induced hypophagia (-50%, $p<0.056$ vs. controls). These findings suggest an antidepressant-like effect in the Nkx2.1-*Drd1*-cKO mice. We next performed targeted analyses of select genes and signaling pathways in the medial frontal cortex and observed significant reductions in the neurotrophin receptor TrkB by immunoblotting (-27%, $p<0.05$), and in the neurexin contactin-associated protein-like 4 (*Cntnap4*) by *in situ* hybridization. Ongoing experiments are exploring basal and restraint stress-induced changes in blood corticosterone levels and stress-induced differences in depression-related behavior. We are also quantifying additional changes in gene expression patterns. Our studies to date suggest that D1 receptor loss within Nkx2.1-derived GABAergic neurons modulate brain circuits involved in mood regulation and perhaps in stress responses. These data may lead to the identification of new cellular mechanisms through which behavioral resiliency and antidepressant-like effects could be generated.

Disclosures: N. Delva: None. **D.L. Graham:** None. **B. Noble:** None. **G.D. Stanwood:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.07/LL13

Topic: F.03. Stress and the Brain

Support: Iowa Osteopathic Education and Research Foundation

Title: Estrogen as a Causal Link for voluntary Exercise Behavior: Insights from a Rodent Model

Authors: *V. MATHIS, M. MOHAMED, K. TEFFT, S. CLAYTON, L.-L. YUAN;
Des Moines Univ., West Des Moines, IA

Abstract: Exercise is widely recognized for its beneficial effects on physical and mental health, whereas a sedentary lifestyle poses a risk for various chronic health conditions. Although the physiological mechanisms underlying the positive effects of exercise are well-established, the factors influencing exercise behavior and the motivation to maintain regular physical activity remain largely unknown. In this study, we investigated the role of estrogen in exercise behavior using a rodent model of voluntary wheel running (VWR) and uncovered intriguing sex differences. While female rats exhibited higher levels of average VWR activity compared to males, their running patterns also displays a rhythm that coincides with the peaks and troughs of the estrogen level across the estrus cycle. Ovariectomy (OVX) significantly reduces running activity and eliminates the rhythm. We recently discovered that subcutaneous injection of estradiol benzoate (EB) at 1.5 µg in ovariectomized (OVX) female rats reliably revives VWR to similar levels before OVX. Importantly, individual differences are preserved (e.g., high runners before OVX are high responders to EB, and low runners before are low after). However, this EB-induced behavioral response took more than 24 hours to manifest, suggesting a temporal arrangement and activation of estrogen/estrogen receptor-mediated genomic signaling cascades with VWR responses. By investigating the molecular events prior to the behavioral manifestation, we began to identify estrogen-dependent molecular mechanisms that drive running behavior. Understanding these underlying mechanisms holds significant promise for unraveling the complexities of exercise behavior and may inform strategies to promote physical activity in humans.

Disclosures: V. Mathis: None. **M. Mohamed:** None. **K. Tefft:** None. **S. Clayton:** None. **L. Yuan:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

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Program #/Poster #: PSTR555.08/LL14

Topic: F.03. Stress and the Brain

Support: NIH R01MH125898

Title: Exercise Hormone Irisin Enables Stress Resistance

Authors: *S. M. MELLERT;
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Abstract: Exercise Hormone Irisin Enables Stress Resistance
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Supported by NIH R01MH125898 Adverse life events are often associated with negative implications for physical and mental health; therefore, it is critical to identify processes that foster stress resistance. Inescapable stress produces depressive- and anxiety- like behaviors in rodents, including reduced social exploration and increased shock-elicited freezing. Prior exercise prevents the outcomes of inescapable stress, although the underlying mechanisms are not well understood. Irisin is a hormone secreted from skeletal muscle during exercise that crosses the blood-brain barrier and has been implicated in the effects of exercise on mood and cognitive function. Therefore, the current study sought to determine if an increase in peripheral irisin is sufficient to enable resistance against the behavioral consequences of inescapable stress, as does exercise. Adult, male C57/BI6 mice were given peripheral tail vein injections of either AAV8-Irisin or AAV8-GFP control. After six weeks, subjects (n=10/group) were assigned to either inescapable stress consisting of 100 x 0.3mA tail shocks (5s duration) or no shock. All subjects were tested for social exploration and shock-elicited freezing twenty-four hours later. AAV8-Irisin injections successfully increased plasma levels of Irisin-Flag (mean = 238 ng/ml) compared to AAV8-GFP (mean = 0 ng/ml), measured six weeks after viral injections. AAV8-GFP control mice exposed to stress demonstrated social avoidance and exaggerated fear compared to their home cage counterparts. These effects were prevented with AAV8-Irisin pretreatment, such that AAV8-Irisin stressed mice behaved similarly to the home cage groups. These findings demonstrate that a single peripheral injection of AAV8-Irisin is sufficient to enable behavioral stress protection in sedentary subjects.

Disclosures: S.M. Mellert: None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.09/Web Only

Topic: F.03. Stress and the Brain

Support: Iowa Osteopathic Education and Research Foundation

Title: The gut microbiome and its role in regulating voluntary running behavior in rats

Authors: A. KARIM, M. RUSLING, V. MATHIS, *L.-L. YUAN;
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Abstract: Exercise is integral to the livelihood of almost all species. It has physiological, psychological, and evolutionary benefits. Exercise is a spectrum of activities that differ in distance and time, and understanding what influences varying degrees of activity warrants investigating. It is well-known that the impact of the gut microbiome (GMB) goes beyond the gastrointestinal system to include disease pathogenesis and recovery. Inspired by the many roles of the GMB, we explore the relationship between voluntary wheel running behavior in male Sprague-Dawley rat models and GMB composition. The running group (n=8) had full access to a running wheel, and the sedentary group (n=3) did not. There were four weeks of running. Over the last week, differences in running distance stratified the running group into high (n=3) and low (n=5) runners, creating high, low, and sedentary rungroups. We used QIIME2 to run alpha and beta diversities and ANCOM statistical analyses. Next, we used R to assess the relationship between the abundance of taxa and rungroup, time, and final 7 day average for running. We then identified which bacterial features best influence these outcomes and created a step-wise regression model. There was a negative association between Actinobacteria and time, as well as final 7 day average (MLM, $p < 0.001$, $p = 0.016$, respectively). Mixed effects GLS models showed significantly increased Ruminococcacae and *R. bromii* in high versus low runners. Actinobacteria maintain gut homeostasis by regulating immune responses and gut permeability. *R. bromii* ferments resistant starches into short-chain fatty acids, which play a beneficial role in gut homeostasis by modulating gut barrier integrity and inflammatory/immune responses. These findings demonstrate a relationship between the relative abundance of bacterial species and factors such as rungroup, time exposed to running, and distance traveled. Further delving into these relationships and extrapolating the function of the taxa involved is necessary to understand the impact of these changes on the organism as a whole.

Disclosures: A. Karim: None. **M. Rusling:** None. **V. Mathis:** None. **L. Yuan:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.10/LL15

Topic: F.03. Stress and the Brain

Title: Single-nuclei RNA-sequencing: heterogeneity of striatal astrocytes and the effects of stress and exercise

Authors: *B. B. PEREIRA[1], T. J. BUHR[1], M. G. CONNOLLY[2], L. CHU[3], J. S. RHODES[4], Z. V. JOHNSON[5], P. J. CLARK[3], E. M. MCNEILL[1];
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Abstract: Physical inactivity has reached epidemic proportions worldwide and has been linked to the development of the most debilitating chronic diseases. A growing number of studies suggest that exposure to adverse experiences (e.g., psychological traumas) can lead to lifelong impairments in physical activity engagement. However, the biological factors that contribute to stress-related persistent physical inactivity remain unclear. Our group has shown that exposing rats to a single episode of unpredictable tail shocks can result in a persistent reduction in physical activity, outlasting well-characterized depression- and anxiety-like behaviors by at least month. This mode could represent an important tool to understand the biological underpinnings by which adverse experience can persistently impair physical activity engagement. To that end, young adult male SD rats were housed in cages with locked running wheels for one week. Half of the rats were exposed to a single episode of 100 uncontrollable tail shocks (stress) and the remaining half were left undisturbed in home cages (no stress). Forty-eight hours later, the running wheels were unlocked for half of the rats in the acute stress (stress running, n=6) and no stress conditions (no stress running, n=6), which received free access to running wheels for 42 days. Running wheels remained locked for the other half of the rats in the acute stress (stress sedentary, n=6) and no stress conditions (no stress sedentary, n=6). On the final day of wheel access, rats were sampled. Single-nuclei RNA-sequencing (snRNA-seq) was performed in rat striatum to identify cell-specific molecule events and multi-cellular biological networks that could be involved in persistent reductions in motivation for physical activity following acute stress. The striatum was targeted due to its documented involvement in the acquisition and maintenance of wheel running behavior. Here, we performed a detailed analysis of changes to the expression of genes within the astrocyte cell population, as astrocyte-specific molecular changes contribute to the emotional and behavioral effects of stress. Data collection is ongoing. Preliminary results suggest that six genetically disparate populations of astrocytes can be derived in the striatal area. Within these populations, 108 differentially expressed genes (DEGS) were found due to stress exposure in sedentary animals, and 60 DEGs were found due to exercise. Future directions include considering individual variation in running distances across stressed and unstressed rats as covariates to identify DEGs that may be driving persistent reductions in motivation for physical activity.

Disclosures: B.B. Pereira: None. **T.J. Buhr:** None. **M.G. Connolly:** None. **L. Chu:** None. **J.S. Rhodes:** None. **Z.V. Johnson:** None. **P.J. Clark:** None. **E.M. McNeill:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.11/LL16

Topic: F.03. Stress and the Brain

Title: Striatum-area microglial transcriptomic responses to stress exposure and voluntary wheel running

Authors: *J. TERRILL[1], M. G. CONNOLLY[2], T. J. BUHR[3], B. B. PEREIRA[5], L. CHU[1], Z. V. JOHNSON[6], E. M. MCNEILL[7], J. S. RHODES[8], P. J. CLARK[4];
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Abstract: A sedentary lifestyle significantly increases the risk of some of the most devastating chronic diseases and mental health disorders. However, a large proportion of individuals fail to meet physical activity recommendations across society. Exposures to adverse experiences are linked to the development of sedentary lifestyles, but the neurobiological underpinnings of chronic physical inactivity following stress exposure are poorly understood. The striatum is a component of the natural rewards circuit that is sensitive to stress and may play a critical role in mediating exercise behavior. This study used single nuclei RNA-sequencing (snRNA-seq) to identify biological pathways in striatal area microglia that become persistently disrupted by stress exposure and may contribute to chronic physical inactivity. To that end, young adult male SD rats were either exposed to a single episode of uncontrollable tail shock (stress) or left undisturbed in home cages (no stress). Half of the rats in the stress and no stress groups then received free access to running wheels for 42 days (running) or were housed in cages without wheel access (sedentary). On day 42 of wheel access running, sedentary (no stress n=6, stress n=6) and running (no stress n=6, stress n=6) rats were sampled. snRNA-seq was completed on the striatal area including the septal nuclei. Results show that stress exposure caused a four to sixfold decrease in daily running distance for months, without impacting overall food consumption or body weight. A snRNA-seq cell cluster containing microglia was identified, and further divided into three subclusters using genetic composition. Preliminary data considering only the stress versus no stress groups showed that of the 544 differentially expressed genes that were detected in the microglia, 3% were upregulated in the no-stress compared to the stress group. This was due to shifting of microglia from inactivated to activated type in terms of global gene expression in the cell. Further work is needed to determine whether the inactivated microglia phenotype that occurs in the long-term contributes to reduced motivation for physical activity or whether these two phenomena are unrelated.

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Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.12/LL17

Topic: F.03. Stress and the Brain

Support: College of Human Sciences Collaborative Research Seed Grant

Title: Possible central and peripheral mechanisms mediating stress-induced persistent physical inactivity.

Authors: *T. BUHR[1], C. REED[2], O. WEE[3], J. LI[2], L.-L. YUAN[6], M. FLESHNER[7], R. J. VALENTINE[4], P. J. CLARK[5];
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Abstract: Physical inactivity is detrimental to health, increasing the risk of obesity and cardiovascular disease, as well as anxiety and depression. Exposure to psychological stress has also been linked to the development of many of the same negative health outcomes that occur as a result of prolonged sedentary lifestyles. Moreover, a growing body of literature suggests that prior stress exposure may play a role in the development of sedentary lifestyles. However, the precise neurobiological mechanisms by which stress exposure contributes to prolonged physical inactivity are not well understood. The purpose of this study was twofold. First, to identify the effects of acute stress exposure on voluntary wheel running (VWR) behavior in rodents. Adult male SD rats were exposed to a single episode of 0, 50, or 100 uncontrollable tail shocks (stress). Stress caused a two to fourfold decrease in daily VWR distances that was inversely proportional to shock number and persisted months beyond well-characterized anxiety- and depression-like behaviors. Second, to identify stress-induced alterations to central monoamine neurotransmitters and peripheral muscle physiology that may be maladaptive to exercise output. Rats were exposed to 100 uncontrollable tail shocks (stress) or left undisturbed in home cages (unstressed). Eight days later, monoamine neurochemicals were assessed by ultra-High Performance Liquid Chromatography (uHPLC) across brain reward, motor, and limbic structures immediately following a bout of treadmill exercise controlled for duration and intensity. Additionally, protein markers of oxidative stress, inflammation, and metabolic activity were assessed in the gastrocnemius muscle by western blot. Results show that stress exposure blunted the exercise-induced enhancement of DA turnover in the prefrontal cortex and hippocampus, and potentiated 5HT turnover in the hypothalamus and remaining cortical area. However, stress exposure also caused several monoaminergic changes independent of exercise that could be relevant to impaired motivation for physical activity, including a mild dopamine deficiency in the striatal area. Finally, stress potently increased HSP70 and lowered SOD2 protein concentrations in the gastrocnemius muscle. These data point to possible central and peripheral mechanisms by which exposure to adverse experiences may chronically impair physical activity engagement.

Disclosures: T. Buhr: None. **C. Reed:** None. **O. Wee:** None. **J. Li:** None. **L. Yuan:** None. **M. Fleshner:** None. **R.J. Valentine:** None. **P.J. Clark:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.13/LL18

Topic: F.03. Stress and the Brain

Support: Iowa Osteopathic Education and Research Foundation

Title: Individual and sex difference in stress-induced persistent physical inactivity

Authors: *M. MOHAMED[1], V. MATHIS[1], T. BUHR[2], C. REED[2], K. TEFFT[1], P. CLARK[2], S. CLAYTON[1], L.-L. YUAN[1];
[1]Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA; [2]Food Sci. and Human Nutr., Iowa State Univ., Ames, IA

Abstract: A sedentary lifestyle is a major contributor to chronic and metabolic diseases, with significant economic implications for healthcare systems worldwide. While psychological stress exposure has been implicated as a risk factor for the sedentary lifestyle, little is known about how stress alters physiology in manners that make individuals more prone to chronic inactivity, especially long after the stressor is no longer present. To address this gap, we utilized a rodent model of voluntary wheel running (VWR) to investigate the persistent effects of a single stress episode on physical activity. In our study, young adult rats exposed to 100 tail shocks showed a sustained reduction in daily VWR distance for weeks following the stress, extending beyond the timeframe of anxiety and depression-like behaviors. Notably, individual differences in response to stress caused a wide-ranging variation in wheel running distance. Additionally, intriguing sex differences emerged, as female rats exhibited no or a milder detrimental effect on VWR following the same stress. Depending on the types of running wheels, the stress even led to an enhancement of VWR activity, particularly among low-running females, suggesting a potential protective role of estrogen against stress-induced physical inactivity. To further explore these findings, we are currently investigating estrogen and estrogen receptor-dependent molecular events in brain regions associated with exercise motivation and execution. Understanding the physiological mechanisms underlying individual and sex differences is crucial not only for comprehending the long-lasting effects of acute stress on physical activity but also for identifying innovative interventions to combat sedentary lifestyles.

Disclosures: M. Mohamed: None. **V. Mathis:** None. **T. Buhr:** None. **C. Reed:** None. **K. Tefft:** None. **P. Clark:** None. **S. Clayton:** None. **L. Yuan:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.14/LL19

Topic: F.03. Stress and the Brain

Support: NIH Grant MH099851
Penn State University

Title: Elucidating mechanisms underlying stress resilience in mice with disinhibited SST^+ neurons

Authors: *M. SHAO[1], J. BOTVINOV[1], D. BANERJEE[2], A. SEBASTIAN[2], I. ALBERT[2], S. GIRIRAJAN[2], B. LUSCHER[1];
[1]Dept. of Biol., Pennsylvania State Univ., University Park, PA; [2]Dept. of Biochem. and Mol. Biol., Pennsylvania State Univ., UNIVERSITY PARK, PA

Abstract: Chronic stress is a key environmental factor contributing to various neuropsychiatric disorders, particularly major depressive disorder (MDD). Clinical and preclinical research in mice indicates that while some individuals develop a maladaptive vulnerability to chronic stress, others may adapt and exhibit resilience. This suggests that exploring the mechanisms underlying stress resilience may facilitate the discovery of innovative antidepressant therapies. Both MDD and chronic stress are associated with impairments in GABAergic synaptic inhibitory transmission. In particular, transcriptomic changes point to a key role of somatostatin (SST)-positive neurons, a major subclass of mostly dendrite targeting GABAergic interneurons. We previously showed that mice with disinhibited SST^+ neurons, achieved through the deletion of the γ2 subunit of $GABA_A$ receptors specifically from these neurons (SSTCre:$\gamma2^{f/f}$ mice), exhibit enhanced GABAergic synaptic inhibition of pyramidal cells, mimic behavioral changes of anxiolytic and antidepressant drug treatment, and show resilience to the anxiogenic-like consequences of chronic mild stress. Here we used a more severe stress protocol known as chronic variable stress (CVS) to extend these studies to reward-related behavior. We found that SSTCre:$\gamma2^{f/+}$ and SSTCre:$\gamma2^{f/f}$ mice are resilient to CVS-induced neophobia in the Open Field Test and show abnormal CVS-induced reductions in anxiety-like behavior in the Novelty Suppressed Feeding Test. Male SSTCre:$\gamma2^{f/f}$ mice were also resilient to CVS-induced reductions in hedonic drive in the Female Urine Sniffing Test. Comparison with previously published results suggests that the degree of stress resilience induced by SST^+ neuron disinhibition scales with the intensity of stress and with the degree of disinhibition of SST^+ neurons. To explore the underlying molecular mechanisms, we performed RNA-Seq analysis of bulk media prefrontal cortex (mPFC) from male mice, which revealed greater numbers of CVS-induced differentially expressed genes (DEGs) in SSTCre than SSTCre:$\gamma2^{f/f}$ mice. RNA-Seq of live SST^+ cells purified by fluorescent activated cell sorting from dissociated mPFC revealed that SST mutant cells were entirely resilient to CVS, in contrast to cells from SSTCre controls. Moreover, CVS-induced DEGs observed in SSTCre control mice overlapped with genotype-induced DEGs in stress naïve SSTCre:$\gamma2^{f/f}$ vs. SSTCre mice, revealing putative natural stress resilience genes that are similarly induced by stress and hyperactivity in SST cells.

Disclosures: M. Shao: None. **J. Botvinov:** None. **D. Banerjee:** None. **A. Sebastian:** None. **I. Albert:** None. **S. Girirajan:** None. **B. Luscher:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.15/LL20

Topic: F.03. Stress and the Brain

Support: NIH Grant MH099851
Pennsylvania State University

Title: Chronic activation of somatostatin-positive GABAergic interneurons reverses the behavioral effects of chronic stress in a brain region- and sex-specific manner

Authors: *T. JIANG, A. HUTSELL, Y. GUO, B. LUSCHER;
Biol., Pennsylvania State Univ., State College, PA

Abstract: Chronic stress is a major risk and precipitating factor for virtually all psychiatric disorders Including especially major depressive disorder. However, individuals differ significantly in their vulnerability to stress, pointing to individual differences in their capacity to adapt to stress and resilience to stress. Clinical and preclinical research by us and others has identified somatostatin (SST)-positive GABAergic interneurons as key elements regulating the vulnerability and resilience to stress as well as for major depressive disorder. In particular, we previously showed that globally disinhibiting SST neurons through cell type-specific deletion of an essential subunit of $GABA_A$ receptors from these neurons (SSTCre:$\gamma 2^{f/f}$ mice) results in increased excitability of SST neurons and behavioral and biochemical resilience to the anxiogenic-like effects of uncontrolled chronic mild stress (Fuchs et al. 2017, Jefferson et al. 2020). In the context of a more severe chronic stress protocol referred to as chronic variable stress (CVS) we recently showed that these same mice are also resilient to the detrimental effects of stress on hedonic drive. Here we used AAV- hM3Dq mediated chronic chemogenetic activation of SST neurons to map antidepressant (AD) drug-like changes in positively and negatively motivated behavior to specific brain regions and to test whether the same manipulations could reverse prior stress-induced changes in motivated behavior in adulthood. AAV-hM3Dq mediated chronic activation of SST neurons in the prelimbic cortex (PLC) mimicked the effects of AD-drug induced behavior in male mice in multiple tests but not in female mice. Analogous manipulation of the ventral hippocampus (vHPC) had such effects in female but not male mice. AAV-hM3Dq-mediated chronic activation of SST neurons in the PLC reversed prior CVS induced defects in motivated behavior of males whereas the same manipulation was ineffective in females. Nevertheless, activation of SST neurons in the vHPC effectively reversed CVS-induced behavioral alterations in female mice. These data predict that the GABAergic microcircuits controlling AD-drug-induced behavior and reversal of detrimental stress effects are sex and brain region specific. Notably, analyses of brain sections for c-Fos immunoreactivity of CVS exposed mice revealed that chronic activation of SST neurons resulted in significant activation of pyramidal cells, suggesting that increasing GABAergic inhibition to pyramidal cell dendrites effectively rescues stress-induced defects in excitability of these cells.

Disclosures: **T. Jiang:** None. **A. Hutsell:** None. **Y. Guo:** None. **B. Luscher:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.16/LL21

Topic: G.01. Fear and Aversive Learning and Memory

Support: NRF of Korea 2018R1A3B1052079
NRF of Korea 2018M3C7A1024152
KBRI basic research program through KBRI funded by the Ministry of Science and ICT(23-BR-04-02)

Title: Dopaminergic modulation of posterior basolateral amygdala circuits mediates the transition of defensive behavior

Authors: *J. PYO[1], S. LEE[1], S. CHOI[1], K. PARK[1], E. CHO[2], J. YOON[2], J.-H. KIM[1];
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Abstract: Animals confronted with threats display either passive or active defensive behaviors as survival strategies. While recent reports have disclosed the neural circuits mediating passive freezing behavior and active avoidance behavior, the neural substrate orchestrating the transition from passive to active defensive behavior has yet to be clarified. This study aims to reveal the neural circuits mediating this transition. In employing an auditory two-way active avoidance paradigm, we found that 75% of the tested mice, classified as Good Performers (GPs), demonstrated high avoidance behavior (80%) and low freezing levels after learning. In contrast, the remaining 25% of mice, categorized as Poor Performers (PPs), consistently exhibited low avoidance behavior (20%) and high freezing levels. We observed lower dopamine concentration in the pBLA of PPs compared to GPs, suggesting a crucial role in the deficit in active avoidance learning. To study how dopamine contributes to these differing responses, we utilized techniques including viral tracing, fiber photometry, pharmacological interventions, and opto- and chemogenetics. Our findings emphasize the critical role of dopaminergic transmission from the ventral tegmental area (VTA) to the posterior part of the basolateral amygdala (pBLA) in the learning of active avoidance. In this study, we also use Ppp1r1b-Cre mice, which express Cre recombinase in the pBLA. We modulated DRD1 signaling using Opto-D1 and measured and modulated the activity of Ppp1r1b (+) pBLA neurons to show the necessity of pBLA Ppp1r1b (+) neurons for the active avoidance behavior. We propose dopaminergic transmission and activity of pBLA neurons as the neuronal substrate mediating the transition of defensive behaviors.

Disclosures: **J. Pyo:** None. **S. Lee:** None. **S. Choi:** None. **K. Park:** None. **E. Cho:** None. **J. Yoon:** None. **J. Kim:** None.

Poster

PSTR555. Stress-Modulated Pathways: Motivational Drive Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR555.17/LL22

Topic: F.03. Stress and the Brain

Support: Hacettepe University Scientific Research Projects Coordination Unit TSA-2020-18753

Title: Do synaptic input distributions from different brain regions onto mPFC differ between stress susceptible and resilient mice?

Authors: *T. SOLAKOGLU[1], S. ERDENER[1], O. GLIKO[2], A. CAN[3], U. SÜMBÜL[2], E. EREN-KOCAK[1];
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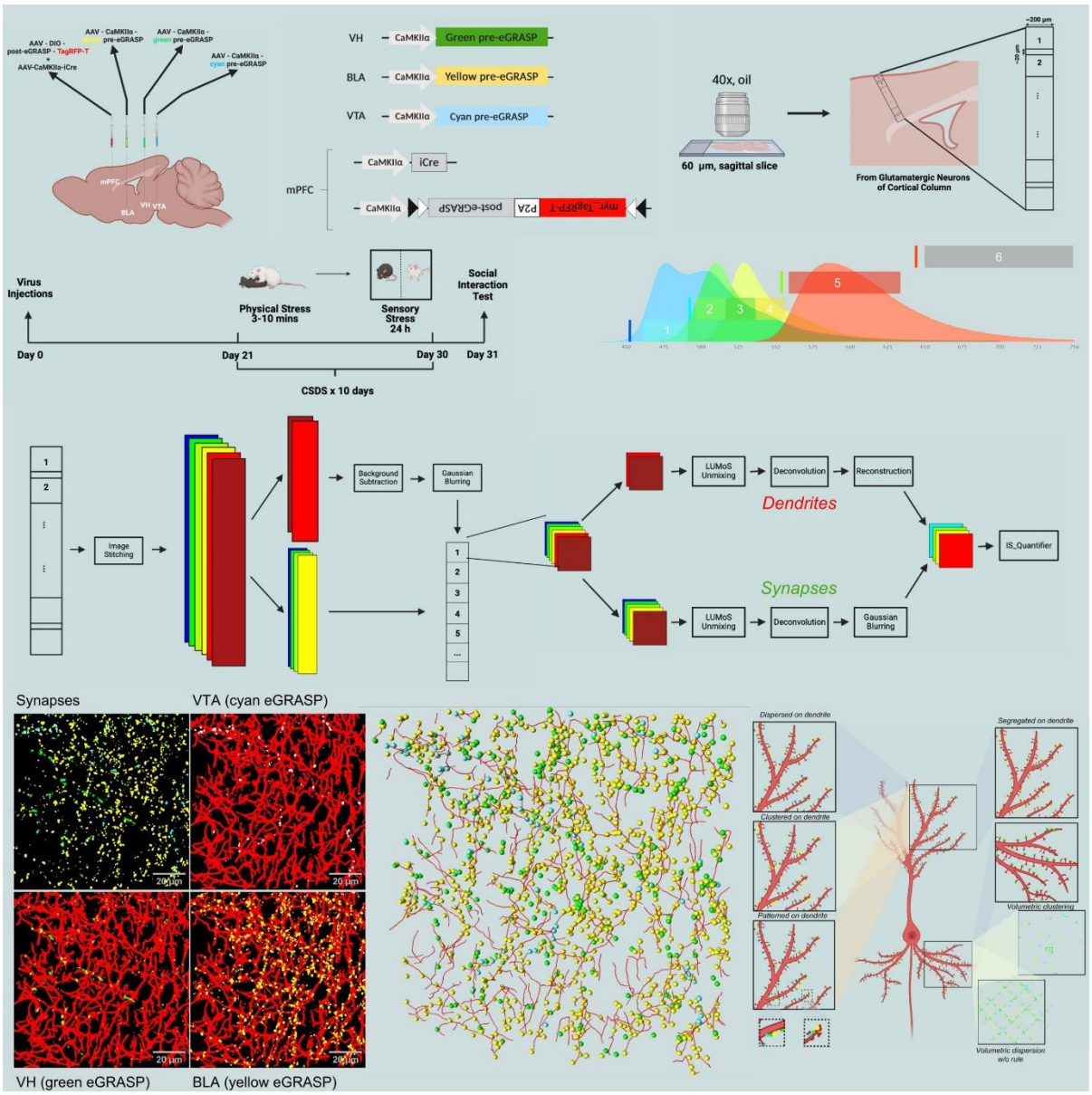
Abstract: Introduction: Stress decreases branching and spine numbers in apical dendrites of layer II/III and layer V pyramidal neurons of the medial prefrontal cortex (mPFC). In light of recent studies suggesting dendritic branches as the basic functional unit of neural computation, it is important to know from which brain regions these atrophied dendrites receive input. We mapped distribution of synaptic inputs from ventral tegmental area (VTA), ventral hippocampus (VH) and basolateral amygdala (BLA) on mPFC pyramidal neurons and investigated the differences between stress susceptible (SS) and stress resilient (SR) mice.
Methods: Synapses were labelled with the eGRASP (enhanced green fluorescent protein reconstitution across synaptic partners) technique in 12 C57BL/6 male mice. Briefly, AAV1 expressing cyan, green and yellow pre-eGRASP under the CAMKII promoter were injected into VTA, VH and BLA, respectively. AAV1 expressing post-eGRASP under the CAMKII promoter was injected into the mPFC. 3-weeks later mice were exposed to chronic social defeat stress for 10 days. Mice were divided into SS and SR groups by social interaction test scores performed on day 11. High resolution, frame sequential, 3D tile scans were acquired from the mPFC column by confocal microscopy. Images were then stitched and fluorophores were unmixed with the LUMoS plug-in of FIJI. Signals were deconvolved and dendritic signals were reconstructed. For synapse quantification, the Synapse Detector plug-in of Vaa3D was used.
Results: We have successfully unmixed multicolored synapses throughout the cortical column. We are currently analysing the numbers and distribution of VTA-mPFC, BLA-mPFC and VH-mPFC synapses. Differences in synaptic input numbers and spatial distribution between SS and SR mice will be presented and discussed.
Conclusion: We will identify the differences in the distribution of inputs from different brain regions on mPFC in response to chronic stress between SS and SR mice. This will help to gain insight into the pathophysiology of stress related disorders and stress susceptibility.



Disclosures: **T. Solakoglu:** None. **S. Erdener:** None. **O. Gliko:** None. **A. Can:** None. **U. Sümbül:** None. **E. Eren-Kocak:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.01/LL23

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Elucidating the role of the extracellular matrix in behavioral dysregulation in hyperglycemia

Authors: *A. MENDEZ[1], M. JODEIRI FARSHBAF[3], J. L. ABLES[2];
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Abstract: Diabetes (DM) is highly comorbid with neuropsychiatric disorders such as depression. Yet, little research has been focused on understanding the biological mechanisms by which DM is able to inflict changes on the brain to alter reward-related behavior. To investigate this, we injected mice with streptozocin (STZ), a chemical compound that destroys pancreatic beta cells, and models type-1 DM in humans. We then performed targeted purification of polysomal mRNA (TRAP-Seq) in cholinergic neurons of the medial habenula (mHb) in diabetic (n=4; M=3, F=1) and non-diabetic (n=11; M=6, F=5) mice. We collected tissue from the mHb as cholinergic neurons from the mHb that project to the interpeduncular nucleus (IPN) have been shown to be involved in the regulation of both reward-related behavior and blood glucose. We found that several pathways involved in the regulation of the extracellular matrix (ECM) such as the activation of matrix metalloproteinases (p=0.0127), collagen biosynthesis (p=8.7E-6), and ECM organization (p=7.1E-5) were upregulated in mice with DM. Thus, we wanted to further explore the ECM as a possible mechanism linking neuropsychiatric disorders and diabetes. To investigate this, we used biotinylated lectins to visualize and quantify the effects of DM on the ECM of the mHb and IPN. We found that the area (p=0.0397), total length (p=0.034), branch points (p=0.047), and endpoints (p=0.043) of the ECM of the IPN, but not the mHb, were significantly decreased after chronic diabetes (12 weeks; control IPN n=4, STZ IPN n=3, control mHb n=5, STZ mHb n=2; all M) but not subchronic diabetes (6 weeks; control IPN n=2, STZ IPN n=3, control mHb n=2, STZ mHb n=2; all M). Next, we sought to understand how diabetes impacts susceptibility to sub-threshold mild variable stress in male diabetic (non-stressed n=15, stressed n=15) and non-diabetic (non-stressed n=15, stressed n=15) mice. Surprisingly, we found that diabetic mice do not display increased susceptibility to stress as measured by the elevated plus maze (p=0.14) or social interaction task (p=0.14). Given these findings, we chose to investigate how DM alters reward-related learning through the use of the progressive ratio task in diabetic (n=15) and non-diabetic (n=15) male mice. We found that while mice with DM are slightly quicker at learning the task, they are not more motivated for reward as measured by the breakpoint (p=0.182). Together, our data suggests that DM causes a decrease in the ECM of the IPN and that DM drives alterations in reward learning. Together, these findings identify the ECM as a novel therapeutic target to prevent incidences of neuropsychiatric disorders in individuals with DM.

Disclosures: A. Mendez: None. **M. Jodeiri Farshbaf:** None. **J.L. Ables:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.02/LL24

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: TIFR-DAE Grant RTI4003

Title: Substrain-dependent effects of physical exercise on brain mitochondrial and neurotrophic measures in C57Bl/6N-derived mouse substrains

Authors: *A. SINGLA[1], P. R. CHAUDHARI[1], M. HINGMIRE[1], D. KAPRI[1], S. E. FANIBUNDA[2], S. T. SURYAVANSHI[1], U. KOLTHUR-SEETHARAM[1], P. GOVINDARAJ[3], V. A. VAIDYA[1];
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Abstract: Physical exercise elicits several beneficial changes in the brain, including bioenergetic and neurotrophic ones, the mechanistic understanding for which has emerged primarily from rodent studies. Recent reports indicate that mouse strains such as the widely used C57Bl/6J and C57Bl/6N, respond differently to metabolic interventions, with implications for the study of the mechanistic targets of physical exercise. Further, hundreds of C57Bl/6N-derived mouse substrains across the globe are used in neuroscientific research with limited focus on their genetic heterogeneity. Differential responses to physical exercise in such closely related mouse substrains have not been investigated thus far. Here, we compared responses to voluntary physical exercise in two C57Bl/6N-derived mouse substrains, namely C57Bl/6NCrl (NCrl) andC57BL/6NCrlCri (NCrlCri), which were both obtained from Charles River Laboratories, five years ago and forty years ago, respectively. Adult male NCrl and NCrlCri mice were housed with (Exercise) or without (Non-Exercise) running wheels for seven days, after which serum and hippocampi were obtained. We report the effects noted in male mice in this study, and we are currently addressing the same in female mice. Results from both the substrains were analyzed independently on unpaired Student's t-test. We first assessed circulating levels of BDNF, and as compared to Non-Exercise-NCrl mice (100% ± 4.5, mean ± SEM), Exercise-NCrl mice showed a significant increase (248.5% ± 18.8, $p < 0.05$), which was not observed in Exercise-NCrlCri mice (109.1% ± 8.2) as compared to Non-Exercise-NCrlCri mice (100% ± 16.6). In the hippocampus, we examined L-lactate levels, wherein a significant increase in Exercise-NCrl mice (213.6% ± 17.5, $p < 0.05$) was noted as compared to Non-Exercise-NCrl mice (100% ± 16), but no significant difference was observed in Non-Exercise- and Exercise-NCrlCri mice (100% ± 11.3 and 113.4% ± 15.4, respectively). NCrl mice also showed an increase in mitochondrial-DNA levels, ATP levels, mitochondrial respiration, and Bdnf expression in the hippocampus upon exercise, whereas these effects were not observed in NCrlCri mice. Our findings indicate differential responses to voluntary physical exercise in two C57Bl/6N-derived mouse substrains. Whole genome sequencing analyses to find potential genetic mutations causing this effect are underway as this may shed light on the mechanisms that underlie the observed differential responses to exercise. It is intriguing to observe divergent exercise responses in C57Bl/6N-derived mouse substrains, underscoring the significance of strain differences in rodent models used in research.

Disclosures: **A. Singla:** None. **P.R. Chaudhari:** None. **M. Hingmire:** None. **D. Kapri:** None. **S.E. Fanibunda:** F. Consulting Fees (e.g., advisory boards); S.E.F. serves as a consultant to Beckley Psytech which is not relevant to the current work.. **S.T. Suryavanshi:** None. **U. Kolthur-Seetharam:** None. **P. Govindaraj:** None. **V.A. Vaidya:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.03/LL25

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NSTC 110-2326-B-038-002-MY3
CGMH Grant CMRPD1M0122

Title: The Impacts of NPFFR2 Overexpression in the Hypothalamic Arcuate Nucleus on Metabolic Symptoms Induced by Type 2 Diabetes

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Abstract: NPFFR2, the cognitive receptor of NPFF, has been reported to regulate thermogenesis and glucose homeostasis. Both NPFF and NPFFR2 are predominately expressed in the hypothalamus, including the arcuate nucleus (ARC). Located in the mediobasal hypothalamus, the ARC serves as a pivotal brain region for receiving peripheral signals such as insulin and leptin, which in turn regulate energy homeostasis. Our previous studies have demonstrated that NPFFR2 deletion in mice ameliorated metabolic symptoms in models of type 1 and type 2 diabetes. Moreover, we have shown that NPFF inhibits insulin downstream signaling in the hypothalamus. In the current study, we aim to investigate the involvement of NPFFR2 in hypothalamic ARC and its modulation of energy metabolism. Specifically, we overexpressed NPFFR2 in the bilateral ARC of mice and subsequently induced type 2 diabetes by administering a high-fat diet in combination with streptozotocin (STZ) injection. We monitored the changes in food consumption, growth curves, blood sugar levels, and various metabolic parameters, including circulating lipids, glucose tolerance, and insulin sensitivity. Through this research, we seek to underscore the significance of ARC NPFFR2 in regulating metabolic symptoms and energy metabolism.

Disclosures: Y. Lin: None. **K. Wu:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.04/LL26

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: AMED JP21zf0127005
CREST JPMJCR1655

Title: Capturing and manipulating neurons activated by exhaustive exercise

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Abstract: Exhaustive exercise elicits a range of physiological responses, such as increased core body temperature, glycogen depletion, and hypoglycemia, collectively referred to as "fatigue". However, the neural mechanism underlying these phenomena remains largely unknown. To identify the neurons activated during exhaustive exercise, we employed the Targeted Recombination in Active Populations 2 (TRAP2) system. In this study, we utilized *TRAP2*mice, which express Cre^{ERT2} under the *c-fos* promoter. Through crossing these mice with *Rosa26-CAG-lsl-hM3Dq-mCherry* or *Rosa26-CAG-lsl- hM4Di-mCherry* mice, we obtained *TRAP2;Rosa26-CAG-lsl-hM3Dq-mCherry* or *TRAP2;Rosa26-CAG-lsl-hM4Dq-mCherry* mice. We trained them to run on a motor-driven treadmill in order to capture neurons activated during exhaustive exercise in the brain. To achieve this, we administrated tamoxifen intraperitoneally. Our findings revealed that exhaustive exercise induced the expression mCherry in several brain regions, including the anteroventral periventricular nucleus (AVPe), medial preoptic area (MPA), dorsomedial hypothalamus (DMH), paraventricular hypothalamic nucleus (PVN), paraventricular hypothalamus (PH), Pons, and periaqueductal gray (PAG). Subsequent reactivation of these neurons through intraperitoneal injection of Clozapine-N-Oxide (CNO) resulted in a significant reduction in surface body temperature and energy expenditure, accompanied by severe immobility. Conversely inhibiting these neurons didn't induce any changes in these parameters. Furthermore, we observed a decrease in body temperature around brown adipose tissue during exhaustive exercise on the treadmill. To identify specific brain regions controlling these phenomena, we locally injected Cre-dependent AAV carrying hM3Dq-mCherry into the AVPe, MPA, PVN, DMH, and PH of *TRAP2* mice. We observed that chemogenetic re-excitation of AVPe and MPA neurons which were activated during exhaustive exercise, strongly decreased surface body temperature. In contrast, the excitation of PH neurons increased energy expenditure and surface body temperature, accompanied by tail vasodilation. These results suggest that neurons activated during exhaustive exercise exhibit differential effects on body temperature and energy expenditure.

Disclosures: S. Yamada: None. **S. Soya:** None. **T. Matsui:** None. **T. Sakurai:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.05/LL27

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Grant-in-Aid for Early-Career Scientists (23K14130)
JST FOREST Program, Grant Number JPMJFR2066

Title: The poly(A) sequence at the Qrfp gene strongly affects the behavioral pattern of a hibernation-like state in mice

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Abstract: The 3′ end sequence of an RNA molecule modulates mRNA levels and, thereby, protein expression. Stimulation of QRFP-positive neurons in the anteroventral periventricular nucleus (AVPe) of mice (Q neurons) triggers a hypometabolic state similar to hibernation, denoted as QIH (Q neurons-induced Hypometabolism). The QC mouse features an iCre-SV40-poly(A) construct introduced at the starting codon of the *Qrfp* gene. The iCre knock-in of QC mice results in a *Qrfp* knock-out on the same allele. Microinjection of AAV9-hSyn-DIO-hM3Dq-mCherry into QC mouse AVPe facilitates DREADD receptor expression on Q neurons, and subsequent intraperitoneal administration of CNO induces a QIH.
This study compared behavioral effects produced by different poly(A) sequences downstream of the *Qrfp* gene or the absence thereof. The behavior was evaluated using QB mice (with bGH-poly(A)) and QD mice (without poly(A)). Additionally, we investigated the QIH phenotype when the iCre allele preserved the *Qrfp* gene by inserting the construct just before the stop codon with a T2A sequence or after the stop codon with IRES sequence (QT and QI mice).
All mice in the study showed comparable basal metabolic rates. Upon QIH induction, a significant VO_2 reduction was observed before and after 12 h of CNO administration (the first (Q1), second (Q2), and third (Q3) quartiles of the data are presented as [Q1, Q2, Q3]): QC mice dropped from [3.40, 3.67, 4.16] to [0.76, 0.90, 0.95] ml/g/h (n = 7); QB mice shifted from [3.22, 3.47, 3.59] to [1.47, 2.16, 2.92] ml/g/h (n = 17); interestingly, QD mice (lacking poly(A)) were closer to QC mice, moving from [3.14, 3.32, 3.46] to [0.79, 0.96, 1.17] ml/g/h (n = 8). QI and QT mice manifested QIH phenotypes akin to QB mice with 12-h post-CNO VO_2 levels of [1.81, 2.07, 2.38] (n = 12) and [1.84, 2.49, 2.98] ml/g/h (n = 11), respectively. Histological evaluation revealed a correlation between mCherry expression and QIH phenotype in all strains.
Our data emphasize the poly(A) sequence's profound impact on phenotypic expression. QC and QB mice exhibited notable differences in QIH. Further, QD mice demonstrated a stronger QIH phenotype than QB mice, despite lacking a poly(A) sequence, presumably due to the endogenous poly(A). Our findings stress the importance of careful poly(A) sequence design for knock-in animal models.

Disclosures: H. Ono: None. **G. Sunagawa:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.06/LL28

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: P01-DK119130 (NIDDK)
Ruth L. Kirschstein Postdoctoral NSRA (NIDDK)
Ruth L. Kirschstein Predoctoral NSRA (NIMH)
Graduate Research Fellowship (NSF)

Title: Hypothalamic neurons drive metabolic adaptations following exercise.

Authors: *M. KINDEL[1,2], R. POST[2], J. CARTY[2], N. GOLDSTEIN[2], R. VILLARI[2], H. KERN[2], B. SKELLY[2], L. RICHIE[2], J. BETLEY[2];
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Abstract: Physical exercise is one of the most effective lifestyle practices in preventing metabolic, neurological, and psychiatric diseases. However, the neural mechanisms underlying these effects are unknown. The ventromedial hypothalamus (VMH) maintains energy balance by controlling components of energy metabolism. The expression of steroidogenic factor 1 (SF1), which is necessary for the development of VMH neurons, is important for endurance capacity and exercise-induced metabolic adaptations (Fujikawa et al., 2016). We sought to explore the physiology and function of VMH^{SF1} neurons during exercise. We find that activity of VMH^{SF1} neurons increases following exercise. This activity is amplified by repeated exercise training. Importantly, augmenting this activity leads to an increased exercise capacity. These findings suggest that exercise increases VMH^{SF1} neural activity and that this activity mediates metabolic and physiological adaptations that lead to increased physical fitness.

Disclosures: M. Kindel: None. **R. Post:** None. **J. Carty:** None. **N. Goldstein:** None. **R. Villari:** None. **H. Kern:** None. **B. Skelly:** None. **L. Richie:** None. **J. Betley:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.07/MM1

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NRF Grant 2022R1A4A5034121

Title: Hypothalamic mGluR1 signaling that controls appetite and energy expenditure

Authors: *H. YANG[1], B. PARK[1], Y. KIM[1], Y. KIM[1], S. LEE[2], S. YANG[3], J. KIM[1];
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Abstract: Hypothalamic neuronal circuits play a crucial role in regulating whole-body energy metabolism. Recent research has identified mGluR1 as an active participant in the function of

hypothalamic neurons involved in the regulation of energy homeostasis. However, further studies are necessary to elucidate the underlying mechanism by which glutamate and mGluR1 coupling control appetite and energy expenditure. Homer1a and Pin1 have been found to modulate the activity of mGluR1 and mGluR5 signaling by detecting their intracellular domain. Therefore, we aimed to investigate whether alterations in the activity of the mGluR1-Homer-Pin1 signaling axis could lead to changes in metabolic phenotypes. To test our working hypothesis, we primarily employed mGluR1 transgenic mice, which retained modifications in the activity of mGluR1 and Homer coupling, enabling us to conduct gain-of-function and loss-of-function studies. We assessed energy intake and expenditure patterns using an indirect calorimetry system. Additionally, we conducted patch clamp studies on brain slices containing hypothalamic neurons to identify the target neurons mediating the effects of altered mGluR1-Homer-Pin1 signaling on metabolic control. Our findings revealed that mGluR1 transgenic mice with enhanced mGluR1 activity (gain-of-function) exhibited increased food intake and energy expenditure. Consistent with these observations, intracerebroventricular administration of a mGluR1 positive allosteric modulator led to an elevation in appetite. Furthermore, we determined that hypothalamic AgRP neurons responded to changes in mGluR1 signaling activity. Collectively, our observations suggest that the activity of mGluR1 signaling, regulated by Homer and Pin1 adaptor molecules, is involved in the hypothalamic control of energy metabolism.

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Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.08/MM2

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: FACEPE APQ-0765-4.05/10
FACEPE APQ-1026-4.09/12
CNPq Universal-408403/2016
CAPES – Finance Code 001

Title: Effects of serotonergic manipulation in the brainstem in overnourished rats.

Authors: M. T. B. DE LEMOS[1], V. L. DE SOUZA[1], O. H. DOS SANTOS-JÚNIOR[1], S. DE ANDRADE SILVA[1], T. R. D. OLIVEIRA[1], A. DA SILVA[2], ***C. LAGRANHA**[3];
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Abstract: Introduction: Epidemiological data demonstrated a direct relationship between childhood overnutrition/obesity with neurodegenerative and chronic diseases in adulthood. **Aim:**

Investigate the effects of serotonergic manipulation by Fluoxetine in the brainstem of adult rats submitted or not to overnutrition during lactation. **Methodology:** Male Wistar rats were divided on the third day of life into two groups: Normonourished (n=9), Supernourished (n=3), according to the ethics committee (n°0036/2022). The groups were subdivided at 39 days of life according to saline (NaCl, 0.9%) or fluoxetine (10mg/kg) pharmacological treatment. At 60 days, we collected the tissue to analyze: malondialdehyde-MDA, carbonyl content, Superoxide dismutase-SOD, Catalase-CAT, and Glutathione-S-Transferase-GST activity, levels of total thiols, RT-PCR for PGC1a, TFAM, OPA1, FIS1. **Results:** We observed an increase in MDA, a decrease in SOD, and total thiols in the overnourished group. At the same time, Fx treatment in overnourished rats induces a decrease in MDA and carbonyl, and an increase in SOD, CAT, GST-activity, and GSH levels. Additional to the oxidative balance data, we observed at mRNA levels that overnutrition decreases PGC1a and increases FIS-1. On the other hand, Fx-treatment in overnourished animals increases PGC1a, TFAM, and OPA; and decreases FIS-1. **Conclusion:** Our data suggest that, at least in the brainstem, the serotonin modulation in overnourished/obese animals positively affects oxidative balance and mitochondrial dynamics.

Disclosures: M.T.B. de Lemos: None. **V.L. de Souza:** None. **O.H. dos Santos-Júnior:** None. **S. de Andrade Silva:** None. **T.R.D. Oliveira:** None. **A. Da Silva:** None. **C. Lagranha:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.09/MM3

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: R21 AG080248
R21 HD098498

Title: Metabolism and mitochondrial protein turnover

Authors: *C. FLORES[1], E. BOMBA-WARCZAK[2], J. N. SAVAS[2], E. F. FORNASIERO[3]; [1]Northwestern Univ., Evanston, IL; [2]Northwestern Univ., Chicago, IL; [3]Neuro- and sensory physiology, Univ. Med. Ctr., Goettingen, Germany

Abstract: Mitochondria are known for their unique ability to renew, reshape, and remodel themselves in order to ensure a cell's survival. Mitochondrial replenishment within the heart and brain plays a pivotal role throughout a cell’s lifespan up until death. The replenishment of mitochondria is essential for post-mitotic cell types such as neurons. As neurons cannot replace themselves, mitochondrial protein turnover is essential for maintaining normal function of processes within the brain. Mitochondria and metabolic rate have a direct relationship, as metabolic rate refers to the amount of energy used by an organism to maintain normal processes within the brain and heart. The mitochondria then use oxygen to convert glucose to adenosine

triphosphate (ATP), which is an energy storing molecule. Using a metabolic stable isotope (15N), we previously found a discrete subset of mitochondrial proteome in the mouse brain escapes the classical first-order degradation kinetics and persists for at least 4 months (Bomba-Warczak et al., *JCB 2021*). Mitochondrial long-lived proteins, mt-LLPs, are found mostly in the oxidative phosphorylation (OxPhos) and mitochondrial contact site (MICOS) complexes within the inner mitochondrial membrane. This demonstrates that mt-LLPs are co-preserved within the same organelle and suggests the capability of 'older' mt-LLPs to isolate themselves from new organelles in need of mitochondrial cell replenishment. However, it is unclear if these mt-LLPs are influenced by varied metabolic activity. Furthermore, this suggests the idea that the lifespan of mt-LLPs is influenced by metabolic activity within organelles. To investigate this, we placed mice in an environment-based behavioral paradigm to ensure different metabolic activity. Nine mice were placed within an enriched environment and another group of nine mice were placed within a controlled environment. Within each environment, we pulse-chased labeled groups for timepoints of 5, 14, and 21 days. Dissections of the liver, cerebellum, hippocampus, striatum, anterior and posterior cortex were performed at each indicated timepoint. Mitochondrial isolation was performed, producing a homogenate that was followed by quantitative proteomic analysis. From this, we found that the environment enriched group at 14 days had an increased mitochondrial turnover compared to the controlled group at 14 days. This shows a direct relationship between increased metabolic activity and increased mitochondrial protein turnover within the brain. Taken together, our work implicates increased metabolic activity as a method for modulating protein turnover and the mitochondrial dysfunction in neurons.

Disclosures: C. Flores: None. **E. Bomba-Warczak:** None. **J.N. Savas:** None. **E.F. Fornasiero:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

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Program #/Poster #: PSTR556.10/MM4

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Korea National Institute of Health(2023-NS-003-00)

Title: High glucose changes subsets of metabolic gene expression levels alongside p21 and bmal1 genes in the mHypo-N46 cell line

Authors: *E. KIM, Y. KIM;
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Abstract: High glucose (HG) or high lipid impairs normal cellular functions, including neuron cells. The hypothalamic area in the brain has been known to regulate whole-body energy metabolism. Previous studies have shown that hypothalamic MC4R, Htr2a, OrexinR1, and NPY cells play critical roles in energy homeostasis. Using the mouse hypothalamic cell line, mHypo-

N46, we examined the direct effects of HG or palmitic acid (PA) on hypothalamic gene expression changes related to senescence and circadian and energy metabolism, including MC4R and NPY. We found that the levels of MC4R and Htr2a gene expression significantly decreased with either HG or PA after 6 h of incubation in N46 cells. In contrast, NPY gene expression increased, while OrexinR1 gene expression showed no changes. Interestingly, levels of the senescence gene, p21, significantly increased with HG alongside Bmal1 gene expression levels. The levels of gene expression changes with PA were very mild compared to that of HG. Another senescent gene, p16, showed no changes in expression levels either with HG or PA. There were no changes in the levels of clock gene expression with HG, even after 24 h of incubation. These data suggest that direct treatment of HG or lipid changes only selected genes during energy metabolism alongside changes in the expression of p21 and bmal1 genes, without p16 and clock1 genes in the mHypo-N46 cell line.

Disclosures: E. Kim: None. **Y. Kim:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.11/MM5

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Chronic administration of L-Lactate alleviates locomotor deficit in a mouse model for Glut1 deficiency syndrome

Authors: S. BURLET-GODINOT[1], H. FIUMELLI[2], M. TANG[3], U. MONANI[3], D. DE VIVO[3], *J.-L. MARTIN[1], **P. MAGISTRETTI**[2];
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Abstract: Mutations in the SLC2A1 gene that encodes for glucose transporter type 1 (Glut1) Glucose transporter results in deficient glucose transport into the brain and is characterized by early onset epilepsy, complex movement disorders and cognitive impairments in Human. Recently, we have shown that, in mice, this mutation has also an early and marked impact on brain energy metabolism not only on glucose levels but also on other important energy substrates including lactate and glycogen. Consistent with the Astrocyte Neuron Lactate Shuttle model, lactate provided by astrocytes plays an active role in supporting neuronal function, making it a potential therapeutic candidate as an alternative fuel for the brain to alleviate symptoms of Glut1 deficiency syndrome (Glut1DS).
To test this hypothesis, we evaluated the locomotor performance of Glut1DS mice after chronic administration of L-Lactate starting at 2 weeks old, prior to the end of the weaning period. A coat hanger test and a rotarod test were conducted after 6 and 7 weeks respectively of daily injection of L-Lactate at a dose of 1g/kg intraperitoneally (i.p.). Using biosensors, we previously showed that this dose increases lactate levels in the hippocampus (Carrard et al, 2018). Twenty-

four hours after the final injection, mice were sacrificed, and standard biochemical assays were used to measure glycogen, lactate, and glucose levels in the cerebral cortex, hippocampus, and thalamus. All measurements were performed in both male and female WT and GLUT1+/- mice treated with either Vehicle or Lactate.
Consistent with previous findings (Wang et al, 2006), locomotor performance was impaired in GLUT1+/- mice compared to WT mice, with no observed changes in muscular strength. Chronic peripheral administration of L-Lactate significantly increased locomotor performance in GLUT1+/- mice compared to vehicle-treated GLUT1+/- mice, as evidenced by increased time spent on the rotarod and decreased latency to escape in the coat hanger test. However, L-Lactate administration did not induce a long-lasting change on glucose, lactate, and glycogen levels in the brain in any of the groups.
Together, these data suggest that L-Lactate, might be a promising therapeutic agent for the treatment of the locomotor deficits in Glut1DS patients. As of today, we cannot determine whether its beneficial action is linked to its role as a signaling molecule or as an energy substrate (Magistretti and Allaman, 2018) in this paradigm.The molecular mechanisms of L-Lactate action will be further characterized through the analysis of target genes regulated by chronic L-lactate administration in different brain regions.

Disclosures: **S. Burlet-Godinot:** None. **H. Fiumelli:** None. **M. Tang:** None. **U. Monani:** None. **D. De Vivo:** None. **J. Martin:** None. **P. Magistretti:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.12/MM6

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIGMS Grant 1SC1GM144190-01
Texas Woman's University internal funding mechanisms: Research Enhancement Program Awards, Chancellor’s Research Fellowships, small grant and startup funds

Title: Pomc-specific knockdown of mecp2 leads to adverse phenotypes in mice chronically exposed to high fat diet

Authors: *P. FRAYRE[1], K. PONCE-RUBIO[2], J. FRAYRE[2], J. MEDRANO[2], E. NA[3]; [1]Texas Women's Univ., Denton, TX; [2]Texas Woman's Univ., Denton, TX; [3]Texas Woman's Univ., Aubrey, TX

Abstract: Methyl-CpG binding protein 2 (MeCP2) is an epigenetic factor associated with the neurodevelopmental disorders Rett Syndrome and *MECP2* duplication syndrome. Previous studies have demonstrated that knocking out MeCP2 globally in the central nervous system leads to an obese phenotype and hyperphagia, however it is not clear if the hyperphagia is the result of

an increased preference for food reward or due to an increase in motivation to obtain food reward. Here, we show that mice deficient in MeCP2 specifically in pro-opiomelanocortin (POMC) neurons of the arcuate nucleus have an increased preference for high fat diet as measured by conditioned place preference but do not have a greater motivation to obtain food reward using a progressive ratio task, relative to wildtype littermate controls. We also demonstrate that POMC-Cre MeCP2 knockout (KO) mice have increased body weight after long-term high fat diet exposure as well as elevated plasma leptin and corticosterone levels compared to wildtype mice. Collectively, these data suggest that knockdown of MeCP2 in arcuate POMC neurons leads to an increase in the reward value of food as well as changes in hormones associated with body weight homeostasis and stress.

Disclosures: P. Frayre: None. **K. Ponce-Rubio:** None. **J. Frayre:** None. **J. Medrano:** None. **E. Na:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.13/MM7

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: The impact of age on intermittent fasting

Authors: *G. BAINS, E. LOHMAN, N. DAHER, L. BERK;
Loma Linda Univ., Loma Linda, CA

Abstract: Introduction: Obesity affects nearly 78 million adults in the United States. Caloric reduction is a popular method of weight loss. Intermittent Fasting is an alternative method to caloric reduction. The purpose of the study was to determine the role of aging on the acute effect of four weeks of Intermittent Fasting on body composition, stress levels, sleep quality, and hunger levels. **Methods**: Sixteen participants with mean age 34.0 ± 11.7 years (nine less than 30 years and seven greater or equal to 30 years), completed a four-week session of Intermittent Fasting. Participants fasted for 16 hours from the time of their last meal of the evening to start of their first meal the following day. Participants consumed their normal daily caloric intake within an 8-hour period. Body composition was determined using InBody 770. The Pittsburgh Sleep Quality Index, Cohen Perceived Stress Scale, and a hunger level survey were administered at baseline and post 4 weeks. **Results**: There were no significant differences in mean weight (kg), body fat %, BMI (kg/m2), visceral fat, trunk fat, body fat mass, SMM, stress, sleep, and hunger level at baseline between subjects < 30 years old and those ≥ 30. Results of the mixed model ANOVA showed that there was a significant reduction in mean trunk fat and hunger level over time ($p=0.001$, and $p=0.009$ respectively), however, this change did not differ by age group as determined by p-value for age x time interaction ($p>0.05$). Results of the paired t- test showed that there was a significant reduction in stress level among subjects ≤30 years old post 4 weeks versus at baseline (10.8±1.9 versus 17.7±2.5, $p=0.03$ ($d=0.7$)), however, this change was not

significant in subjects ≥30 (11.3±2.1 versus 10.9±2.8, p=0.57). On the other hand, there was a significant reduction in hunger level among subjects ≥ 30 years old post 4 weeks versus at baseline (23.6±5.7 versus 60.3±13.8, p=0.028 (d=1.3)), however, this change was not significant in subjects <30 (48.3±8.9 versus 65.8±6.7, p=0.23). Among all participants, there was a reduction in mean weight, BMI, and visceral fat hunger level ($\eta 2 = 0.19$, 0.11, and 0.12, respectively) but not statistically significant ($p>0.05$). **Conclusion**: There is no gender bias in 16:8 Intermittent Fasting program's benefits on changes in trunk fat and hunger level. Intermittent fasting can be an effective option for all ages and thus can be incorporated into whole person wellness programs. Further research is needed to expand these positive findings.

Disclosures: G. Bains: None. **E. Lohman:** None. **N. Daher:** None. **L. Berk:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.14/MM8

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Modeling, evaluating obese and binge-eating disorder in non human primates

Authors: *Y. JUNG[1,2], H.-G. YEO[1], W. CHOI[1], S. PARK[1], E. JEON[1], G. BAE[1], K.-J. JEONG[1], Y.-H. KIM[1], J.-W. HUH[1], J. MIN[1], S. BAEK[1], Y. LEE[1], H. CHOI[2];
[1]Korea Res. Inst. of Biosci. and Biotechnololy, Cheoungju-si, Korea, Republic of; [2]Seoul Natl. Univ. collge of medicine, Seoul, Korea, Republic of

Abstract: •Obesity and binge eating are . So we tried to find out how we can drive such models and how those types of diets can change eating behaviors. In that case, the present study suggests procedures for studying binge eating and obesity using two different feeding methods in nonhuman primates. •One cynomolgus monkey has access to chocolate bars for 30minutes two days per week. Other two cynomolgus monkeys have access to ad libitum high-fat-high-sucrose diets. •They participated in chocolate sugar-covered candy (M&M's®) feeding test. Both monkeys fed with ad libitum high-fat-high-sucrose diets showed no behaviors of regarding M&Ms, which they did behave the most before the high fat high sucrose ad libitum diet. •The monkey with intermittent high sucrose diet was video recorded every other months on the day of the diet, and the amount they ate in 30 minutes were counted. The amount has increased each month from 495kcal on the first to 1634kcal. •We measured the serum levels of metabolic factors (i.e. insulin, glucose, triglyceride, total cholesterol, alkaline phosphatase). •We also evaluated relationships between adipose tissue density with the types of diets. •Thus, free access to high-fat-high-sucrose diet successfully decreased chocolate feeding, though periodic access to highly palatable candy food successfully engendered large amounts of candy consumption in a single meal by non human primates, providing a behavioral baseline for assessing the effects of manipulations on obese and binge eating in the same animals.

Disclosures: **Y. Jung:** None. **H. Yeo:** None. **W. Choi:** None. **S. Park:** None. **E. Jeon:** None. **G. Bae:** None. **K. Jeong:** None. **Y. Kim:** None. **J. Huh:** None. **J. Min:** None. **S. Baek:** None. **Y. Lee:** None. **H. Choi:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.15/MM9

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R35 NS116824
NIH P01 NS097197

Title: Defining the Impact of Glycan-Associated Monosaccharides on Brain Metabolism and Behavior

Authors: ***A. R. CANTRELL**[1], M. COLPAERT[1], L. E. A. YOUNG[3], H. CLARKE[1], B. ARNETT[3], R. C. SUN[1,2], M. S. GENTRY[1,2];
[1]Biochem. and Mol. Biol., [2]Ctr. for Advanced Spatial Biomolecule Res., Univ. of Florida, Gainesville, FL; [3]Mol. and Cell. Biochem., Univ. of Kentucky, Lexington, KY

Abstract: Introduction: Glycosylation is a critical post-translational modification to ensure proper protein folding and function. Unsurprisingly, alterations in glycosylation have devastating downstream consequences that are observed in multiple neurological diseases, such as congenital disorders of glycosylation (CDGs) and Alzheimer's disease. Strikingly, recent evidence suggests glycogen in the CNS acts as a glucosamine (GlcN) cache for glycan synthesis. Additionally, mouse models for Lafora disease, a childhood dementia and neurological glycogen storage disease (nGSD), exhibit a hypo-glycosylation phenotype further supporting the relationship between glycogen metabolism and glycan synthesis. However, protein glycosylation in other nGSDs, e.g., adult polyglucosan body disease (APBD) and Pompe disease, have not been fully explored and there are currently no therapeutic options for the neurological phenotypes associated with these disorders. Therefore, we hypothesized that supplementing diets with monosaccharides would significantly impact cerebral metabolism and glycosylation. In the current study, we delivered ~2g/kg of either galactose, mannose, fucose, GlcN or N-acetyl-glucosamine (GlcNAc) to C57BL/6 mice and quantified metabolic changes compared to water-treated controls using mass spectrometry, lectin blots, and matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) techniques to establish baseline metabolic changes. **Methods/Results:** In the current study, we defined the baseline metabolic consequences of feeding WT mice glycan-associated monosaccharides. We report significant changes in central carbon metabolic pathways (e.g., glycolysis, TCA, and pentose phosphate pathway) in the brain of mice treated with some sugars, as determined by gas chromatography mass spectrometry (GC-MS). Due to their importance in glycosylation, we interrogated the effect of dietary monosaccharides in glycosylation and observed no significant changes in either N-

linked or O-linked protein glycosylation. Interestingly, despite no significant changes in glycosylation, we observed significant behavioral changes with open field tests and tube aggression paradigms due to monosaccharide supplementation. **Conclusions:** Dietary monosaccharides can significantly alter brain metabolism and behavior in WT mice without significantly affecting glycosylation. Future experiments will explore the metabolic and behavioral consequences of supplementing glycan-associated monosaccharides in mouse models of nGSDs to determine if monosaccharide supplementation would be an efficacious treatment option.

Disclosures: A.R. Cantrell: None. **M. Colpaert:** None. **L.E.A. Young:** None. **H. Clarke:** None. **B. Arnett:** None. **R.C. Sun:** None. **M.S. Gentry:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.16/MM10

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Regulation of the Melanocortin-4 Receptor (MC4R) by Attractin like protein 1 (ALP1).

Authors: *P. BUSCAGLIA[1], J. A. SEBAG[2];
[1]Mol. physiology and biophysics, Univ. of Iowa, Iowa City, IA; [2]Mol. physiology and biophysics, Univ. of Iowa, iowa city, IA

Abstract: The Melanocortin-4 Receptor (MC4R) plays a central role in the regulation of energy homeostasis. Mutations in MC4R are responsible for up to 6% of early onset obesity in humans and deletion of MC4R in mice results in severe obesity due to hyperphagia. MC4R is a GPCR expressed in different region of the brains including the paraventricular nucleus of the hypothalamus. Stimulation of MC4R by its agonist αMSH and inhibition by its endogenous inverse agonist AGRP result in inhibition or stimulation of food intake respectively. MC4R is known to interact with two single transmembrane proteins, the Melanocortin Receptor Accessory Protein 2 (MRAP2) and Attractin Like Protein 1 (ALP1). Whereas MRAP2 has been shown to promote MC4R signaling in vitro and in vivo, the pharmacological and physiological relevance of ALP1 for MC4R signaling and actions is not known. To determine if ALP1 alters MC4R signaling, we measured αMSH-stimulated cAMP production in cells transfected with MC4R alone or with either ALP1 or MRAP2. We found that ALP1 increases MC4R signaling to a larger extent than MRAP2. We also showed that ALP1 inhibits β-arrestin recruitment to MC4R, thus likely preventing desensitization of the receptor. To assess the physiological importance of ALP1, we generated an $ALP1^{Flox}$ mouse and bred it to $MC4R^{CRE}$ mice. Deletion of ALP1 in MC4R neurons resulted in increased body weight compared to $MC4R^{CRE}$ control on standard diet. This increase in body weight was further exacerbated when animals were fed a high fat diet. In conclusion our results suggest that ALP1 regulates MC4R signaling and that deletion of ALP1 from MC4R neurons causes weight gain that may be due to decreased MC4R activity.

Disclosures: **P. Buscaglia:** None. **J.A. Sebag:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.17/MM11

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

Support: NIH Grant NS066019
NIH Grant MH104318
NIH Grant EY027881
NIH Grant AAG070766

Title: Reprogramming of glucose utilization may underlie heightened vulnerability to excitotoxic injury observed in the CA1 region in hippocampal slices from the neuronal GLT-1 knockout mouse

Authors: *P. A. ROSENBERG[1], J. WANG[2], J. V. ANDERSEN[3], B. I. ALDANA[3], E. V. PROCHOWNIK[4], S. LI[5];
[1]Neurol., [2]Boston Children's Hosp., Boston Children's Hosp., Boston, MA; [3]Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [4]Div. of Hematology/Oncology, UPMC Children's Hosp. of Pittsburgh, Pittsburgh, PA; [5]Ann Romney Ctr. for Neurologic Dis., Brigham and Women's Hosp., Boston, MA

Abstract: GLT-1 is the major glutamate transporter of the brain and is predominantly expressed in astrocytes. 5-10% of GLT-1 is expressed in neurons, primarily in excitatory terminals. It's generally assumed that glutamate clearance and homeostasis is primarily achieved through the operation of astrocytic GLT-1. We generated a conditional GLT-1 knockout mouse with the aim of understanding the cell-specific functions of GLT-1, using GFAP-Cre/ERT to inactivate GLT-1 in astrocytes (gfapGLT-1 KO), and synapsin-Cre to inactivate GLT-1 in neurons (synGLT-1 KO). We've previously reported a failure of functional recovery in the CA1 region of hippocampal slices from the synGLT-1 KO due to heightened vulnerability to excitotoxicity. Remarkably, slices from the gfapGLT-1 KO showed normal recovery [(2021) *Frontiers Cell Neurosci* 15:788262]. GLT-1 in axon terminals promotes the utilization of glutamate by synaptic mitochondria [(2019) *J Neurosci* 39:4847-4863]. We hypothesize that changes in synaptic mitochondrial metabolism contribute to the heightened vulnerability to excitotoxicity observed in hippocampal slices from the synGLT-1 KO. We here combined biochemical, molecular, metabolic tracing, and metabolomic studies with electrophysiology in hippocampal slices to understand the basis for the heightened vulnerability to excitotoxic injury in the synGLT-1 KO. We found impaired flux of carbon from ^{13}C-glucose into the TCA cycle in synGLT-1 KO slices. Glycogen content of slices is known to collapse over the first hour after cutting and recover by 2-3 hours. Glycogen recovery in slices from synGLT-1 KO animals was significantly impaired, even in the presence of MK-801, which promotes functional recovery of the slices.

Supplementing ACSF during recovery with 20 mM D-glucose normalized glycogen recovery but had no effect on functional recovery of slices; in contrast 20 mM non-metabolizable sucrose or L-glucose substantially improved slice recovery, suggesting that D-glucose itself is toxic. Using 20 mM sodium L-lactate as the metabolic substrate did not promote recovery, suggesting mitochondria as the source of the toxicity. Consistent with this hypothesis, pyruvate dehydrogenase phosphorylation, which is inhibitory, was increased in WT slices but not in synGLT-1 KO slices. In summary, we have identified 3 parameters of glucose utilization in hippocampal slices that are altered in slices from the synGLT-1 KO mouse. In addition, we have obtained evidence that glucose is toxic to synGLT-1 KO slices. We hypothesize that reprogramming of glucose utilization contributes to the increased vulnerability to excitotoxicity of slices from synGLT-1 KO mice.

Disclosures: P.A. Rosenberg: None. **J. Wang:** None. **J.V. Andersen:** None. **B.I. Aldana:** None. **E.V. Prochownik:** None. **S. Li:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.18/MM12

Topic: C.06. Neuromuscular Diseases

Title: Loss of Miro1-mediated mitochondrial dynamics from cortical excitatory neurons leads to impaired motor coordination

Authors: *C. A. RIVELL[1], M. CASE[1], N. D. KERN[1], S. SPRINGER[2], J. GIDICSIN[1], A. K. MYERS[2];
[1]Neurosci., [2]Psychology, Hamilton Col., Clinton, NY

Abstract: Mitochondrial dysfunction is implicated in a broad range of neurological diseases and can include neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's Disease, and Parkinson's Disease. However, the mechanisms underlying these disorders are poorly understood. Miro1, a critical protein for mitochondrial transport, plays a pivotal role in both anterograde and retrograde mitochondrial dynamics and calcium regulation. Previous studies examining the loss of *Miro1* in the cerebral cortex and spinal cord have found neurodegenerative features such as Bunina bodies in the brainstem and spinal cord, rigidity, and kyphosis. In this study, we conditionally ablated *Miro1* from excitatory neurons of the mouse forebrain using an *Emx1-cre*. We examined both gross and fine motor coordination in *Miro1* conditional mutant (CKO) mice using a battery of tests. *Miro1* conditional mutant mice were similar to controls in hind base and stride length measurements, indicating retained gross motor coordination while walking. However, the rotarod test, a measure of motor coordination and balance, was abnormal in the *Miro1* CKO mice and more severe in males than females. Additionally, *Miro1* conditional mutant mice exhibited decreased forepaw grip strength and increased foot slips compared to controls in the grip strength test and wire grid tests respectively,

evidence of fine motor deficits. Examination of tissue sections from *Miro1* conditional mutant cortices revealed deficits in layer 2/3 and 5/6 as well as morphology changes in neurons of the motor cortex when compared to control tissue. These data further support a role for MIRO1 in motor coordination and further implicates mitochondrial transport in neurodegenerative diseases.

Disclosures: C.A. Rivell: None. **M. Case:** None. **N.D. Kern:** None. **S. Springer:** None. **J. Gidicsin:** None. **A.K. Myers:** None.

Poster

PSTR556. Cellular Metabolism, Brain Energy, Diabetes, and Hyperglycemia

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR556.19/MM13

Topic: F.06. Autonomic Regulation

Support: NIH Grant NS114478
NIH Grant NS107342
NIH Grant EY027202
ZIA MH002964

Title: Light modulates glucose homeostasis and energy balance via the sympathetic nervous system

Authors: *X. CHEN[1], M. HAGHIGHATIAN[1], S. HATTAR[2], R. KURUVILLA[1], H. ZHAO[1]; [1]Biol. department, Johns Hopkins Univ., Baltimore, MD; [2]Natl. Inst. of Hlth., Bethesda, MD

Abstract: Exposure to a regular light-dark cycle is important for mammals to regulate many essential physiological processes, including glucose metabolism and energy balance. Aberrant light conditions are known to impair glucose homeostasis, increasing the risk of metabolic diseases like diabetes. However, the neural mechanisms underlying the effects of light on metabolic functions remain incompletely understood. In the retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) provide the conduit to relay light signals to brain regions involved in non-image-forming functions. Further, emerging evidence suggests that ipRGCs relay light information to peripheral tissues through the sympathetic nervous system. Multiple glucose-regulatory peripheral organs are densely innervated by sympathetic nerves, which is critical for maintaining metabolic homeostasis. In this study, we investigate how light modulates glucose homeostasis and energy balance through a retina-hypothalamus-sympathetic pathway. We found that dark-reared (DD) male mice exhibited metabolic defects by 6-8 weeks of age, which are consistent with blunted sympathetic activity. Notably, sympathetic neural activity in response to a sympathetic stimulus (cold exposure) was significantly attenuated in sympathetic ganglion in the DD mice compared to animals raised in a normal 12:12 light-dark cycle (LD). The metabolic defects were exacerbated with age, with older animals exhibiting insulin resistance, impaired glucose tolerance, and increased body weight at 6-8 months. Currently, ongoing multi-synaptic anterograde and retrograde tracing and neural activity measurements are

being used to define the underlying neural circuits by which light signals impinging on the retina are relayed to the periphery to influence glucose metabolism. These studies will lead to new knowledge about how light chronically modulates glucose metabolism and the potential impact of environmental light on metabolic diseases.

Disclosures: X. Chen: None. **M. Haghighatian:** None. **S. Hattar:** None. **R. Kuruvilla:** None. **H. Zhao:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.01/MM14

Topic: F.07. Biological Rhythms and Sleep

Support: Brown University SPRINT-UTRA award (to MLG)

Title: Interaction between GPCR signaling and Notch regulation of sleep in C.elegans.

Authors: *M. LAMELA, A. HART;
Brown Univ., Providence, RI

Abstract: Sleep is an essential behavior observed across species and is characterized by behavioral quiescence, including reduced sensory responsiveness and decreased activity. In C. elegans, Notch signaling regulates developmentally timed sleep (DTS) which occurs in the transition between developmental stages during periods known as lethargus. A double knock-out of the two Notch co-ligands (osm-7 and osm-11) leads to a decrease in DTS, whereas overexpression of OSM-11 in adult C. elegans results in anachronistic sleep, a temporary, reversible behavioral quiescence similar to lethargus sleep (Singh 2011). With this knowledge, a forward genetic screen was undertaken to identify genes whose perturbation disrupts anachronistic sleep in C. elegans, and goa-1, a gene that encodes a G-alpha(o), was identified (Huang 2017). However, the GPCR receptors that may interact with GNAO1 to regulate sleep are still unknown. Notably, the orthologous human gene GNAO1 was associated with insomnia in a large-scale, human genome-wide association study, suggesting GNAO1 may be important in sleep across species (Lee 2019). To study this receptor in C. elegans, I (1) optimized the sleep assay for assaying anachronistic sleep presented in Huang et al. 2017, (2) crossed individuals containing the osm-11 overexpression transgene with a loss of function allele, (3) tested this double mutant strain for sleep defects in the osm-11 overexpression animals. The results will provide insight into interactions between GPCR and Notch signaling in regulating sleep, demonstrate conservation of GPCRs in C. elegans, and may provide mechanistic insights into the importance of GPCR signaling in sleep and insomnia.
Huang H, Zhu CT, Skuja LL, Hayden DJ, Hart AC. Genome-Wide Screen for Genes Involved in Caenorhabditis elegans Developmentally Timed Sleep. G3 Genes|Genomes|Genetics. 2017;7(9):2907-2917. doi:10.1534/g3.117.300071

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Singh K, Chao MY, Somers GA, et al. C. elegans Notch Signaling Regulates Adult Chemosensory Response and Larval Molting Quiescence. Current Biology. 2011;21(10):825-834. doi:10.1016/j.cub.2011.04.010

Disclosures: **M. Lamela:** None. **A. Hart:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.02/MM15

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R35GM142490
NIH Grant R00NS101065
BrightFocus Foundation
Whitehall Foundation

Title: Microscale instability of neural dynamics drives metaplasticity in controlling circadian regulation of sleep in Drosophila

Authors: A. HUTSON[1], ***B. CHONG**[1], Y. ZHANG[2], Y. XIE[1], E. PAUL[1], L. ZUKOWSKI[1], E. FAULK[1], M. TABUCHI[3];
[1]Case Western Reserve Univ. Dept. of Neurosciences, Cleveland, OH; [2]Interdepartmental Neurosci. program, Univ. of California, Irvine, Irvine, CA; [3]Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: Explaining macroscale system dynamics using microscale biophysical dynamics can provide an essential understanding of the implications for quantitative processes that shape internal brain states. Here, by using the Drosophila circadian network regulating sleep, we provide evidence that the usually neglected subtle microscopic biophysical dynamical instability induced by environmental noise plays a key role in regulating the macroscopic dynamics of neuronal activity to regulate behavioral sleep. Circadian clock neurons regulate sleep through neuronal firing patterns. In Drosophila, specific activity patterns in a subset population of circadian clock neurons, DN1, underlie the circadian regulation of sleep quality. During the nighttime, DN1ps spike patterns are precisely structured by the active regulation of several clock-output molecules, and this high-fidelity regularity contributes to increased sleep quality during nighttime. In the present study, we find that dynamical stability and instability in DN1s membrane potential have dualistic functions in determining the direction of learning rules of synaptic plasticity, depending on circadian cycles and environmental light. We performed a large-scale screening and identified Rabphilin (Rph) as a molecule to stabilize DN1 membrane

potential dynamics to increase nighttime sleep quality. During the nighttime, stabilized DN1s membrane potential dynamics can be destabilized by environmental light pollution, leading to synaptic potentiation, but the light pollution effects can be mitigated by synaptic depression by Rph. In contrast to nighttime, DN1ps membrane potential dynamics are highly unstable by the presence of light and by the diminished Rph. Together, these results suggest that stability of the spiking dynamics in sleep regulatory circuits enhances the reliability of spike timing precision and suppresses synaptic plasticity, whereas dynamic instability increases the uncertainty of spike timing precision to generate neural plasticity. These findings also highlight the importance of finding the circumstances under which vulnerable phenotypes emerge in the current trend of neglecting small and variable phenotypes in favor of large, easily understood phenotypes, as well as the biophysical characterization of said microscale neuronal changes may provide a potential mechanism for large-scale brain network instability.

Disclosures: A. Hutson: None. **B. Chong:** None. **Y. Zhang:** None. **Y. Xie:** None. **E. Paul:** None. **L. Zukowski:** None. **E. Faulk:** None. **M. Tabuchi:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.03/MM16

Topic: F.07. Biological Rhythms and Sleep

Title: Discovery of genetic loci underlying daily patterns of sleep behavior using collaborative cross mice

Authors: *S. GRIZZARD[1], J. S. LORD[2], T. KAFRI[3], G. H. DIERING[4];
[1]Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [2]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]UNC Chapel Hill, Chapel Hill, NC; [4]Univ. of North Carolina - Chapel Hill, Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Sleep is an essential physiological process seen in all members of the animal kingdom. It is widely believed that sleep behavior has both genetic and environmental determinants. Our project set out to investigate the genetic basis underlying the daily expression of sleep behavior. We examined baseline sleep behavior in 12 strains of mice from the Collaborative Cross, a genetically diverse collection of recombinant inbred mouse strains, and observed a wide range of sleep phenotypes and daily patterns. We selected two mouse strains based on their distinct sleep phenotypes (CC036, “Rudolph” - highly regulated sleep with strong daily rhythmicity and separation of sleep between the light and dark phases; CC057, “Run” - highly divergent sleep patterns with seemingly no daily rhythmicity). We then generated F1 and subsequent F2 hybrid generations from the parental Rudolph and Run strains, to collect a robust population of mice (F2: N=271; all females) with a wide range of sleep phenotypes to enable genomic mapping of quantitative trait loci (QTLs) based on sleep measures. Sleep/wake behavior for each mouse was recorded for a 5-day period using PiezoSleep recording technology

from Signal Solutions. We collected a wide range of sleep phenotypes, including percent time and mean bout lengths for REM, NREM, and wake and daily rhythmic behavior for each mouse. These phenotypes were then used for QTL mapping, to potentially identify a chromosomal region likely to contribute to the differences in sleep phenotypes between mice in this population. QTL mapping results identified Chromosome 7 as a likely genetic locus responsible for the variation in many of the examined sleep phenotypes, such as light cycle and NREM-specific phenotypes. This suggests that one or multiple genes in this chromosome could play a role in the differential expression of sleep behavior across members of this population. Furthermore, we found evidence for maternal effects on the sleep patterns of the F1 generation, suggesting that environmental factors may also be involved in the observed phenotypical variation.

Disclosures: **S. Grizzard:** None. **J.S. Lord:** None. **T. Kafri:** None. **G.H. Diering:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.04/MM17

Topic: F.07. Biological Rhythms and Sleep

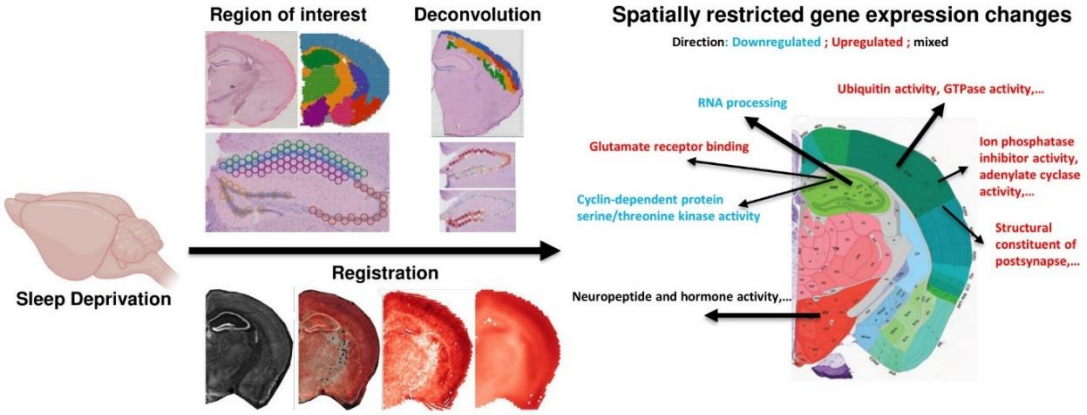
Support: 1R01 AG 062398
P50 HD 103556

Title: Spatial transcriptomics reveals unique gene expression changes in different brain regions after sleep deprivation

Authors: *Y. VANROBAEYS[1,2,3], Z. J. PETERSON[3,4], E. N. WALSH[1,3,5], S. CHATTERJEE[1,3], L.-C. LIN[1,3,6], L. C. LYONS[7], T. NICKL-JOCKSCHAT[1,3,4], T. ABEL[1,3]; [1]Neurosci. and Pharmacol., [2]Interdisciplinary Grad. Program in Genet., [3]Iowa Neurosci. Inst., [4]Dept. of Psychiatry, [5]Interdisciplinary Grad. Program in Neurosci., [6]Dept. of Neurol., Univ. of Iowa, Iowa City, IA; [7]Program in Neurosci., Florida State Univ., Tallahassee, FL

Abstract: Sleep deprivation has extensive effects on both the brain and behavior, impacting memory, attention, and metabolism. Previous studies have primarily investigated changes in gene expression within individual brain regions. However, the uniformity or heterogeneity of sleep loss's effects on the brain remains unclear. In this study, we employ spatial transcriptomics to assess the impact of short-term sleep deprivation on the entire brain of male mice. Our findings reveal substantial differences in gene expression across the brain because of sleep deprivation, with the most significant alterations observed in the hippocampus, neocortex, hypothalamus, and thalamus. Using a rank-sum test like Kruskal-Wallis and stringent thresholds (FDR < 0.001 ; fold-change > |1.2|), differentially expressed genes and their regulatory direction exhibited significant variation among the different regions. Notably, the hippocampal region exhibited the highest sensitivity, characterized by a substantial decrease in gene expression,

especially in RNA processing-related molecular functions. Interestingly, the neocortex demonstrated the second highest sensitivity, displaying significant and robust upregulation of gene expression, primarily associated with transcription factor binding, ubiquitin ligase activity, and protein kinase activity. Additionally, we conducted deconvolution analysis using reference scRNA-seq datasets to investigate the gene expression profiles in individual cortical layers (L2-3, L4, L5, and L6) and specific hippocampal subregions (CA1, CA2, CA3, Dentate Gyrus, stratum radiatum, and stratum oriens). Importantly, we have developed bioinformatic tools enabling the registration of tissue sections and gene expression data into a unified anatomical space, facilitating a comprehensive comparison of gene expression patterns across the entire brain. Our results suggest that distinct molecular mechanisms acting in discrete brain regions underlie the biological effects of sleep deprivation.



Disclosures: **Y. Vanrobaeys:** None. **Z.J. Peterson:** None. **E.N. Walsh:** None. **S. Chatterjee:** None. **L. Lin:** None. **L.C. Lyons:** None. **T. Nickl-Jockschat:** None. **T. Abel:** F. Consulting Fees (e.g., advisory boards); Scientific Advisory Board of EmbarkNeuro, Aditum Bio and Radius Health.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.05/MM18

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant AG062398

Title: Acute sleep deprivation results in sex-specific differences in gene regulation

Authors: ***N. A. STORCH**[1], Y. VANROBAEYS[2], W. G. PLEDGER[1], T. ABEL[2], L. C. LYONS[1];
[1]Dept. of Biol. Science, Program in Neurosci., Florida State Univ., Tallahassee, FL; [2]Dept. of Neurosci. and Pharmacology, Iowa Neurosci. Inst., Univ. of Iowa, Iowa City, IA

Abstract: Sleep deprivation represents a significant public health problem, with more than one third of U.S. adults experiencing sleep deprivation on a regular basis. Sleep deprivation results in significant decrements in memory and performance, as well as increasing the risk for many diseases including metabolic and neurological disorders. The hippocampus is particularly susceptible to the effects of acute sleep deprivation with changes seen in gene expression, cellular signaling, synaptic plasticity and long-term memory. However, there is growing evidence for sex-specific behavioral differences following acute sleep deprivation. To date, almost all of the molecular studies on sleep deprivation have examined changes in males, particularly in rodent studies. Given the differences in sleep between males and females and the behavioral differences following sleep deprivation, we investigated changes in gene expression following acute sleep deprivation in female mice. Following one week of individual housing and pre-handling for three days, mice were sleep deprived using gentle handling (tapping and cage shakes as needed) for five hours and then brain regions were dissected and frozen. Non-sleep deprived mice were dissected at the same time to avoid any confounds in gene expression due to variations in circadian time. The estrus stage of the mouse was determined post-sacrifice using visual and cytological methods and was confirmed independently by at least two individuals. Using an unbiased RNA sequencing approach, we found that females appeared more resilient to changes in gene expression in the hippocampus after acute sleep than similarly aged male mice. Compared to the more than 1100 significant changes in hippocampal gene expression seen in male mice following acute sleep deprivation, we found only 99 genes in the hippocampus exhibited significant differential expression from sleep deprived females compared to non-sleep deprived females. Moreover, in the proestrus stage, female mice had no significant differential gene expression in the hippocampus between sleep deprived and non-sleep deprived animals. Although it is still unknown what the impact of this resilience in gene expression means at the cellular or behavioral levels, these results clearly demonstrate that sleep deprivation induces sex-specific differences and that hormonal changes in female mice provide some resilience to acute sleep deprivation.

Disclosures: **N.A. Storch:** None. **Y. Vanrobaeys:** None. **W.G. Pledger:** None. **T. Abel:** Other; Scientific Advisory Board of EmbarkNeuro and Scientific Advisor to Aditum Bio and Radius Health. **L.C. Lyons:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.06/MM19

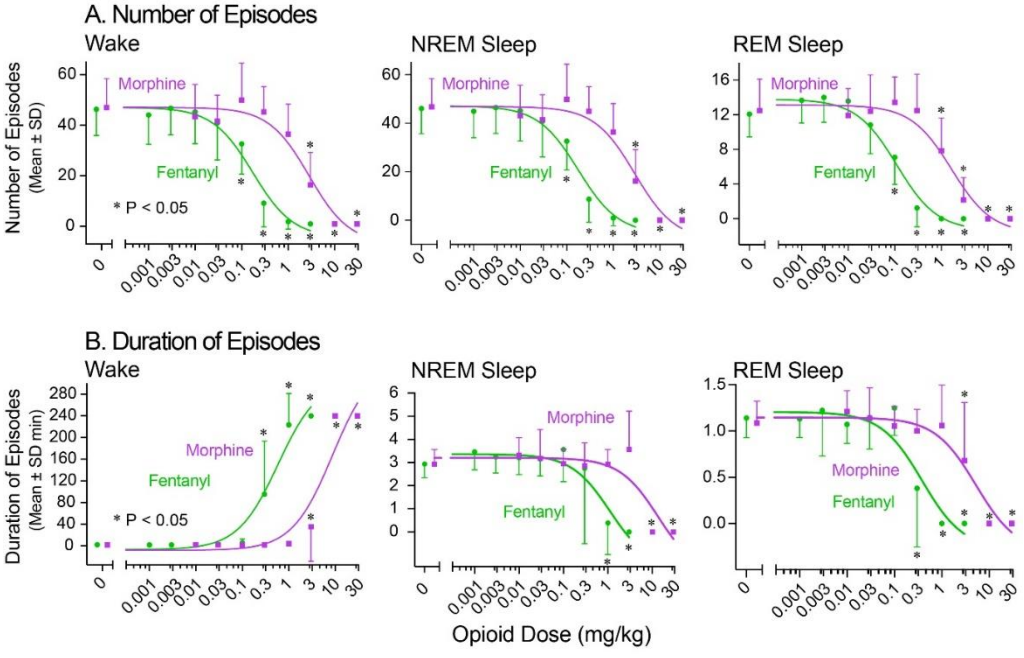
Topic: F.07. Biological Rhythms and Sleep

Support: University of Tennessee

Title: Fentanyl and morphine decrease sleep duration without causing sleep fragmentation in C57BL/6J mice

Authors: N. W. COOPER[1], D. ZEBADÚA UNZAGA[1], C. E. OLSON[1], G. J. O'CONNOR[2], *H. A. BAGHDOYAN[1];
[1]Psychology, [2]Biomed. Engin., Univ. of Tennessee, Knoxville, TN

Abstract: Intrusion of short waking episodes into sleep (fragmentation) is associated with sleep disordered breathing and daytime hypertension in humans (10.1164/ajrccm.162.6.9904008), and with cognitive impairment in humans (10.1038/s41562-020-00964-y) and mice (10.1126/science.abh3021). Opioids disrupt sleep in mice (10.1152/jn.00266.2021), and in humans opioid use is associated with sleep fragmentation (10.1016/j.trsl.2021.03.006). Opioids cause dose-dependent sleep disruption in C57BL/6J (B6) mice that is partially mediated by increased locomotor activity (10.1124/jpet.122.192550). The present IACUC-approved study tested the hypothesis that antinociceptive doses of fentanyl and morphine cause dose-dependent sleep fragmentation in B6 mice. Sleep fragmentation in mice is defined as an increased number of wake and NREM episodes and decreased episode duration during the sleep period (lights on) (10.1126/science.abh3021). Telemeters (DSI HD-X02) permitted 4-h recordings of EEG and EMG from freely moving adult, males (n=12) in their home cages. Subcutaneous injections (0.3 mL) of saline and increasing half-log doses of fentanyl (0.001 to 3 mg/kg) and morphine (0.01 to 30 mg/kg) were administered within 90 min of light onset in a crossover design. Wake, NREM sleep, and REM sleep were assessed in 10-s epochs independently by two investigators, one blinded to treatment condition. Relative to control (0 mg/kg), antinociceptive doses of fentanyl (≥0.1 mg/kg) and morphine (≥3 mg/kg) significantly ($P<0.05$) decreased the number of episodes of all 3 states (Fig A). The two highest doses of fentanyl (1 and 3 mg/kg) and morphine (10 and 30 mg/kg) increased mean duration of wake episodes to 240 min and eliminated NREM and REM episodes (Fig B). Contrary to reports that opioids fragment sleep in humans, fentanyl and morphine did not fragment sleep in B6 mice. These findings identify a difference between humans and mice in the sleep response to opioids.



Disclosures: **N.W. Cooper:** None. **D. Zebadúa Unzaga:** None. **C.E. Olson:** None. **G.J. O'Connor:** None. **H.A. Baghdoyan:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.07/MM20

Topic: F.07. Biological Rhythms and Sleep

Title: The effect of oral nicotine on diurnal sleep patterns and delta homeostasis in Female C57BL/6J Mice

Authors: *S. AKI, M. BROWN, J. STITZEL, H. MATHEWS;
Univ. of Colorado Boulder, Boulder, CO

Abstract: Nicotine use is associated with sleep disturbances. Our lab has previously published on theeffects of nicotine in male C57BL/6J mice, yet nicotine's effects on female mice sleep have yetto be elucidated -- especially since there are known sex differences in sleep patterns andresponses to nicotine. Therefore, this study aims to characterize female C57BL/6J sleep duringa period of nicotine administration and abstinence. Mice (n=11) were implanted with EEG/EMGrecording devices and data was recorded continuously. During the baseline condition, mice hadad libitum access to food and 0.2% saccharin water solution. To produce nicotine dependence,200ug/ml of nicotine was added to the 0.2% saccharin drinking solution for two weeks.Abstinence was induced by removing nicotine from the drinking solution. EEG/EMG data wasscored for two consecutive days of baseline, nicotine administration days 1, 4, 8, 10, andabstinence days 1, 2, 5. EEG data were classified in 4 second epochs as either wake, NREM,or REM. During nicotine administration, total sleep time and NREM percentage were reducedduring the active phase, aligning with nicotine’s stimulatory effects. During the inactive phase,nicotine increased REM bout duration, total sleep stage shifts and sleep bout frequency. Sleepbout duration was also reduced. 24-hour data suggests that nicotine consolidated REM sleep:REM bout frequency was reduced while REM bout duration was increased. During abstinence,total sleep time was reduced during abstinence day 1 and 2. On all abstinence days, sleep boutfrequency was increased while sleep bout duration was reduced – a sleep fragmentationphenotype replicated from our previous male study. These alterations in sleep patterns indicatea sleep fragmentation phenotype in female C57BL/6J mice when undergoing nicotineadministration and abstinence. These findings are consistent with our previously published maledata in 2019 but are more robust. Comparatively, nicotine consolidated 24-hour REM sleep infemales – this was not seen in male mice.

Disclosures: **S. Aki:** None. **M. Brown:** None. **J. Stitzel:** None. **H. Mathews:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.08/MM21

Topic: F.07. Biological Rhythms and Sleep

Support: R21AG080335 (AP)
P20GM103641-09S1 (JAM)

Title: Kynurenic acid and age-related disruptions in sleep-wake behavior in rats

Authors: *S. MILOSAVLJEVIC[1,3], M. V. PIROLI[3], A. K. SMITH[2], N. T. J. WAGNER[3], B. TURPEAU[3], J. A. MCQUAIL[3], H. VALAFAR[2], J. R. FADEL[3], A. POCIVAVSEK[3]; [2]Dept. of Computer Sci. and Engin., [1]Univ. of South Carolina, Columbia, SC; [3]Dept. of Pharmacology, Physiol. and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Advanced age presents challenges that increase the likelihood of poor quality and reduced duration of sleep. Aging pathophysiology points to dysfunctional cholinergic transmission, which may be driven, in part, by altered regulation of basal forebrain neurons by hypothalamic orexin neurons. We hypothesize that kynurenic acid (KYNA), an endogenous antagonist of cholinergic neurotransmission, plays a mechanistic role in age-related sleep dysfunction and orexin activation. The small molecule KYNA is an astrocyte-derived metabolite of the kynurenine pathway of tryptophan catabolism, synthesized primarily by kynurenine aminotransferase II (KAT II) in the brain. An excess of KYNA in the brain results in dysfunctional sleep architecture and cognitive impairment. We hypothesize that KYNA contributes to age-related alterations in physiological regulation of the lateral hypothalamus (LH). Immunohistochemical analysis of brain sections from male young adult (3-6 months) and aged (24-28 months) Fisher 344×Brown Norway F1 hybrid rats evaluated orexin, astrocytic marker GFAP and KAT II expression. Overlap between GFAP and KAT II confirmed colocalization within astrocytes. Aged rats showed reduced orexin expression, but increased KAT II within the LH. In a separate cohort of young and old rats, we acquired polysomnographic recordings that combine electroencephalogram (EEG) and electromyogram (EMG) to evaluate sleep and arousal. Analysis of vigilance state-related parameters categorized as wake, rapid eye movement (REM) and non-REM (NREM) were assessed for 24 h. Vigilance states were classified by our deep neural network trained to mimic expert scorers. Our model performs with a cross-validated macro F1-score of 85.9%, better than reported on humans (81.7%; Supratak et al. 2017 *IEEE*) and on par with reports on rodents (86.8%; Tezuka et al. 2021 *Sci Rep*). Aged rats had significantly decreased REM duration ($P<0.05$), with also shortened duration of each REM bout ($P<0.01$), denoted as fragmented REM sleep. Wake duration was decreased, while NREM duration increased during the latter half of the dark phase in aged rats (time of day x age: $P<0.05$). During the dark phase, aged rats had shorter periods of wakefulness ($P<0.05$). Taken together, our data demonstrate physiological dysregulation of sleep in aged rats. Future experiments are designed to evaluate the role of KYNA within the aged-brain and inhibition of KYNA synthesis as a novel strategy to combat aging-instigated disruptions in sleep-wake homeostasis.

Disclosures: **S. Milosavljevic:** None. **M.V. Piroli:** None. **A.K. Smith:** None. **N.T.J. Wagner:** None. **B. Turpeau:** None. **J.A. McQuail:** None. **H. Valafar:** None. **J.R. Fadel:** None. **A. Pocivavsek:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.09/MM22

Topic: F.07. Biological Rhythms and Sleep

Support: NIAID/NIH Interagency Agreement AOD22011-001-00000; MOA-AI-21002-01
the Oak Ridge Institute for Science and Education (ORISE) Participation Program DOE contract number DE-SC0014664

Title: Sex-dependent nerve agent REM sleep disruption in adult rats

Authors: A. N. SANTORO, M. B. PETTOVELLO, M. WOODSON, J. N. VIGNOLA, *J. MCDONOUGH;
US Army Med. Res. Inst. Chem Def, Edgewood, MD

Abstract: Nerve agents are organophosphorus compounds that inhibit the enzyme acetylcholinesterase resulting in cholinergic overstimulation that can lead to immediate and long-term detrimental health outcomes. One of the most prominent long-term effects of exposure to nerve agent is disruption to the sleep-wakefulness cycle. Data from mass casualty events and animal paradigms indicate that exposure to organophosphorus compounds result in an increase in rapid eye movement (REM) sleep, the sleep cycle responsible for memory consolidation and emotional processing. However, a robust paradigm for characterizing and treating these impacts has not yet been established. In the current study, 15 male and 15 female Sprague Dawley rats were implanted with subcutaneous telemetry devices to measure EEG and EMG signals. Taken together, these signals were used to quantify three sleep-wakefulness states: wakefulness, slow wave sleep, and REM sleep. Data was collected three days prior to and nine days after low-dose nerve agent exposure. Percent time spent in each state and number of times entering each state during the rats' light (sleep) and dark (wakefulness) cycles were quantified. We found that both male and female rats spend significantly less percent time in the REM state across the 12-hr light cycle post-exposure ($M = 5.48$) compared to baseline ($M = 6.72$), $p < .001$. However, only male rats entered the REM sleep state significantly more times across the 12-hr light cycle post-exposure ($M = 13.15$) compared to baseline ($M = 11.15$), $p < .001$, while female rats did not display this increase. These data illustrate a sex-dependent effect of nerve agent on the sleep states in adult rats. In the next phase of this study, we will administer the FDA-approved drug scopolamine to rats post-exposure to potentially minimize these sleep disturbances. In humans, scopolamine is administered as a transdermal patch to combat nausea, but also has demonstrated effects on REM sleep. In preparation for this phase, we identified that concentrations of

scopolamine, self-administered by rats in their drinking water for a duration of three days, resulted in equivalent steady state plasma levels to that of humans wearing a scopolamine patch (150 pg/mL). A concentration of 0.0065 mg/ml scopolamine in drinking water will be administered to post-exposure rats to decrease REM sleep. With these data, we will be able to understand the impact of nerve agent on sleep outcomes and how to improve the quality of life for survivors of nerve agent exposure and support effective post-exposure recovery.

Disclosures: A.N. Santoro: None. **M.B. Pettovello:** None. **M. Woodson:** None. **J.N. Vignola:** None. **J. McDonough:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.10/MM23

Topic: F.07. Biological Rhythms and Sleep

Support: Harmony Biosciences
NIH/NHNINDS 1R01 NS122589-01

Title: Pitolisant inhibits sleep-active neurons in the ventrolateral preoptic area

Authors: R. DE LUCA, J. CHOI, T. E. SCAMMELL, *E. ARRIGONI;
Neurol., BIDMC and Harvard Med. Sch., Boston, MA

Abstract: Pitolisant is an H3 receptor antagonist/inverse agonist that improves daytime sleepiness in adult patients with narcolepsy. Currently, pitolisant is thought to promote wakefulness by increasing the release of histamine, plus other monoamines and acetylcholine, which then directly activate the cortex and other brain regions to produce alertness. However, the flip-flop model of sleep regulation also posits that full wakefulness requires inhibition of sleep-promoting neurons. This suppression of sleep-promoting neurons would enable sustained alertness without the frequent lapses into sleep that are common in narcolepsy. The preoptic area contains essential sleep-promoting neurons, and the ventrolateral preoptic (VLPO) nucleus contains the highest concentration of these cells. We and others showed that VLPO sleep-active neurons express the neuropeptide galanin and are both necessary and sufficient for normal sleep. We hypothesize that inhibition of VLPO neurons is a key part of pitolisant's mechanism of action. Using in vitro electrophysiology, we tested the effects of pitolisant on VLPO galanin-expressing neurons. We found that pitolisant increases the frequency of GABAergic spontaneous inhibitory post-synaptic currents (sIPSCs) in VLPO galanin neurons. To identify the source of this GABAergic input enhanced by pitolisant, we conducted additional optogenetic experiments. Optogenetic stimulation of local VLPO GABAergic neurons produced short latency opto-evoked IPSCs (oIPSCs) in VLPO galanin neurons, indicating a local inhibitory circuit controlling the sleep-active VLPO galanin neurons. Pitolisant (100nM) increased the amplitude of oIPSCs in ¾ of VLPO galanin-expressing neurons. To test whether pitolisant acts on presynaptic terminals,

we made the recordings in the presence of TTX, and pitolisant still increased the oIPSC amplitude in TTX, indicating a presynaptic effect. These results demonstrate that pitolisant inhibits the VLPO galanin neurons by increasing GABAergic inputs, including that from local GABAergic interneurons. We propose that this inhibition of VLPO sleep-active neurons contributes to pitolisant's wake-promoting effect.

Disclosures: R. De Luca: None. **J. Choi:** None. **T.E. Scammell:** 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.; Harmony Biosciences. **E. Arrigoni:** 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.; Harmony Biosciences.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.11/MM24

Topic: F.07. Biological Rhythms and Sleep

Support: NS078410

Title: Sleep Deprivation Reduces BMAL1 in skeletal muscle tissue in mice

Authors: *N. LIBERTY[1], A. TUCKER[1], A. VENKATARAMAN[1], M. MADANI[1], I. NICHOLS[2], K. PAUL[1];
[1]Integrative Biol. and Physiol., UCLA Chapter, Los Angeles, CA; [2]Dept. of Biol., Spelman Col., Atlanta, GA

Abstract: Sleep deprivation has become a prevalent public health issue within the last decade due to the observed deficits in cognition, mental health, and physical health. The consequences associated with sleep deprivation reveal a crucial role for sleep in maintenance of both health and cognition. Understanding the mechanism through which sleep deprivation impairs health is essential for developing therapies for sleep disorders. Brain and muscle ARNT like 1 (Bmal1) is a target circadian transcription factor that has been shown to influence sleep homeostasis. Our lab has found that BMAL1 expression in skeletal muscle plays a significant role in sleep regulation. Two molecular components: brain-derived neurotrophic factor (BDNF) and peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) are potential substrates of BMAL1 that may be involved in the ability of skeletal muscle to influence sleep. BDNF serves as a myokine that modulates sleep regulatory mechanisms in the brain. PGC-1α is a common substrate for BMAL1 and plays a key role in coupling the mammalian clock and energy metabolism. The goal of our study was to determine the effects of sleep deprivation on BMAL1, PGC-1α, and BDNF levels in a mouse model that overexpresses BMAL1 exclusively in skeletal

muscle. Adult male and female BMAL1 overexpression mice and wildtype controls were sleep-deprived for 12 hours during the light phase. Afterward, skeletal muscle tissue samples were collected at Zeitgeber (ZT) 0 and ZT 12, the coinciding peak and trough of the BMAL1 circadian rhythm in skeletal muscle. Western blot analysis revealed that BMAL1 overexpression in skeletal muscle was associated with higher levels of PGC-1α (Welch's t-test; $p<0.05$) but lower levels of BDNF (Welch's t-test; $p<0.05$). Additionally, sleep deprivation decreased BMAL1 (Welch's t-test; $p<0.05$) in the skeletal muscle of overexpression and wildtype mice. However, its effects on PGC-1α, and BDNF were not significant. These results suggest that sleep deprivation is associated with the modulation of circadian genes in skeletal muscle. Our next goal is to determine if this modulation is circadian-dependent.

Disclosures: N. Liberty: None. **A. Tucker:** None. **A. Venkataraman:** None. **M. Madani:** None. **I. Nichols:** None. **K. Paul:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.12/MM25

Topic: F.07. Biological Rhythms and Sleep

Title: Acute sleep deprivation drives distinct changes in the synapse and behavior of developing and mature mice

Authors: *S. M. GAY[1], E. CHARTAMPILA[2], N. BARKER[3], A. L. MORDANT[3], A. MILLS[3], A. PREVETTA[3], L. HERRING[3], G. H. DIERING[4];
[1]Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [2]Univ. of North Carolina Chapel Hill, Univ. of North Carolina Chapel Hill, Carrboro, NC; [3]Univ. of North Carolina, Chapel Hill, NC; [4]Univ. of North Carolina - Chapel Hill, Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Sleep is essential for cognitive function and brain maturation. The importance of sleep during brain development is suggested by the comorbidity of sleep disruptions in numerous neurodevelopmental disorders. Recent studies have shown that chronic sleep loss early in life can have long lasting effects on adult social behavior. However, it is unclear how developing mammals respond to sleep loss and if sleep loss differently affects developing and mature mammals. To address this, we systematically analyzed the behavioral and molecular response to acute 4-hour sleep deprivation (SD4) in juvenile (postnatal, p21-28), adolescent (p42-45), and adult (p90+) mice of both sexes. As previously described, we observe that adult mice respond to SD4 with increased sleep amount in the subsequent active period, called sleep rebound, and with a clear induction of immediate early gene HOMER1a, the "molecular marker of sleep need". Juvenile and adolescent mice showed an absent or severely blunted sleep rebound and HOMER1a induction response to SD4. The lack of appropriate homeostatic response to SD (sleep rebound and HOMER1a induction), suggests that developing mice may be uniquely vulnerable to the negative effects of sleep loss. Consistent with this idea we find that memory

performance in the novel object recognition task was completely impaired by SD4 in juveniles, whereas adults were resilient. Next, we investigated the molecular consequence of sleep loss across development. As the synapse is remodeled during sleep, impacted by sleep deprivation, and many neurodevelopmental disorders are synaptopathies, the synapse is a prime locality to determine the effects of SD in developing mice. We used sub-cellular fractionation to isolate synapse fractions from the mouse cortex and hippocampus and analyzed the samples using quantitative proteomics and phosphoproteomics. SD4 treatment drove profound and unique effects in the juvenile and adolescent synapse proteome and phosphoproteome, whereas adults were again resilient. Importantly, in juveniles and not adolescents or adults we find that SD4 has immediate effects on key aspects of brain maturation such as synaptogenesis and axon-pathfinding, revealing nodes of vulnerability to sleep loss in the developing brain. Next, we treated adult mice with 6 hours of SD to determine if a more severe SD treatment would promote synaptic changes. We observed that SD6 drove hundreds of phosphorylation changes, similar to the SD4 treatment on adolescent mice. Our study shows that developing mice are sensitive to the effects of sleep loss, which suggests how early life sleep loss could contribute to neurodevelopmental disorders.

Disclosures: S.M. Gay: None. **E. Chartampila:** None. **N. Barker:** None. **A.L. Mordant:** None. **A. Mills:** None. **A. Prevetta:** None. **L. Herring:** None. **G.H. Diering:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.13/NN1

Topic: F.07. Biological Rhythms and Sleep

Support: I01 BX001404
R01 NS119227
I01 BX006105
R21 NS079866
I01 BX004673
R21 MH125242

Title: Role of basal forebrain glutamatergic neurons in rapid arousal and homeostatic sleep response

Authors: C. YANG[1], E. L. HODGES[3], T. J. SPRATT[2], J. T. MCKENNA[4], R. E. BROWN[5], *R. BASHEER[6];
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MA; [6]VA Boston Healthcare System-Harvard Med. School-Boston VA Res. Inst., West Roxbury, MA

Abstract: The glutamatergic neurons in basal forebrain (BF) are wake active. Optogenetic stimulation of BF glutamatergic neurons causes rapid and long-lasting arousal in mice. These neurons project locally to neighboring neurons, as well as to distant arousal and reward-associated areas. To assess the contribution of the local vs distant glutamate effects, we performed optodialysis and pharmacological intervention, to examine the role of local glutamatergic projections in modulating arousal and post stimulation homeostatic sleep response (HSR). Male vGluT2-cre mice were injected with AAV5-ChR2-EYFP viral vectors into the BF, implanted with the optodialysis probe targeting the BF for optostimulation and drug infusion, and with the electroencephalogram (EEG)/electromyography (EMG) headmount for sleep recording. The mice were subjected to the following recording paradigm: baseline day (no stimulation), optostimulation day (20Hz 5s/min optostimulation from ZT3-7), drug infusion day (ZT3-7 with the ionotropic glutamatergic receptor antagonists 200 µM DNQX and 500 µM D-AP5) and the optostimulation+drug infusion day (ZT3-7 with both 20Hz optostimulation and infusion of the glutamatergic receptor antagonists). We also performed sleep deprivation (SD) by gentle handling and examined the changes in post-deprivation NREM delta activity (0.5-4.0 Hz), a marker of HSR.Our data (N=10) show that consistent with previously reported findings, wakefulness was significantly increased with the optogenetic stimulation of BF vGluT2 neurons (wake% optostimulation vs baseline: 93.3.3±1.2% of total time vs 30.9±2.0%, $p<0.0001$, paired-t-test). The post-stimulation (ZT7-10) NREM delta activity increased significantly (+17.1±3.5%, $p<0.001$). This increase was comparable ($p=0.1689$) to that observed following 4h of SD (N=7; +24.7±3.8%). The infusion of glutamatergic receptor antagonists during stimulation partially attenuated the optostimulation-induced-time spent awake (optostimulation+drug infusion: 76.6±4.6%, $p=0.001$ compared to optostimulation). Drug infusion without optostimulation had no effect on spontaneous wakefulness (drug infusion wake%: 29.2±4.6%, $p=0.7101$ compared to baseline). Interestingly, with drug infusion the post-stimulation NREM delta showed a marginal reduction to post-stimulation alone, but remained significantly higher (+10.2±4.6%, $p<0.05$) when compared to baseline. Our findings here suggest that while local interactions are involved, extra-BF projections of vGluT2 neurons, perhaps those to the lateral hypothalamus, ventral tegmental area and lateral habenula, also play a central role in arousal promotion and HSR.

Disclosures: C. Yang: None. **E.L. Hodges:** None. **T.J. Spratt:** None. **J.T. McKenna:** None. **R.E. Brown:** None. **R. Basheer:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.14/Web Only

Topic: F.07. Biological Rhythms and Sleep

Support: VA BX000798
VA 1K6BX004216
NIH AA 1R01-029129

Title: Unilateral Optogenetic Stimulation of Lhx6 Neurons in the Zona Incerta Increases REM sleep

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Abstract: To determine how a waking brain falls asleep researchers have monitored and manipulated activity of neurons and glia in various brain regions. In imaging GABA neurons in the zona incerta (ZI) we found a subgroup that anticipates onset of NREM sleep. To differentiate the GABA subtype we now image and optogenetically manipulate the ZI neurons containing the transcription factor, Lhx6. In the first study Lhx6-cre mice (n=5; female=4) were given rAAV-DJ-EF1a-DIO-GCaMP6$_M$ into the ZI (isofluorane anesthesia), a GRIN lens implanted, and 21d later sleep and fluorescence in individual Lhx6 neurons were recorded for 4h. Calcium fluorescence was detected in 132 neurons. 45.5% of the Lhx6 neurons were REM-max; 30.3% were Wake-max; 11.4% were wake+REM max; 9% were NREM-max; and 3.8% had no change. The NREM-max group of neurons fluoresced 30s ahead of sleep onset. The second study tested the effects of unilateral optogenetic stimulation of the ZI Lhx6 neurons (n=14 mice) (AAV5-Syn-FLEX-rc[ChrimsonR-tdTomato]. Stimulation at 1 and 5Hz (1 minute on- 4 minutes off) significantly increased the percent REM sleep during the 4h stimulation period (last half of day cycle). The typical experimental approach is to stimulate neurons in both hemispheres, but here we found that low-frequency stimulation of ZI Lhx6 neurons in one hemisphere is sufficient to shift states of consciousness. Detailed mapping combined with mechanistic testing is necessary to identify local nodes that can shift the brain between wake-sleep states.

Disclosures: P. Shiromani: None. **A. Vidal-Ortiz:** None. **C. Blanco-Centurion:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.15/NN2

Topic: F.07. Biological Rhythms and Sleep

Support: R01 HL127673
P01 HL084207

Title: Leptin engages lateral hypothalamus to ventral tegmental area circuit to affect sleep-wake behavior

Authors: *U. SINGH[1], B. A. TOTH[3], G. DENG[1], J. JIANG[1], O. MUSTAFA[1], R. LI[2], G. F. BUCHANAN[2], D. ATASOY[1], *H. CUI[1];
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Abstract: Sleep and energy metabolism are inextricably linked and mutually affect one another, and any disruptions in these regulatory processes could lead to adverse health outcomes. The literature supports that insufficient sleep and poor sleep quality can lead to overweight and obesity, whereas obesity has been recognized as an independent risk factor for various sleep disorders, including sleep fragmentation, poor sleep quality, and excessive daytime sleepiness. Adipocyte-derived metabolic hormone, leptin, is a key regulator of metabolic homeostasis and exhibits a diurnal rhythm in circulation. However, whether and how leptin signaling might directly affect sleep-wake cycles remains elusive. Here we demonstrate that leptin acts on a subset of lateral hypothalamic area (LHA) GABAergic neurons to affect sleep-wake behavior. We found that selective loss of leptin receptors (LepRs) in the LHA causes sleep fragmentation without altering total sleep time, whereas severe sleep fragmentation observed in obese LepR-null mice can be normalized by selective restoration of LepRs expression in the LHA. In vivo calcium imaging revealed that LHA LepR+ neurons are wake- and REM sleep-active, and chemogenetic activation of LHA LepR+ neurons leads to near-complete sleep disruption. Optogenetic functional circuit mapping further identified the ventral tegmental area as a downstream target through which LHA LepR+ neurons disrupt normal sleep-wake behavior. Collectively, our results identify the LHA as a key node whereby leptin acts to maintain normal sleep architecture. Given the common appearance of leptin resistance in obese patients, our findings may provide novel insights into the neural mechanisms underlying poor sleep quality associated with obesity.

Disclosures: U. Singh: None. **B.A. Toth:** None. **G. Deng:** None. **J. Jiang:** None. **O. Mustafa:** None. **R. Li:** None. **G.F. Buchanan:** None. **D. Atasoy:** None. **H. Cui:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.16/NN3

Topic: F.07. Biological Rhythms and Sleep

Support: R35NS097966 NIH/NINDS
P01AG009973 NIH/NIA

Title: Sleep and circadian rhythm disruption by NPTX2 loss of function

Authors: *S.-E. ROH[1], M. XIAO[1], A. DELGADO[2], C. KWAK[1], A. SAVONENKO[3], H. KWON[1], M. WU[4], P. WORLEY[1];

[1]Neurosci., [2]Cell. and Mol. Med., [3]Neuropathology, [4]Neurol., Johns Hopkins Med. Institutions, Baltimore, MD

Abstract: Sleep and circadian rhythm disruption (SCRD) are commonly observed in neurodegenerative diseases such as Alzheimer's disease (AD). NPTX2, an immediate early gene, is down-regulated in AD individuals and the levels in CSF predicts cognitive impairment in individuals with MCI and AD. NPTX2 has also been implicated in sleep-related circuits and sleep disorders. However, its specific involvement in sleep architecture and circadian rhythm regulation remains unclear. In this study, we investigated the impact of NPTX2 loss of function on sleep and circadian rhythm in mice using EEG recordings, spectral analysis, and wheel-running behavior assessment. Our results revealed altered time allocation across vigilance states, including reduced wake time and increased NREM and REM sleep in NPTX2 knockout (KO) mice. Sleep fragmentation was evident, characterized by alterations in event numbers and average duration of vigilance states, particularly around transition times. The number of sleep transitions were overall increased in NPTX2 KO mice, particularly between Wake and NREM. EEG spectral analysis indicated significant shifts in power across various frequency bands in wake, NREM, and REM states, suggesting disrupted neuronal synchrony. Additionally, NPTX2 KO mice exhibited disrupted circadian rhythm with irregular onset times and increased covariance of onset time over days. These mice also displayed increased wheel-running activity during the light phase, indicating difficulties in maintaining sleep. The phenotype of NPTX2 KO mice bears resemblance to the phenotype of narcoleptic condition. These findings suggest that NPTX2 plays a crucial role in sleep and circadian rhythm regulation, highlighting its potential as a mediator of sleep disruption in narcolepsy and neurodegenerative diseases.

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Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.17/NN4

Topic: F.07. Biological Rhythms and Sleep

Support: NRF/ 2021R1A2C1093712
KSN/ 20223304

Title: Acupuncture stimulation promoted anti-inflammation signaling in the medial septum and restored caffein induced hyperarousal in the rats

Authors: *S. SEO[1], Y. RYU[2], M. CAI[2];
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Abstract: Aims: Acupuncture is used to control various emotional disorders. HT7 stimulation has been found to improve insomnia, improve depression, and modulate the inflammatory cytokines. The study's objective was to evaluate the potential of HT7 for modulating the inflammatory response in the ms region in rats in which insomnia was induced by administration of caffeine for 2 weeks.**Main methods:** Rats were randomly assigned to 4 groups: Control (DW), Caffeine (Caff), Caffeine + Acupuncture (HT7), and Caffeine + Non-Acupuncture (NA). Wake, REM, and nREM sleep durations were evaluated using a rat wireless EEG measurement device. The behavioral patterns of rats were measured through an open field test. Anti- and pro-imflammatory cytokines were measured in the medial septum region using a cytokine array. Microglia type 2 (arginase-1) in the MS region was confirmed through immunofluorescence staining. IL-10 and IL-4 were measured by ELISA assay. Finally, the expression levels of pSTAT6/STAT6 and p-P38/P38 were estimated by western blot assay.**Key findings:** HT7 stimulation restored the arousal time increased by caffeine. HT7 stimulation increased microglia type 2 expression and increased anti-imflammatory cytokien in the medial septum. In addition, increased expression of lL-4 regulated the phosphorylation of STAT6 and P38, which are sub-signaling pathways.**Significance:** HT7 stimulation may improve sleep patterns by regulating the cholinergic anti-inflammatory pathway and increasing the expression of anti-inflammatory markers.
Funding: *This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (No. 2021R1A2C1093712). And This study was supported by a grant from the Korea Institute of Oriental Medicine, Korea (No. KSN20223304).*

Disclosures: S. Seo: None. **Y. Ryu:** None. **M. Cai:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.18/NN5

Topic: F.07. Biological Rhythms and Sleep

Support: 5RO1HL129138
5T32GM008181-34

Title: The role of median preoptic nucleus astrocytes in estradiol's modulation of sleep

Authors: *C. ONWUKWE[1], S. VIECHWEG[2], C. BYRD[2], J. A. MONG[2];
[1]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Women are twice as likely as men to experience sleep disruptions. These differences are most pronounced around menarche and menopause, suggesting a role for estrogens in sleep disorders like insomnia. Our previous work has identified the median preoptic nucleus (MnPO) as a key site regulating estradiol (E2) effects on sleep-wake behavior. We have also shown that

E2 markedly increases extracellular adenosine in the MnPO. Astrocytes are a major source of adenosine in the central nervous system, and cortical astrocytes have been shown to regulate sleep-wake behavior. Thus, we hypothesize that MnPO astrocytes play a key role in the estrogenic mechanisms regulating sleep-wake behaviors. To clarify the role of astrocytes in E2-modulation of sleep, we expressed Gi- or Gq-DREADDs in MnPO astrocytes of ovariectomized rats. Rats were treated with subcutaneous Oil injections (baseline) followed by injections of low (5ug) and high (10ug) dose E2, 24 hours apart. Rats also received Vehicle or Clozapine-N-oxide (CNO; 1.7mg/kg) at the time of their Oil/E2 injections. EEG/EMG recordings were acquired throughout treatment. In Oil-treated animals, CNO activation of Gq-DREADD increased wake and decreased sleep, mimicking E2's effects on Sleep-Wake behavior. Unexpectedly, CNO activation of Gi-DREADD produced similar effects, increasing wake and decreasing sleep. These observations seem to support literature findings that Gi-DREADDs, while inhibitory in neurons, may enhance activity in astrocytes. Thus, Gi-DREADDs may not be an ideal candidate for inhibiting astrocytic activity. To inhibit Ca^{2+} signaling, and, ultimately, gliotransmission in astrocytes, we used a viral approach to express Pleckstrin Homology domain of Phospholipase C (PLC)-like protein p130 (p130PH) in MnPO astrocytes. p130PH is an IP_3 buffer that prevents IP_3 from initiating intracellular Ca^{2+} release and was previously shown to reduce astrocytic Ca^{2+} signaling and gliotransmission (PMID: 20736051). Experimental (p130PH) and Control (mCherry) animals were then treated with the Oil/E2 paradigm described above. Following E2 treatment, p130PH animals (n=3) exhibited less wake and more NREM sleep than animals injected with Control virus (n=3). These preliminary findings suggest that E2 may increase wake by enhancing activity of MnPO astrocytes and highlights a need for further investigation.

Disclosures: **C. Onwukwe:** None. **S. Viechweg:** None. **C. Byrd:** None. **J.A. Mong:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.19/NN6

Topic: F.07. Biological Rhythms and Sleep

Support: T32NS063391
5RO1HL129138

Title: The Effects of Estradiol on Regulating Adenosinergic Signaling in the Median Preoptic Nucleus

Authors: *K. KRUK[1], C. BYRD[2], S. S. VIECHWEG[2], J. A. MONG[2];
[1]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Studies have shown that women report more sleep difficulties than men and that women's sleep disturbances are more likely to occur during times of hormonal fluctuations,

including pregnancy and menopause. This indicates that sex hormones likely play a role in a woman's sleep-wake cycle, but the mechanisms by which sleep is disrupted by estradiol (E2) are largely unknown. Our work utilizes a rodent model to investigate estrogenic mechanisms that regulate sleep-wake behaviors. We have shown that E2 increases wake and decreases NREM sleep in female rats and that E2 action in the median preoptic nucleus (MnPO), a major sleep center in the brain, is necessary and sufficient to induce sleep-wake behaviors. Additionally, in the MnPO, E2 increases extracellular adenosine, which is a marker for sleep pressure, and is known to induce NREM sleep via activation of the A_{2A} adenosine receptor ($A_{2A}R$). Here, we investigate whether E2-mediated changes in adenosine signaling underly its effects on sleep. Our previous work demonstrated that infusion of an $A_{2A}R$ agonist into the MnPO increases NREM sleep and decreases wake in rats, but in the presence of E2, this effect on sleep-wake states is eliminated. The current work investigates mechanisms through which E2 attenuates $A_{2A}R$ signaling in the MnPO. An orphan receptor, GPR37, has been shown to form heteromers with $A_{2A}R$ in the striatum and decrease $A_{2A}R$'s surface expression and function in that region. Preliminary RNA-Seq data from our lab shows that GPR37 mRNA is upregulated in the presence of E2 in the MnPO, and we hypothesize that GPR37 is also binding to and inhibiting $A_{2A}R$ in the MnPO in a similar way to the striatum. To examine GPR37 mRNA and protein expression as well as $A_{2A}R$ mRNA expression, ovariectomized female rats were treated with E2 or oil for 2 days and sacrificed on the third day. The brains were fixed and sectioned for RNAscope to examine mRNA expression or collected fresh frozen for Western Blot to examine protein levels. We found that E2 increases GPR37 mRNA ($p=0.0256$) and protein ($p=0.0212$) expression while decreasing $A_{2A}R$ mRNA expression ($p=0.0344$) in the MnPO. We also have evidence that demonstrates that GPR37 mRNA and $A_{2A}R$ mRNA colocalize in the same cells in the MnPO in the presence of E2. Overall, E2 appears to have an effect on the sleep/wake phenotype through adenosinergic signaling and expression, potentially through E2-induced upregulation of GPR37 reducing $A_{2A}R$ signaling and expression. Understanding more about how sex hormones act to influence sleep can help us develop targeted treatments for women who suffer from sleep disorders.

Disclosures: **K. Kruk:** None. **C. Byrd:** None. **S.S. Viechweg:** None. **J.A. Mong:** None.

Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.20/NN7

Topic: F.07. Biological Rhythms and Sleep

Support: JSPS KAKENHI Grant 21K07538
JSPS KAKENHI Grant 22K07571

Title: Measurement of QRFP concentration in cerebrospinal fluid of hypersomnia patients: a possible new disease index for hypersomnia

Authors: S. CHIBA[1,3,4], H. ISHIDO[1], A. IMANISHI[5], A. HIRANO[1,2], Y. IRUKAYAMA[6], S. FUKUSUMI[1], T. KANBAYASHI[1,3], *M. KIMURA[1];
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Abstract: Pyroglutamylated RFamide peptide (QRFP) is a protein that is specifically expressed in the hypothalamus of rodents and humans. Nerve cells expressing QRFP (QRFP neurons) are known to have axonal projections to areas involved in the regulation of behavior related to rewards, emotions and stress responses. Recently, a new neural circuit containing QRFP has been identified as Q neuron (quiescence-inducing neurons). Activation of Q neuron in mice leads to a state similar to hibernation, in which feeding ceases. Several hibernation-like diseases are known in humans, one of which is a sleep disorder called Kleine-Levin syndrome (KLS). In KLS, periodic somnolence lasting for a few weeks frequently occurs. These periods are sometimes accompanied by behavioral abnormalities such as overeating and hypersexuality. Investigation of whether QRFP neurons are involved in diseases with hypersomnia, including KLS, is essential to advance our understanding of their mechanism. Cerebrospinal fluid (CSF) levels of QRFP and orexin were measured in 89 patients. All patients gave prior consent to lumbar puncture for study participation. The patients were classified into four disease groups for analysis: narcolepsy type 1 (NT1, 25 patients), narcolepsy type 2 (NT2, 25 patients), idiopathic hypersomnia (IHS, 25 patients) and KLS (14 patients). All of them exhibited hypersomnia as one of clinical manifestations. QRFP and orexin levels were determined in intact CSF samples (0.5 or 0.1 ml, respectively) by using a commercially available RIA kit (Phoenix Pharmaceutics, Calif.) The measured QRFP concentrations ranged from 1.0 to 24.1 pg/ml, with an overall average of 6.9 pg/ml. The mean values for each group were 8.4, 6.6, 7.5 and 3.7 pg/ml for NT1, NT2, IHS and KLS, respectively. Compared between disease groups, KLS samples showed significantly lower QRFP levels than those in the other disease groups ($p<0.05$). Orexin concentrations ranged from 40 to 548 pg/ml, and mean values for each disease group were 48.8, 338.5, 331.2, and 242.8 pg/ml for NT1, NT2, IHS, and KLS, respectively. Between groups, the levels of orexin were significantly lower in the NT1 group than in the other disease groups ($p<0.05$). Although the precise etiology of KLS is still unknown, approximately 40% of cases are reported to have a preceding episode of an infectious disease such as influenza, thus autoimmune mechanisms are thought to be implicated. Previous autopsy reports described perivascular lymphocytic infiltration in the thalamus and hypothalamus of patients with KLS. Our results for the first time suggest that some QRFP neurons might have been affected by the activated immune system prior to the onset of KLS.

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Poster

PSTR557. Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR557.21/NN8

Topic: F.07. Biological Rhythms and Sleep

Title: Samelisant (SUVN-G3031): Topline results from the phase-2 proof-of-concept double blind, placebo controlled study in patients with narcolepsy

Authors: R. NIROGI, *A. SHINDE, V. BENADE, J. RAVULA, P. JAYARAJAN, V. GOYAL, S. JETTA, V. JASTI;
Suven Life Sci., HYDERABAD, India

Abstract: Samelisant (SUVN-G3031) is a potent and selective histamine 3 receptor inverse agonist with a Ki of 8.7 nM (human). Samelisant exhibited minimal affinity for other tested receptors and transporters. Samelisant exhibited desired pharmacokinetic properties and brain penetration in nonclinical studies. Samelisant blocked R-α-methylhistamine-induced water intake and increased tele-methylhistamine levels in brain and cerebrospinal fluid in rats. A single oral administration of samelisant produced significant increase in histamine, dopamine and norepinephrine levels in the cortex of rat brain. In orexin knockout mice, samelisant produced wake-promoting and anticataplectic effects suggesting its potential therapeutic utility in the treatment of narcolepsy. In lateral hypothalamic lesioned rats using neurotoxin hypocretin-2-saporin, samelisant produced significant increase in wakefulness with concomitant decrease in rapid eye movement sleep. Samelisant did not affect dopamine levels in striatum and nucleus accumbens and did not cause behavioral sensitization, suggesting no abuse or addiction liability (nonclinical observations). Safety and tolerability studies in animals and healthy human volunteers (phase-1 studies) suggested a favourable risk/benefit profile for samelisant. Wake promoting like effects were also observed in phase-1 study. Contingent on the observation from animal models and healthy humans, samelisant was evaluated as a monotherapy in a double blind randomized controlled trial for treatment of excessive daytime sleepiness in narcolepsy patients with or without cataplexy (ClinicalTrials.gov Identifier: NCT04072380).The primary efficacy endpoint is change from baseline in the mean Maintenance of Wakefulness Test (MWT) score at Day 14. Key secondary endpoint is change from baseline in the mean total Epworth Sleepiness Scale (ESS) score at Day 14. Safety evaluations include the reporting of adverse events, vital signs, ECG, physical and neurological examinations, laboratory investigations, and assessments of suicidality. The enrolment for the study has been completed and data readout from the study is expected in August 2023. Phase-2 study results covering efficacy and safety data from this study will be presented during the Neuroscience 2023 meeting.

Disclosures: **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **J. Ravula:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Jetta:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Jasti:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.01/NN9

Topic: F.07. Biological Rhythms and Sleep

Support: VA Merit Award BX-005167 (N. Alam)

Title: Chronic Inflammation of the Ventrolateral Preoptic Area Causes Aging-like Changes in the Sleep-Wake Architecture in Young Mice

Authors: A. KOSTIN[1], M. ALAM[2], *N. ALAM[1];
[2]Res. Service, [1]VA Greater Los Angeles Healthcare Syst., North Hills, CA

Abstract: INTRODUCTION: Sleep disturbance is a significant problem of advancing age, characterized by frequent awakenings, decreased nonREM and REM sleep amounts, and nonREM slow wave activity during the night, and increased daytime sleepiness. Animal and human studies also indicate that the brains of older subjects are in a heightened inflammatory state, even in the absence of overt disease. Moreover, evidence suggests that chronic inflammatory processes play a role in cellular aging including neuronal aging. We examined the effects of slowly progressing chronic inflammation induced by viral vector-mediated TNF-alpha production in astrocytes localized in the sleep-promoting ventrolateral preoptic area (VLPO) on the sleep-wake organization in young mice. METHOD: Under surgical anesthesia and aseptic conditions, nine mice (3-4 months old) were: i) injected with AAV5-GFAP-TNF-IRES-mCherry (2 males and 3 females) or its control (AAV5-GFAP-mCherry) viruses (2 males and 2 females) into the VLPO; and ii) implanted with EEG and EMG electrodes for recording their sleep-wake profiles. After 10-12 days of recovery, animals' sleep-wake profiles were continuously recorded for 4 weeks. At the end of the experiments, the site of injections and the spread of viral vectors were confirmed. RESULTS: Preliminary analysis of the data from the last 3 days of each week suggests that mice with TNF-alpha production in the VLPO began exhibiting signs of sleep disruption starting from the second week. The data analyzed for the 4th week suggest that compared to control, mice with chronic TNF-alpha production in the VLPO, exhibited a trend towards decreased nonREM (57.9 ± 3.7% vs. 50 ± 3%) and REM sleep (9.7 ± 0.3% vs. 4.2 ± 1.2%) and increased sleep fragmentation as marked by increases in the number of waking intrusions (42.6 ± 4.3 vs. 59.5 ± 4.3) and frequent awakenings (11 ± 2 vs. 16 ± 6). Conversely, during the dark phase (active period) waking was reduced (68.8 ± 4.1% vs. 47.3 ± 1.3%), accompanied by increased nonREM (29.0 ± 4.0 vs. 45.8 ± 2.9%) and REM sleep (2.2 ± 0.4 vs. 4.2 ± 1.2%). Preliminary immune-histochemical examination of the injection sites suggests that the viral vectors had spread into the VLPO and adjacent areas, and microglia in these regions exhibited signs of activation.
CONCLUSION: Chronic inflammation of the sleep-regulatory VLPO, induced by viral vector-mediated TNF-alpha production, leads to sleep-wake disruptions that resemble those observed in aging. Although the precise underlying mechanisms remain unclear, these initial results imply that chronic inflammation of sleep-regulatory VLPO may contribute to sleep disturbances that accompany aging.

Disclosures: **A. Kostin:** None. **M. Alam:** None. **N. Alam:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

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Program #/Poster #: PSTR558.02/NN10

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01NS094571
NIH Grant R35NS122181

Title: Need-dependent plasticity of an excitatory thalamic nucleus encodes homeostatic sleep drive

Authors: *S. LEE[1], Q. LIU[1], T. KIM[2], I. PALMER[1], K. PARK[1], H. MUNZBERG[3], S. BLACKSHAW[2], M. N. WU[1,2];
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Abstract: Prolonged wakefulness drives the persistence of deep sleep, but the underlying mechanisms remain unclear. We found that sleep need-dependent NREM persistence of mice is driven by the activity of excitatory neurons in the nucleus reuniens (RE) of the thalamus. Optogenetic activation of RE neurons elicited persistent NREM sleep with improved sleep quality and promotes natural sleep following pre-sleep behaviors. RE neurons are activated by elevated sleep pressure and the activity of these neurons are sufficient and necessary for driving homeostatic sleep. Glutamatergic RE projection to $Lhx6^{+}$ zona incerta (ZI) is responsible for driving NREM persistence. The RE-ZI circuit undergoes morphological and functional plastic changes by the degree of sleep need. Moreover, we found that CaMKII signaling is required for homeostatic NREM sleep and the need-dependent structural plasticity of this RE-ZI circuit. Our findings propose a novel neural circuit and underlying plasticity mechanism encoding homeostatic sleep drive.

Disclosures: **S. Lee:** None. **Q. Liu:** None. **T. Kim:** None. **I. Palmer:** None. **K. Park:** None. **H. Munzberg:** None. **S. Blackshaw:** None. **M.N. Wu:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.03/NN12

Topic: F.07. Biological Rhythms and Sleep

Support: Department of Veterans Affairs Merit Research Award I0BX002661
NIH GRANT AA028175-01
MU RESEARCH COUNCIL (URC-22-006)

Title: Blockade of A2a receptor in the accumbal core region attenuated alcohol-induced sleep in C57BL/6Jmice.

Authors: *M. THAKKAR[1], R. SHARMA[2], M. PARIKH[3], A. CHISCHOLM[3];
[1]Neurol., HSTMV Hospital/University of Missouri, Columbia, MO; [2]Neurol., Univ. of Missouri, Columbia, MO; [3]Neurol., Univ. of Missouri, Columbia, Columbia, MO

Abstract: Background: Alcohol use disorder (AUD) is a medical condition characterized by persistent alcohol use despite adverse social, occupational, or health consequences. The initial alcohol use/abuse is motivated by the pleasurable and/or rewarding effects which is regulated by the brain's reward circuitry, including the nucleus accumbens (NAc), especially the core region (NacC), and dopamine. On the other hand, alcohol promotes sleep which actually limits the use of alcohol and thus considered as its "aversive effect". This explains the concurrent use of alcohol and caffeine where caffeine inhibits A2a receptors (A2aR; nonspecifically) and promotes wakefulness. However, the role of A2aR in mediating alcohol-induced sleep is unclear. The reward circuitry consists of two efferent pathways from the NAc: direct striatonigral (projecting to substantia nigra), with GABAergic medium spiny neurons (MSNs) possessing D1 (D1R) and A1 (A1R) receptors (D1R-MSNs), and indirect striatopallidal (SP; projecting to ventral pallidum), with MSNs having D2R and A2aR receptors (D2R-MSNs). Recent research reveals that A2aR expressing SP pathway represents the brain's antireward system and promotes sleep when activated which led to a hypothesis that alcohol promotes sleep via activation of A2a receptor in the NAcC. **Methods**: To test our hypothesis, C57BL/6J mice were implanted with sleep recording electrodes on the skull and bilateral guide cannulas above the NacC. Following the surgery, the mice were allowed to recover and adapt to the sleep recording setup. The experimental procedure commenced 30 minutes before dark onset, during which the mice received bilateral NacC infusions of either ZM241385 (a selective A2aR antagonist; 100pg/500nl/side; N=4) or saline (500nl/side; N=4) through the cannulas. Subsequently, all animals were exposed to a 20% ethanol for the next 12 h, utilizing a modified intermittent access 2-bottle choice (IA2BC) paradigm. After 12h, blood alcohol concentration (BAC) was measured, mice were euthanized, and their brains were processed for histology. **Results**: All animals consumed similar amount of alcohol and showed similar BAC. Intra-NAcC administration of ZM241385 prior to alcohol self-administration caused a significant ($p < 0.01$) reduction in the amount of time spent in NREM sleep as compared to saline treatment. **Conclusions**: Our results suggest that A2aR present in the NacC mediates alcohol-induced sleep. This is first step towards understanding mechanistic insights into the interactions between the reward circuitry and the sleep-wakefulness, and provide an impetus for the development of novel sleep-focused therapeutics to treat AUD.

Disclosures: M. Thakkar: None. **R. Sharma:** None. **M. Parikh:** None. **A. Chischolm:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.04/NN13

Topic: F.07. Biological Rhythms and Sleep

Support: Natural Sciences and Engineering Research Council of Canada CGS-M
Ontario Graduate Scholarship
Carleton University International Research Seed Grant
European Research Council STG-715933

Title: Activation of sleep deprivation-sensitive basolateral amygdala neurons promotes NREM sleep

Authors: *N. K. KOZIEL LY[1], M. E. OSBORNE[1], C.-L. LEU[2,3], R. H. WILLIAMS[2], M. J. CHEE[1];
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Abstract: Chronic sleep deprivation (SD) is associated with worsened physiological and mental health outcomes. To develop interventions against SD-related deficits, we must delineate the brain regions compromised by sleep loss. Preliminary findings from whole-brain tissue clearing showed elevated expression of the cell activity marker cFOS in the mouse amygdala after SD, but the specific SD-activated amygdalar nuclei were unresolved. Here, we mapped the distribution of SD-activated cells within the amygdala and determined their functional contribution to sleep-wake behaviour. Tissue from male wildtype mice (N = 3 per group) that slept undisturbed (control), underwent 4-h SD, or 4-h SD with 2-h recovery sleep (RS) were immunolabeled for cFOS and parcellated to generate standardized maps of cFOS+ cells in the amygdala. There were more cFOS+ cells in several amygdalar regions, including the central, basomedial, medial, and basolateral amygdala. Interestingly, while RS decreased cFOS activity in most SD-activated cells, those in the anterior basolateral amygdala (BLAa) remained elevated after RS (control: 60 ± 36 cells; SD: 299 ± 8 cells; RS: 218 ± 26 cells). We then performed whole-cell patch-clamp recordings from control or SD-treated mice to assess the functional activation of BLAa cells. There were no differences in the resting membrane potential of BLAa cells in control (-69 ± 2 mV, n = 10) or after SD (-69 ± 3 mV, n = 11). However, BLAa cells were more excitable and needed less current to fire action potentials after SD (control: 73 ± 9 pA, n = 12; SD: 37 ± 7 pA, n = 11). Excitatory synaptic input to BLAa cells also tended to be higher after SD (control: 1.9 ± 0.9 Hz, n = 3; SD: 2.4 ± 0.3 Hz, n = 9). To isolate the behavioural contributions of SD-sensitive cells, we trapped the expression of the chemogenetic, excitatory hM3D(Gq) receptor in BLAa cells of tamoxifen-inducible *Fos*$^{2A\text{-}iCreERT2}$ mice during SD (N = 4). Treatment with clozapine-N-oxide (CNO; 3 mg/kg) at ZT1 in undisturbed mice increased the percent time animals spent in non-rapid eye movement (NREM) sleep (59 ± 6%) compared to vehicle-treated controls (49 ± 5%). Moreover, CNO treatment could also increase NREM sleep in sleep deprived mice (vehicle: 22 ± 9%; CNO: 30 ± 10%). In summary, BLAa cells were more excitable after SD, and the selective activation of SD-sensitive BLAa cells promoted NREM

sleep. Together, these results point to a new amygdalar candidate that contributes to the regulation of sleep-wake behaviour by increasing sleep pressure.

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Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.05/NN14

Topic: F.07. Biological Rhythms and Sleep

Title: The role of the cortical projections to the ventrolateral preoptic nucleus in sleep regulation

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Abstract: Sleep is divided in two neurophysiological states : NREM (non-rapid eye movement) sleep and REM (rapid eye movement) sleep. The mechanisms by which these two states alternate remain poorly understood. Neuroanatomical studies showed that sleep-promoting structures, such as the VLPO (ventrolateral prepotic nucleus), receive projections from the PFC (prefrontal cortex). Thus, higher level structures could play a role in brain states regulation. Here, we used optogenetic tools to specifically activate the PFC-VLPO pathway in mouse brain slices as well as in freely moving mice. In this latter case, animals were implanted with electrodes to record the local field potentials in different brain structures in order to perform a brain states scoring. Our *ex vivo* results highlighted an excitation of the putative VLPO sleep-promoting neurons when the cortical afferents were locally activated by light pulses, demonstrating the functionality of this pathway. Our *in vivo* results showed that the PFC-VLPO pathway activation disrupted the theta oscillation during REM sleep. Moreover, these optogenetic stimulations led to an increase in ripples density, reinforcing the idea of a transition out of REM sleep. In conclusion, our results suggest that the activation of the PFC-VLPO pathway interrupts and fragments REM sleep in favor of NREM sleep. These results are consistent with the established role of the VLPO in promoting NREM sleep.

Disclosures: M. Chouvaeff: None. **T. Gallopin:** None. **K. Benchenane:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

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Program #/Poster #: PSTR558.06/NN15

Topic: F.07. Biological Rhythms and Sleep

Support: JSPS KAKENHI 2KF0048
AMED grant number JP22gm1110008
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Title: Direct evidence of cerebral blood flow upsurge during REM sleep: toward elucidating the refreshing mechanism in the brain during sleep

Authors: *C.-J. TSAI[1], T. NAGATA[2], C.-Y. LIU[1], T. SUGANUMA[1], T. KANDA[1], T. MIYAZAKI[1], K. VOGT[1], M. YANAGISAWA[1], Y. HAYASHI[1,3];
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Abstract: Sleep is generally considered a period of recovery, but how cerebral blood flow (CBF) level changes across sleep/wake states has remained unclear. CBF is critical in maintaining energy-dependent processes and clearing metabolic wastes. Hence, we aim to elucidate the role of sleep in regulating CBF. Till now, conflicting conclusions regarding the dynamics of CBF during sleep/wake have been drawn from different methods (such as positron emission tomography, Doppler methods, near-infrared spectroscopy, and functional magnetic resonance imaging) and often lacked the results of rapid-eye movement (REM) sleep. Hence, we developed an alternative strategy of using 2-photon microscopy to directly measure the movement of individual red blood cells within cortical capillaries, specifically when the mouse was awake or in REM sleep, or in non-REM (NREM) sleep. In our method, we were able to calculate the speed and number of red blood cells flowing into the capillaries, where actual substances are exchanged between blood and brain tissue, during sleep in mice. Across multiple cortical areas, the level of capillary CBF was surprisingly comparable between wakefulness and NREM sleep, while it is almost twice as high during REM sleep. We also found that the adenosine A2a receptor, the target of caffeine in preventing falling asleep, is crucial for the upsurge of capillary CBF during REM sleep, whereas it seemed not to be involved in capillary CBF during other sleep/wake stages. Our findings establish a new paradigm for future studies of the function of REM sleep and the underlying mechanisms. According to previous studies, CBF dysregulation affects numerous biological processes and links to neurodegenerative disorders, such as Alzheimer's disease. Meanwhile, reduced REM sleep showed an increased risk of dementia. Our results of observing the capillary CBF upsurge during REM sleep prompt speculation that the insufficient substances exchange and waste removal during REM sleep results in the deterioration of normal brain function and accumulation of waste products, and finally leads to the development of dementia.

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Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

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Program #/Poster #: PSTR558.07/NN16

Topic: F.07. Biological Rhythms and Sleep

Support: NSF of China 31771195
NSF of China 81790640

Title: Controlling Eye Movements And REM Sleep By Distinct Cholinergic Neurons In Oculomotor Nucleus

Authors: *C. JIANG, X. TAN, B. YAN, J. ZHANG;
Fudan Univ., Shanghai, China

Abstract: ABSTRACT The oculomotor nucleus (OMN) in midbrain is a well-known center for eye movements (EMs). As a paradigmatic behavior, EMs showed distinct activation patterns in rapid eye movement (REM) and non-REM (NREM) sleep stages. Previous studies indicates that OMN may be involved in the regulation of EMs during REM sleep. However, it is still unclear whether the neurons in OMN participated in regulating sleep states and how they modulate different sleep states and EMs separately. In this study, we found that a subsets of cholinergic neurons within the OMN were active during sleep and exhibited increased activity before REM sleep ends, especially during transitions from REM sleep to wakefulness. Through the application of optogenetic manipulation and calcium imaging, we revealed the existence of functional heterogeneity among OMN neurons and identify a subset of cholinergic neurons which did not control EMs could terminate REM sleep. We identified the nucleus papilio (NP) as a potential effector pathway in lighting the OMN for initiation of REM-off. Our results suggested that OMN is a key node that possesses functionally segregated subpopulations capable of parallel, independent control of EMs and sleep.

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Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

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Topic: F.07. Biological Rhythms and Sleep

Support: KEIT20008980

Title: Gabaergic activation by extract of zizyphus seed prolongs sleep and enhances sleep quality in animal models.

Authors: *M. KIM, S. OH;
Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: Sleep is the most basic physiological necessity for human life, and sleep disorders can be a potential cause of neurodegenerative diseases. Due to the various side effects of prescription drugs for sleep disorders such as benzodiazepines, interest in sleep-promoting compounds derived from natural products is increasing. *Zizyphus seed* (Zizy) is traditionally known as an analgesic, sedative, and remedy for insomnia and anxiety. Therefore, this study evaluated the sleep-promoting activity of Zizy extract (100, 200 mg/kg) and its active substance, Jujuboside A, using a pentobarbital-induced sleep model. In addition, the effects of Zizy extract on rapid eye movement (REM) and non-REM sleep were analyzed by electroencephalography (EEG). In a pentobarbital-induced sleep test using mice, oral administration of Zizy extract showed longer sleep duration in a dose-dependent manner than both hypnotic and sub-hypnotic pentobarbital alone administration groups. Zizy extract also attenuated sleep disturbance in mice with caffeine-induced insomnia. Jujuboside A also prolonged sleep time in a dose-dependent manner in a pentobarbital-induced sleep test. Administration of Zizy extract significantly enhanced sleep quality, especially the relative power of low-frequency (delta) waves, compared to the normal group in rat EEG measurements. Jujuboside A and muscimol (a $GABA_A$ receptor agonist) both increased chloride (Cl^-) uptake in the SH-SY5Y human cell line. After oral administration of Zizy extract for 7 days in rats, the expression of $GABA_A$ receptors α1-, and β2-subunits and $GAD_{65/67}$ were regulated in the rat brain. These results suggest that Zizy extract increases sleep duration through GABA receptor activation and gives a chance to become a functional food for improving sleep disorders.

Disclosures: M. Kim: None. **S. Oh:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant 1P01HL149630-01

Title: Inputs to the CGRP/Calca neurons of the parabrachial nucleus

Authors: *M. KORKUTATA, R. DE LUCA, B. FITZGERALD, E. ARRIGONI, T. E. SCAMMELL;
Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: The parabrachial nucleus (PB), located in the dorsolateral pons, primarily contains glutamatergic neurons which relay sensory information related to visceral malaise, taste, temperature, pain, and itch to various forebrain regions, including the thalamus, hypothalamus, and extended amygdala. Among these neurons, specific subpopulations express *Calca*, which encodes for the calcitonin gene-related peptide (CGRP). These CGRP-expressing neurons in the external-lateral PB have been implicated in various homeostatic functions and play a crucial role

in regulating appetite, pain and hypercapnic-induced arousals and transmitting signals related to threats to the extended amygdala. Though some studies in rats and mice have identified afferent projections to the CGRP-expressing neurons within the PB, our understanding of these crucial inputs remains incomplete. We mapped afferent projections to the lateral parabrachial nucleus (PB) of mice using conventional CTb retrograde tracing, and then used conditional rabies virus retrograde tracing to map monosynaptic inputs specifically targeting the CGRP-expressing neurons. To further characterize the neurochemical identity of these inputs, we utilized GABA and glutamate reporter mice. We found that CGRP-expressing PB neurons receive GABAergic afferents from regions such as the lateral capsule of the central nucleus of the amygdala, oval nucleus of the bed nucleus of the stria terminalis, substantia innominata, and the ventral lateral periaqueductal gray. In addition, they receive glutamatergic afferents from the infralimbic and insular cortex, paraventricular hypothalamus, parasubthalamic nucleus, and nucleus of the solitary tract. Finally, we employed channelrhodopsin-2 (ChR2)-assisted circuit mapping (CRACM) to determine whether these inputs monosynaptically synapse onto the CGRP-expressing PB neurons. We found that roughly 60% of CGRP-expressing neurons in the PB are inhibited by the central nucleus of the amygdala through a $GABA_A$-mediated signaling. These findings provide a comprehensive neuroanatomical framework for understanding the afferent projections regulating the CGRP-expressing PB neurons.

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Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

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Program #/Poster #: PSTR558.10/NN19

Topic: F.07. Biological Rhythms and Sleep

Support: Funded by Sanofi

Title: HCRT-MJD model mice show sex-differences in the manifestation of narcolepsy type I (NT1) phenotypes

Authors: *D. D. AGUILAR[1], T. TAKSIR[2], S. GHIASVAND[2], J. BUCCI[3], B. ZHANG[3], D. BANGARI[2], C. MOREL[2], P. PIEPENHAGEN[2], P. SARDI[1], J. DODGE[1], E. JENSEN[1];
[1]Rare & Neurologic Dis. Therapeut. Area, [2]Translational In Vivo Models, [3]Precision Med. and Computat. Biol., Sanofi, Cambridge, MA

Abstract: Narcolepsy Type I (NT1) is a rare neurodegenerative disease characterized by the loss of orexin or orexin-producing neurons. Orexin or hypocretin (HCRT) plays a critical role in regulating central nervous system (CNS) and peripheral functions including the maintenance of wakefulness, food and energy homeostasis, and stress. In NT1 patients, orexin loss leads to excessive daytime sleepiness (EDS), cataplexy, weight gain, hypertension, and many other

psychiatric and cognitive issues. Here, we describe the characterization of narcolepsy-related phenotypes in the HCRT-MJD model of NT1, which are reported to feature orexin neuron loss starting after postnatal day 4. We show progressive weight gain in HCRT-MJD mice starting at 12 weeks of age, consistent with previous characterizations. Furthermore, we observe lower nighttime (active period) activity in the NT1 mice without significant differences in daytime activity. In the open field assay at 12 weeks of age HCRT-MJD mice traveled a shorter distance and were slower. Additionally, we observed reduced orexin A levels in the plasma of the HCRT-MJD mice across multiple time points, a novel observation. Interestingly, upon separation by sex, female HCRT-MJD mice showed more robust statistically significant differences in weight gain, nighttime activity, Open Field activity, and Orexin A plasma levels. These novel findings suggest that there are sex-related differences in the manifestation of the narcoleptic phenotype in this model, supporting the importance of characterizing both sexes in disease models. Currently, we are continuing to characterize HCRT-MJD mice for orexin loss, changes in orexin projections, and narcoleptic sleep phenotypes using electroencephalogram (EEG)/electromyography (EMG). In summary, our results show that the HCRT-MJD mice recapitulate many aspects of narcolepsy and demonstrate the potential of these mice as a preclinical model for the development of novel therapies for NT1, which are urgently needed because current treatments leave many debilitating symptoms unaddressed for NT1 patients. Disclosures: This study was funded by Sanofi. All authors are Sanofi employees and may hold shares and/or stock options in the company. All in vivo studies were conducted according to IACUC approved protocols in an accredited institution following institutional, local, state, and country requirements.

Disclosures: D.D. Aguilar: A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **T. Taksir:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **S. Ghiasvand:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **J. Bucci:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **B. Zhang:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **D. Bangari:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **C. Morel:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **P. Piepenhagen:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **P. Sardi:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **J. Dodge:** A. Employment/Salary (full or part-time):; Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock. **E. Jensen:** A. Employment/Salary (full or part-time):;

Sanofi. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi stock.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.11/NN20

Topic: F.07. Biological Rhythms and Sleep

Support: Academy of Finland
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Title: The mouse sleep cycle is shorter than generally believed

Authors: *H. TANILA, S. HÄKLI, T. BOC, N. JIN;
A. I. Virtanen Inst., Univ. of Eastern Finland, Kuopio, Finland

Abstract: An established way of dividing the mouse sleep-wake cycle into stages is to coin periods with high EMG and low EEG amplitude as wake and the rest as sleep. Periods of very low EMG and high theta/delta ratio are considered REM sleep and the opposite pattern NREM sleep. However, there is prevailing uncertainty about the criteria for NREM substages, especially about the wake - N1 transition and the existence of the N3 state. Further, the onset of REM sleep is not sharp and often a transition period is identified. We aimed to improve identification of different sleep stages in the mouse by recordings with multiple intracerebral electrodes. Nine C57BL/6J male mice were implanted with double or triple wire electrodes into the hippocampus, somatosensory cortex and olfactory bulb, with conventional skull screw electrodes above the frontal cortex, a reference electrode above the cerebellum, and neck EMG electrodes. The mice were trained to sleep in a cardboard cylinder on a light table and plugged to a headstage connected to a light recording cable with a counterweight. Sessions with > 1 h of sleep of the 3-h total recording time were included in the analysis. Wake - N1 transition was identified by attenuation of EMG and disappearance of prominent gamma oscillation in the olfactory bulb. N1-N2 transition was characterized by appearance of positive slow-waves in subgranular cortical layers and sleep spindles in screw channels. We observed N2 to alternate between slow-wave + spindle (N2s) and slow-wave no spindle (N2n) stages. No human N3 like stage was identified. We further divided REM sleep into two substages. During the initial asynchronous stage (aREM), only the hippocampal channels showed regular theta oscillation while the cortical channels showed slow-waves and spindles intermingled with bouts of theta entrainment. During the synchronous stage (sREM) all channels but the olfactory bulb were entrained with the dominant hippocampal theta rhythm. The REM ended suddenly with or without a brief bout of a second aREM. In addition, the NREM epochs showed a repeating pattern of N1 - N2n - N2s - N2n - N1 in cycles that took 80 - 100 s, corresponding to an underlying oscillation of 0.01 -

0.012 Hz. The cycling pattern was interrupted during REM sleep. We provide a new division of mouse sleep into six stages that all have their unique EEG/EMG signatures.

Disclosures: H. Tanila: None. **S. Häkli:** None. **T. Boc:** None. **N. Jin:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

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Program #/Poster #: PSTR558.12/OO1

Topic: F.07. Biological Rhythms and Sleep

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CDA-2 IK2 BX004905
R01 NS119227
R01 NS069777

Title: Chemogenetic activation of mouse basal forebrain Npas1+ neurons promotes wakefulness

Authors: T. TROPPOLI[1], C. YANG[1], D. S. UYGUN[1], S. CHAN[2], J. T. MCKENNA[1], *R. E. BROWN[1];
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Abstract: The basal forebrain (BF) is a key brain area regulating sleep-wake behavior. Chemogenetic activation of all GABAergic neurons in the BF strongly promotes wakefulness, though which subpopulation of BF GABA neurons is responsible remains unclear. Here we report the effect of chemogenetically activating a major subpopulation of BF GABAergic neurons distinct from parvalbumin neurons which express the transcription factor neuronal PAS domain 1 (Npas1). Npas1-cre-2A-TdTomato mice were used for experiments. Npas1 neurons in the BF were bilaterally transduced with the designer receptor hM3Dq and the red fluorescent protein mCherry as a marker of expression. One month later, mice received intraperitoneal injections of saline (vehicle), 0.3 or 1 mg/kg clozapine-N-oxide (CNO) in the light (inactive) period (ZT2) in a within-subject design. Following saline injections (n=9), average sleep latency was <30 min and REM latency was <50 min. In contrast, injections of 0.3 (n=9) or 1mg/kg (n=7) resulted in significantly longer latencies to both non-REM sleep (approximately 100 min) and REM sleep (>150 min). CNO significantly increased wakefulness and suppressed NREM sleep vs. saline within 2 hours of administration. Additionally, REM sleep was significantly suppressed within 3 hours of CNO administration. Latencies and amounts of wakefulness were not significantly different between 0.3 and 1mg/kg doses of CNO and no rebound of NREM or REM was seen during the remainder of the light period. CNO (0.3 mg/kg) alone had no significant effect on sleep-wake behavior or cortical EEG (N=4). In a subset of the mice, animals received saline, 0.3 or 1 mg/kg CNO 2 hours prior to sacrifice. Expression of the immediate early gene product Fos was quantified using immunohistochemistry. In saline treated mice (n=3),

the % of mCherry+ neurons expressing Fos was less than 5%, whereas following 0.3 mg/kg CNO (n=3) or 1 mg/kg CNO (n=2) it was greater than 50%, confirming activation of the Npas1 neurons by CNO. The percentage of neighboring cholinergic (ChAT) and parvalbumin (PV) neurons which expressed Fos also increased following CNO injections, consistent with a wake-active profile, but remained below 20% (PV) or 10% (ChAT). We conclude BF Npas1+ neurons represent a novel subtype of BF wakefulness-promoting neurons, distinct from previously characterized PV, cholinergic and vGluT2 neurons.

Disclosures: **T. Troppoli:** None. **C. Yang:** None. **D.S. Uygun:** None. **S. Chan:** None. **J.T. McKenna:** 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.; JTM received partial salary compensation and funding from Merck MISP but has no conflict of interest with this work. **R.E. Brown:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.13/OO2

Topic: F.07. Biological Rhythms and Sleep

Support: Department of Veterans Affairs Merit Research Award I01BX002661
NIH Grant AA028175-01
MU Research Council (URC-22-006)

Title: Cholinergic interneurons in the shell region of the nucleus accumbens regulate binge alcohol consumption

Authors: *R. SHARMA[1,2], M. PARIKH[2], A. CHISCHOLM-ZUNIGA[2], M. M. THAKKAR[3]; [1]Neurol., Univ. of Missouri, Columbia, MO; [2]Neurol., [3]HSTMV Hospital/University of Missouri, HSTMV Hospital/University of Missouri, Columbia, MO

Abstract: Background: Alcohol use disorder (AUD) is a chronic and relapsing disorder. Notably, the predominant contributor to the development of AUD is binge or heavy episodic alcohol consumption. The involvement of the nucleus accumbens (NAc), particularly the shell region (NAcSh), in binge drinking is well-recognized. However, the precise neuronal mechanism is unclear. The NAc contains several neuronal populations, including the medium spiny GABAergic projection neurons (MSNs; >95%) and cholinergic interneurons (CINs; 2-3%). Although small in numbers, the CINs provide extensive local innervation and regulate the excitability and plasticity of MSNs, and have been recently emerged as key players in modulating reward and addiction processes. While the role of MSNs in reward and addiction has been extensively studied, the specific contribution of CINs in the context of binge alcohol consumption and the development of AUD remains poorly understood. Based on prior studies

highlighting the dynamic nature of reward-related responses in these tonically active CINs, we hypothesized that binge drinking would elicit distinct changes in CIN activity. **Methods**: To test our hypothesis, a viral-mediated gene transfer approach was used to express a fluorescent sensor, GCaMP, in the CIN present in the NAcSh of transgenic male ChAT-cre mice. Additionally, a small, integrated microscope was fixed to the head of the animals to capture the fluorescence signal from the CIN expressing GCaMP. Animals were exposed to alcohol (N=3)/sucrose (N=3) using the 4-Day DID paradigm. During the first three days of alcohol/sucrose consumption, no imaging was conducted. On Day 4, CIN activity was recorded for 4h (last 20 min/hour) during alcohol/sucrose consumption. The fluorescence signal (df/f) was measured as the change in fluorescence intensity relative to the baseline fluorescence signal, which reflects the changes in calcium levels relative to the action potentials. The amount of alcohol or sucrose was measured at the end of 4h. Upon completion, histology was performed to confirm the presence of GCaMP in the CINs and localization of the GRIN lens in the NAcSh. **Results**: A total of 131 individual neurons were imaged. Z-score was calculated using $\Delta F/F0$ values of individual neurons in mice exposed to sucrose ($N = 67$) or alcohol ($N = 64$) which showed that there was a significant ($t = 12.96$, $df = 129$, $p < 0.001$) increase in the activity of the CIN in the NAcSh during alcohol consumption as compared to sucrose. **Conclusions**: Our results provide compelling evidence for the involvement of CINs in the NAcSh in regulating binge alcohol consumption and suggest their potential as a therapeutic target for addressing AUD.

Disclosures: R. Sharma: None. **M. Parikh:** None. **A. Chischolm-Zuniga:** None. **M.M. Thakkar:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.14/Web Only

Topic: F.07. Biological Rhythms and Sleep

Support: Korea Medical Device Development Fund grant, RS-2022-00165378

Title: Pilot Study to Explore the Efficacy and Safety of At-home Transcutaneous Electrical Trigeminal Nerve Stimulation in Insomnia

Authors: *J. KIM[1], S. LIM[1], S. KIM[1], W.-J. LEE[2], J. KIM[2], S. NAM[2], C.-H. YUN[2]; [1]Ybrain, Inc, Seongnam-si, Korea, Republic of; [2]Seoul Natl. Univ. Bundang Hosp., Department of Neurology, Bundang Clinical Neurosci, Korea, Republic of

Abstract: Insomnia patients often exhibit an overactive sympathetic branch of the autonomic nervous system, with hyperarousal models reported as key in the pathophysiology of insomnia. Transcutaneous Trigeminal Nerve Stimulation (TENS) may stabilize the overactive sympathetic nerve and improve insomnia symptoms. This study aimed to investigate the effects and safety of at-home TENS usage for individuals diagnosed with insomnia. A 4-week, single-center,

randomized sham-controlled study was conducted. Twenty-nine individuals (18 females; mean age 49.9±11.0 years), aged between 19 to 65 years and diagnosed with insomnia, were included in the study and randomized into an experimental group (n=14) and a control group (n=15). Each participant administered TENS for 20 minutes daily, using a YPS-401B device (Ybrain Inc., South Korea) before bedtime every night for the duration of the study. The experimental group employed an actual device that applied TENS, including pulse (10kHz), and burst waveform (10Hz) to the trigeminal nerve on the forehead. In contrast, the control group attached an identical-looking device to the same location but received sham stimulation. The effectiveness of insomnia symptom improvement was evaluated using questionnaires, such as the Insomnia Severity Index (ISI), Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), Beck Depression Index (BDI), and Generalized Anxiety Disorder 7-item scale (GAD-7), both pre- (T0) and post- (T1) the four-week TENS intervention. There were no significant interactions between time and groups concerning sleep and mood parameters. However, in the PSQI sub-scale analysis, a notable time× group interaction emerged in habitual sleep efficiency ($p < 0.05$). Subgroup analysis of patients with moderate to severe insomnia (ISI score of 15 or higher) revealed a significant interaction between time and group in the PSQI total score ($p < 0.05$). The experimental group (T0: 14.3±2.9, T1: 9.7±3.4) demonstrated a better improvement in the PSQI score compared to the control group (T0: 13.1±1.5, T1: 10.8±3.4). Furthermore, no serious adverse events were reported in either group. The findings of this study suggest that at-home TENS application can have a beneficial effect on sleep quality, particularly sleep efficiency. However, TENS's effectiveness may be limited in patients with moderate to severe insomnia.

Disclosures: **J. Kim:** A. Employment/Salary (full or part-time):; Ybrain, Inc. **S. Lim:** A. Employment/Salary (full or part-time):; Ybrain, Inc. **S. Kim:** A. Employment/Salary (full or part-time):; Ybrain, Inc. **W. Lee:** None. **J. Kim:** None. **S. Nam:** None. **C. Yun:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.15/OO3

Topic: F.07. Biological Rhythms and Sleep

Title: The Impact of sleep deprivation on human cortical excitability, LTP-LTD like neuroplasticity, and cognitive functions in humans

Authors: *M. SALEHINEJAD[1], E. GHANAVATI[2], M.-F. KUO[4], M. A. NITSCHE[3]; [1]Psychology and Neurosciences, Leibniz-Institut fur Arbeitsforschung an der TU Dortmund, Dortmund, Germany; [3]Psychology and Neurosciences, [2]Leibniz Res. Ctr. for working environment and Human Factors, Dortmund, Germany; [4]Psychology and Neurosciences, Leibniz Res. Ctr. for Working Envrn. and Human Factors, Dortmund, Germany

Abstract: Sleep strongly affects synaptic strength, making it critical for cognition, especially learning, and memory formation. Whether and *how* sleep deprivation modulates human brain

physiology and specifically the parameters that are relevant for human cognition and also cognitive functions is not well understood. Here we examined how overnight sleep deprivation vs overnight sufficient sleep affects (a) cortical excitability, measured by different protocols of transcranial magnetic stimulation (TMS), (b) inducibility of LTP- and-LTD-like plasticity via anodal and cathodal transcranial direct current stimulation (tDCS) respectively, and (c) learning, memory and attention and their electrophysiological correlates. To do so, we recruited 30 healthy, right-handed participants in this randomized, crossover study. All participants attended two experimental sessions after having sufficient sleep (23:00-8:00), or sleep deprivation (23:00-8:00). Using single-pulse and double-pulse TMS protocols, corticospinal and corticocortical excitability were measured after sleep deprivation or sufficient sleep. Additionally, neuroplasticity was induced with anodal and cathodal tDCS in two parallel groups after sufficient sleep and sleep deprivation. Participants performed motor learning, working memory, and attention tasks at the beginning of each experimental session (sufficient sleep vs sleep deprivation) while their electroencephalography was recorded. Our results suggest that sleep deprivation upscales cortical excitability due to enhanced glutamate-related cortical facilitation and decreased and/or reversed GABAergic cortical inhibition. Furthermore, tDCS-induced LTP-like plasticity is abolished while the inhibitory LTD-like plasticity is converted to excitatory LTP-like plasticity under sleep deprivation. This is associated with increased EEG theta oscillations due to sleep pressure. Finally, we show that learning and memory formation, behavioral correlates of plasticity, and working memory and attention, which are associated with cortical excitability, are impaired during sleep deprivation. Our data suggest that upscaled brain excitability, and altered plasticity, due to sleep deprivation, are associated with impaired cognitive performance.

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Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.16/OO4

Topic: F.07. Biological Rhythms and Sleep

Title: Sleep promotes increased brain pulsations compared to narcolepsy

Authors: *M. JÄRVELÄ[1], J. KANANEN[1], V. KORHONEN[2], V. KIVINIEMI[1];
[1]Univ. of Oulu, Oulu, Finland; [2]Oulu Univ. Hosp., Oulu, Finland

Abstract: We have earlier shown with ultrafast 10 Hz whole-brain functional magnetic resonance imaging sequence called magnetic resonance encephalography (MREG) that sleep elicits increased brain pulsations that arise from brain vasomotion and cardiorespiratory rhythms. Our recent study showed that narcolepsy type 1 (NT1) presents decreased cardiorespiratory but increased vasomotor brain pulsations. Yet, no direct comparison of sleep and awake state

narcolepsy has been conducted investigating these pulsations. Combining our earlier work, we investigated if brain pulsations in sleep and NT1 differ by comparing spectral power and entropy between sleeping healthy controls (HC) and awake NT1. We hypothesized that NT1 would present lower vasomotor/cardiorespiratory power and higher entropy when compared with sleeping HC. Twenty-three patients with NT1 (12 females, 27.6 ± 9.03 years) and 13 HC (6 females, 30.5 ± 9.84 years) were recruited. Two NT1 patients were excluded for corrupted data. 5 min of EEG-confirmed sleep (total 95% of N1/N2 sleep) MREG (10 full brain images per second) data acquired with Siemens 3T scanner was used. NT1 group were told to stay at wakeful rest. Standard FSL pipeline was used for MREG preprocessing. MREG spectrum was obtained, and anesthesia and MREG data were then used to estimate subject-wise cardiac- and respiratory frequencies. MREG data was filtered according to individuals' own cardiorespiratory frequency ranges and to very low frequency range (0.008 - 0.1 Hz). Pulsation-wise spectral power and entropy were calculated, and these voxel-wise brain maps were registered to 3 mm MNI152 space. FSL *randomise* was used for statistical inference while controlling for sex and age (NT1 n = 21 and HC n = 13 in all statistical calculations, significance threshold at $p < 0.05$ family-wise error rate corrected). In accordance with our hypothesis, we found that sleep indeed enhances spectral power compared to narcolepsy in full band, and especially in cardiac pulsations. Respiratory pulsations in the thalamic/ventricular area are increased in the null model where no control for sex and age are used. Surprisingly, we observed no significant differences in vasomotor pulsation power between the groups suggesting a compensatory mechanism in awake state NT1. Against our hypothesis, full band spectral entropy was greater in sleep than in NT1 in a location inside/surrounding the right angular gyrus. Our results show that the forces driving the cerebrospinal fluid flow are increased in sleep, but the overall power of the signal is locally more concentrated in the default mode network in NT1.

Disclosures: **M. Järvelä:** None. **J. Kananen:** None. **V. Korhonen:** None. **V. Kiviniemi:** None.

Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.17/OO5

Topic: F.07. Biological Rhythms and Sleep

Support: National Natural Science Foundation of China (81571296, 32170983)
Shanghai Science and Technology Committee (21ZR1408000)
National Major Project of China Science and Technology Innovation 2030 for Brain Science and Brain-Inspired Technology (2021ZD0203400）

Title: The role and circuits of the rostromedial tegmental nucleus in regulating sleep

Authors: *S. YANG, Y.-N. ZHAO, J.-B. JIANG, Y. ZHANG, W.-M. QU, Z.-L. HUANG; Fudan Univ., Shanghai, China

Abstract: Neuropsychiatric diseases such as anxiety, depression, and Parkinson's disease are often accompanied by sleep disorders, which exacerbate the symptoms of these diseases. Dopamine produced by neurons in the midbrain plays a key role in processing reward, aversive, cognitive signals, and wakefulness. Therefore, abnormal dopamine is closely associated with these neuropsychiatric disorders. The rostromedial tegmental nucleus (RMTg), a structure in the midbrain, is primarily composed of GABAergic neurons. The axons from the RMTg densely project to midbrain dopaminergic neurons, acting as an inhibitory brake for dopamine neurons. Moreover, the RMTg sends projections to other sleep/awakening-related brain regions such as the dorsal raphe nucleus, and the lateral dorsal tegmental nucleus (LDT). We propose that the RMTg is involved in sleep-wake regulation. In this study, we manipulated the activities of RMTg neurons using chemogenetics and optogenetics and recorded electroencephalogram and electromyogram in freely behaving animals. Optogenetics combined with patch clamp was employed to assess functional connectivity between RMTg neurons and the targeted neurons *in vitro.* We found that activation of RMTg neurons by chemogenetics promoted non-rapid eye movement (Non-REM, NREM) sleep in rats. EEG power spectrum analysis revealed that the rats with activation of RMTg neurons exhibited higher slow-wave activity (SWA, an indicator of sleep depth). Conversely, rats after neurotoxic lesions showed decreased NREM sleep with reduced SWA at lights on. Moreover, specific activation of RMTg GABAergic neurons in vesicular GABA transporter (VGAT)-Cre mice during REM sleep immediately converted REM sleep to arousal and then initiated NREM sleep. In contrast, laser-mediated inactivation completely converted NREM to REM sleep and prolonged the duration of REM sleep. Using neural tracing and RNA interference techniques, we found that RMTg GABAergic neurons converted REM sleep to wakefulness and NREM sleep through indirect disinhibition of glutamatergic neurons in the LDT and direct inhibitory projections to the lateral hypothalamus, respectively. In all, these findings reveal an essential role of RMTg GABAergic neurons in the promotion of NREM sleep and suppression of REM sleep. The results may have important implications for the treatment of sleep disorders.

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Poster

PSTR558. Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR558.18/OO6

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R35GM142490
NIH Grant R00NS101065
BrightFocus Foundation
Whitehall Foundation

Title: Dynamical instability of the brain shaped by aging

Authors: *D. NGUYEN[1], A. HUTSON[1], Y. ZHANG[2], Y. XIE[1], S. D. DANIELS[1], E. PAUL[1], B. CHONG[1], E. M. SNYDER[1], F. S. FERRY[1], J. A. WALKER, Jr.[3], M. TABUCHI[4]; [1]Case Western Reserve Univ. Dept. of Neurosciences, Cleveland, OH; [2]Interdepartmental Neurosci. program, Univ. of California, Irvine, Irvine, CA; [3]Morehouse Col., Atlanta, GA; [4]Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: As sleep homeostatic regulatory mechanisms deteriorate with aging, sleep becomes more irregular and fragmented. Deleterious effects of disrupted sleep underlie neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases. While the circadian clock has been well-studied at the molecular level, the sleep homeostasis model is less understood. We focused on the R5 ring neurons within the ellipsoid body in Drosophila to assess sleep drive influence on sleep quality across the lifespan. Sleep homeostasis reflects the pressure for sleep that builds up with longer periods of wakefulness. Sleep pressure is altered by membrane potential fluctuations. Brain activity measured by local field potential revealed that network coordination is disturbed in aged flies compared to young, which may lead to an age-dependent reduction in sleep drives. RNAi screening was performed to locate age-inducing molecules affecting the structure of R5 neurons. Several young RNAi samples were clustered with aged control samples, indicating that the knockdown of these molecules modified young neurons to have similar morphology as aged neurons. Ly6, a protein that participates in sleep modulation, is one of the molecules that display this change.An RNA sequencing analysis of young and aged flies characterized various Ly6 genes as downregulated and upregulated. In congruence with the downregulation of genes involved in metabolism and protein synthesis during aging, we postulate that the loss of some Ly6 genes may also promote aging characteristics, and therefore the restoration of these genes will intervene with aging effects. On the other hand, the upregulation of Ly6 genes may be a compensatory response, revitalizing lost sleep regulation functions by increasing the expression of related genes. Overall, the dysregulation of Ly6 gene expression contributes to sleep instability. By identifying how Ly6 gene expression alters R5 neuronal structure and function, we can determine the consequences of structural deterioration on sleep quality and quantity and understand how sleep disruption aggravates neurodegenerative disorders. This insight may contribute to the development of a remedy to prevent or mitigate disease progression during aging.

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Poster

PSTR559. Drugs of Abuse: Reward and Motivation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR559.01/OO7

Topic: G.03. Motivation

Support: Swedish Research Council, VR project number 2018-02320 (to Eric Augier)

Title: Sex differences in aversion-resistant oxycodone intake and choice of oxycodone over healthy rewards

Authors: *S. ERIKSSON SOLANDER, V. SCHWABL, A. M. ANANIADOU, M. ATUDOREI, G. AUGIER, E. AUGIER;
BKV, CSAN, Linköping Univ., Linköping, Sweden

Abstract: Opioid misuse continues to be a major concern and a rapidly evolving public health crisis that requires innovative scientific approaches. Opioid addiction leads to a progressively increased choice of drugs over healthy rewards. However, the availability of alternative non-drug rewards has so far been largely overlooked in preclinical models of opioid use disorder (OUD). We recently used a procedure in which about 15% of outbred rats choose alcohol over an alternative high-value reward, a rate similar to human addiction, and identified that decreased expression of the GABA transporter GAT-3 within central amygdala (CeA) was causal for alcohol choice behavior and translated to humans. Whether choice of alcohol over a sweet reward translates to oxycodone is currently unknown. In the present study, we therefore evaluated choice between oral oxycodone and an alternative reward (sweet or social), as well as aversion-resistant oxycodone drinking, in both male and female rats. Using operant conditioning, we first trained an equal number of male and female Wistar rats (n=32 per group) to self-administer oxycodone orally. We found that males earned more oxycodone reinforcers and showed a higher motivation for oxycodone. Blood oxycodone metabolites concentrations were in addition strongly correlated with oxycodone reinforcers earned during self-administration. We then used an exclusive choice-based method to identify both male and female rats that continue to self-administer oxycodone at the expense of a high-value natural reward, a sweet solution or social interaction with a cagemate. We found that, in agreement with our previous work with alcohol, only a minority of outbred rats (10% or less) choose oxycodone over an alternative high-value reward. Prevalence of choosing oxycodone over the sweet reward was also higher in male rats. In marked contrast, we found that female rats drank more oxycodone despite adverse consequences, as modeled using quinine adulteration. Together, our results indicate profound sex differences in oxycodone-related behaviors and indicate the importance of developing more personalized treatment strategies to treat the different aspects of oxycodone use disorder.

Disclosures: S. Eriksson Solander: None. **V. Schwabl:** None. **A.M. Ananiadou:** None. **M. Atudorei:** None. **G. Augier:** None. **E. Augier:** None.

Poster

PSTR559. Drugs of Abuse: Reward and Motivation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR559.02/OO8

Topic: G.03. Motivation

Support: Swedish Research Council, VR project number 2018-02320 (to Eric Augier)

Title: Role of the GABA transporter GAT-3 in cocaine and oxycodone use disorders

Authors: A. LGUENSAT, G. AUGIER, V. SCHWABL, G. BOUHANICH, ***E. AUGIER**; Linköping University, BKV, Linköping, Sweden

Abstract: Among addictive disorders, cocaine use disorder (CUD) inflicts some of the greatest emotional and economic cost to the individual, families, and society. Despite this impact of CUD on individuals and society, no medications are currently approved for its treatment, and psychosocial treatments with evidence supporting their efficacy, such as contingency management or cognitive behavioral therapy-based relapse prevention have effect sizes in need of improvement. Thus, bringing forward CUD medications is a major unmet medical need. We recently identified that impaired GABAergic transmission, due to decreased expression of the GABA transporter GAT-3, within central amygdala (CeA) was causal for alcohol choice behavior and translated to humans. Whether this mechanism also operates for CUD is currently unknown. Supporting this hypothesis, the $GABA_B$ receptor agonist baclofen has been shown to also attenuate the reinforcing effects of cocaine and motivation to self-administer this drug in rodents. Our main objectives were therefore to investigate whether GAT-3 is also decreased in a rat model of CUD and whether experimentally impairing the function of GAT-3 in the CeA would promote escalation of cocaine intake and other CUD-like behaviors in rats. Using intravenous self-administration, we first trained Wistar rats to self-administer cocaine under an extended access of 6 hours (Long Access, LgA), a regimen that promotes escalation of drug intake and increased motivation to obtain cocaine. We found that GAT-3 expression was robustly decreased in the CeA of animals that escalated their cocaine intake compared to animals with stable intake (Short Access, ShA). We then investigated the functional role of GAT-3 in both male and female Wistar rats, using CeA injections of an AAV-shRNAi targeting GAT-3, or a scrambled control vector. GAT-3 KD in the CeA potently promoted escalation of cocaine intake and increased motivation for cocaine and cocaine craving, irrespective of sex. In marked contrast, we found that GAT-3 KD didn't affect oxycodone-related behaviors in a preclinical model of opioid use disorder (OUD). Finally, using RNAscope in situ hybridization, we investigated whether GAT-3 in the CeA is expressed in neurons, astrocytes, or both. All together, these results provide evidence that the GABA transporter GAT-3 may also play a role in cocaine-related behaviors and indicate that rescuing impaired GABA clearance due to suppressed GAT-3 expression might be a successful therapeutic mechanism in CUD.

Disclosures: A. Lguensat: None. **G. Augier:** None. **V. Schwabl:** None. **G. Bouhanich:** None. **E. Augier:** 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.; Eric Augier is PI of a research contract with Indivior Inc to evaluate novel candidates for AUD, which is not related to the present work.

Poster

PSTR559. Drugs of Abuse: Reward and Motivation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR559.03/OO9

Topic: G.03. Motivation

Support: Swedish Research Council, VR project number 2018-02320 (to Eric Augier)

Title: Social status matters: subordinate rats choose oxycodone over social interaction compared to dominant counterparts.

Authors: *A. BORRUTO, A. COPPOLA, H. KARLSSON, G. AUGIER, S. ERIKSSON SOLANDER, E. AUGIER;
Dept. of Clin. and Exptl. Med. (BKV), Linköping Univ., Linköping, Sweden

Abstract: Impaired social behavior is a prevalent characteristic observed across numerous psychiatric disorders, including substance use disorders. Animals that are socially subordinate or experience social stress often exhibit a heightened vulnerability to drug addiction. For instance, several studies conducted on monkeys and rats have shown that social status can influence the reinforcing effects of cocaine in an operant setting. However, it is still unclear whether social rank is associated with other traits that contribute to addiction susceptibility and whether social status can similarly predict oxycodone self-administration in both male and female rats. To investigate this, we first screened male and female Wistar rats (n=16 per sex) for their dominance- and subordination-like behavior using the confrontation tube test. We then used the recently developed operant model of choice of drugs over social rewards (in which social interaction consistently reduces self-administration and craving for stimulants and opioids) to study the impact of social status on choice of oxycodone over social interaction. Here, we showed that male subordinate rats, but not females, exhibited heightened reactivity to novelty without displaying an anxious phenotype when compared to dominant rats. Additionally, we found that male and female rats did not differ in their acquisition of oxycodone and social self-administration or in their motivation for the drug or social reward, irrespective of their social status. By contrast, subordinate male rats showed a higher preference for oxycodone over social interaction compared to dominant male animals, an effect that was not observed in females. Altogether, these findings highlight a sex-dependent link between social status and oxycodone preference, providing valuable insight into the development of more effective prevention and personalized treatment strategies for people with oxycodone use disorder.

Disclosures: A. Borruto: None. **A. Coppola:** None. **H. Karlsson:** None. **G. Augier:** None. **S. Eriksson Solander:** None. **E. Augier:** None.

Poster

PSTR559. Drugs of Abuse: Reward and Motivation

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR559.04/OO10

Topic: G.03. Motivation

Support: R00DA041493
R21DA053462
5T32DA018926-18

Title: Relationships between fentanyl self-administration and risk-taking behavior in rats.

Authors: *A.-R. WHEELER[1], L. M. TRUCKENBROD[2], M. GARNER[3], C. A. ORSINI[3]; [1]Univ. of Texas at Austin, Austin, TX; [2]Neurosci., Univ. of Texas, Austin, TX; [3]Psychology, The Univ. of Texas at Austin, AUSTIN, TX

Abstract: Individuals with opioid use disorder (OUD) display impaired decision-making behavior and elevated risk taking. To understand the relationship between opioid use and elevated risk taking, our lab employs a rodent model of decision making involving risk of explicit punishment (Risky Decision-making Task; RDT). In this task, rats choose between a small, safe food reward and a large food reward that is accompanied by increasing risk of mild footshock punishment. In Experiment 1, we examined whether individual differences in risk preference in the RDT predicted aspects of self-administration of the synthetic opioid fentanyl. Male Sprague-Dawley rats were characterized on the RDT and then underwent fentanyl self-administration (6 hours/day) for 21 days. During self-administration, rats escalated their fentanyl intake, but neither the rate of escalation nor overall fentanyl intake correlated with risk preference in the RDT. These data suggest that increased risk taking in individuals with OUD is likely a consequence of drug exposure. To explore this possibility, male and female rats were trained on the RDT until stable behavior emerged and then underwent fentanyl self-administration (6 hours/day) or sucrose self-administration for 14 days. Following the cessation of self-administration, rats remained undisturbed for 3 weeks and were then re-tested on the RDT to assess fentanyl-induced changes in risk taking. Relative to performance before self-administration, rats that self-administered fentanyl displayed an increase in risk taking, an effect that was absent in sucrose control rats. Increased risk taking in rats that self-administered fentanyl could not be attributed to impaired behavioral flexibility, augmented motivation to work for food or diminished sensitivity to footshock. These findings support our hypothesis that the high rates of risk taking in individuals with OUD are a direct result of chronic opioid exposure. Future work will determine whether there is a similar lack of relationship between drug-naïve risk taking and aspects of fentanyl self-administration in females and will also focus on identifying the neurobiological mechanisms that contribute to fentanyl-induced increases in risk taking. Collectively, this work will provide important insight into the neurobehavioral mechanisms underlying the relationship between opioid use and altered risk taking.

Disclosures: A. Wheeler: None. **L.M. Truckenbrod:** None. **M. Garner:** None. **C.A. Orsini:** None.

Poster

PSTR559. Drugs of Abuse: Reward and Motivation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR559.05/OO11

Topic: G.03. Motivation

Support: NIH Grant AA028602
Miami University

Title: Selective genetic deletion of Oprm1 in FoxP2-expressing neurons reduces aversion-resistant reward seeking

Authors: *H. M. CARVOUR[1], M. ROBERTSON[1], N. PARSADANYAN[1], M. MILLER[1], D. P. UNDERWOOD[1], C. ROEMER[1], E. P. ECCLES[1], T. W. PERRY[2], K. D. REAM[1], A. K. RADKE[2];
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Abstract: Mu-opioid receptors (MORs) in the amygdala and striatum are important in addictive and rewarding behaviors. For the current series of studies, we characterized the behavior of mice with genetic deletion of the MOR gene *Oprm1* in FoxP2-expressing neurons (FoxP2-Cre/Oprm1$^{fl/fl}$). FoxP2 is expressed in intercalated cells of the amygdala and a subset of striatal medium spiny neurons, both of which express MORs in wild-type mice. *Oprm1* deletion was first verified using fluorescent *in situ* hybridization. FoxP2-Cre/Oprm1$^{fl/fl}$ mice were tested for aversion-resistant alcohol consumption using an intermittent access (IA) task, conditioned place aversion (CPA) to morphine withdrawal, and operant responding for a sucrose reward. For IA, mice received the choice of 20% ethanol and water for 24 hours three days a week, with mice receiving only water on intervening days for five weeks. In the fifth week, escalating quinine concentrations at 10, 100, and 200 mg/L were added to the ethanol solution. Results from this study indicated that mice with the MOR-knockout were more sensitive to the quinine in ethanol and less aversion-resistant, as they decreased ethanol consumption from baseline at all quinine concentrations, while control animals did not. For the CPA study, mice received four conditioning days: two with morphine/naloxone injections and two with saline/saline, with 30 minutes between each injection. Following the second injection, mice were confined to one side of the conditioning apparatus. Both wild-type and knockout mice demonstrated withdrawal-induced aversion. For operant conditioning, mice were trained to nosepoke for 10% sucrose before undergoing a fading procedure with decreasing concentrations of 10%, 5%, and 2%. Lastly, mice were tested for aversion-resistance with escalating quinine concentrations of 10, 100, and 200 mg/L in the sucrose. Results from this study will determine whether deletion of *Oprm1* on FoxP2 neurons alters general sensitivity to aversion. Together, these results suggest that MOR expression on FoxP2-expressing neurons is not necessary for alcohol consumption or expression of opioid withdrawal but may be involved in aversion-resistant reward seeking.

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Poster

PSTR559. Drugs of Abuse: Reward and Motivation

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Topic: G.03. Motivation

Support: NIH Grant AA028602

Title: Effect of μ-opioid receptor knockout and sex on context fear extinction

Authors: *J. R. DOWELL, H. T. BECKETT, A. N. REICHERT, A. K. RADKE, J. J. QUINN; Psychology, Miami Univ., Oxford, OH

Abstract: Our understanding of the biological mechanisms underlying fear, trauma, and the development of related disorders like post-traumatic stress disorder (PTSD) is incomplete. The amygdala exhibits maladaptive changes/activity in PTSD patients. The amygdala serves an important role in the acquisition and expression of fear memories. Within the amygdala, the basolateral nucleus (BLA) integrates sensory information, with the central nucleus (CeA) helping to initiate/regulate a subsequent behavioral response. Between the BLA and CeA lie a subset of GABAergic neurons known as the intercalated interneurons (ITCs). They serve to modulate signaling, primarily from the BLA to the CeA. This results in inhibition of the CeA via ITC activation by the BLA. Functionally, ITCs appear to play a significant role in fear memory extinction. These ITC neurons densely express the inhibitory mu-opioid receptor (MOR), and thus are sensitive to both the endorphins/enkephalins and exogenous opioids. In this experiment, we sought to determine a potential role of ITC MORs in fear acquisition and extinction in male and female mice. To do this, we utilized a Cre expression-dependent knockout (KO) of Oprm1 from FoxP2-expressing neurons,which includes but is not selective for ITCs. Male and female Cre+ and Cre- mice were fear conditioned to a novel context using one footshock per day for five consecutive days. The next day, context extinction commenced using daily 30 minute sessions across 10 days. At least 2 days following the final extinction session, mice were tested on a hotplate to assess morphine-induced analgesia. Half of each experimental condition received an injection of saline, while the other half received morphine (10mg/kg/10ml), 60 minutes prior to the hotplate test. Our results demonstrate no significant effects of sex or genotype across context fear acquisition. However, we observed significant sex X session and genotype X session interactions across the 10 days of extinction, with females and Cre+ mice acquiring extinction more slowly compared to males and Cre- mice. Further, we observed no significant effects of sex or genotype on morphine-induced analgesia. These results replicate previous sex differences in extinction of fear and, further, iimply that MORs, possibly those expressed on ITC neurons, contribute to context fear memory extinction. Since the MOR knockout was not selective for ITC neurons, future experiments are needed to determine whether the critical MOR contribution to context fear memory extinction occurs in these ITC neurons.

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Poster

PSTR559. Drugs of Abuse: Reward and Motivation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR559.07/OO13

Topic: G.01. Fear and Aversive Learning and Memory

Title: Modulation of the prelimbic cortex to rostromedial tegmental nucleus pathway in reinstatement of cocaine seeking

Authors: *E. CARLSON[1], C. TOBUREN[2], B. DINU[2], S. PARMAR[2], P. VENTO[2];
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Abstract: Drug addiction is a chronic relapsing disorder characterized by persistent drug use and maladaptive decision-making despite high monetary, physiological, and interpersonal costs. Previous work from our lab and others suggests the rostromedial tegmental nucleus (RMTg), a GABAergic midbrain region with dense projections to dopamine neurons, is capable of modulating both cue- and drug-primed reinstatement of cocaine seeking. While a large body of research has demonstrated the prefrontal cortex to also be critical for reinstatement, far less is known regarding how these cortical and brainstem circuits interact to influence drug seeking. Notably, the prelimbic (PL) region sends an excitatory projection to the RMTg, and emerging findings demonstrate stimulation of this pathway promotes avoidance, while pharmacological inactivation of the PL to RMTg pathway increases cue-induced reinstatement for cocaine. Here, we demonstrate that chemogenetic PL to RMTg stimulation robustly suppresses cue-induced reinstatement of cocaine seeking, and this influence is impressively selective with negligible effects on locomotor activity, FR1 responding for food reward, or progressive ratio for food. Further testing into the PL role in modulating RMTg responses to rewarding and aversive stimuli were conducted using pharmacological and chemogenetic inactivation of the PL or its projections to the RMTg during a cue discrimination task. Specifically, rats were trained to lever press for sucrose in the presence of a reward-associated cue (light), a neutral cue (white noise) paired with no outcome, or an aversive cue (2Khz tone) followed by mild foot shock. Results suggest the PL to RMTg pathway is important for suppressing reward seeking in response to aversive environmental cues.

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Poster

PSTR559. Drugs of Abuse: Reward and Motivation

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Program #/Poster #: PSTR559.08/OO14

Topic: G.03. Motivation

Support: RO1DA044925

Title: Aberrant accumbal encoding of natural and opioid rewards following prenatal cannabis exposure

Authors: *R. YOUNG-MORRISON, M. LUJAN, N. MAIN AFSHAR, J. CHEER;
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Abstract: Pregnancy is a sensitive time for both mother and offspring. Substance use and abuse during pregnancy severely impacts the development of the offspring. Consumption of cannabis during pregnancy was reported in 7% of pregnant women in 2016-2017 (CDC). The antiemetic, analgesic and anxiolytic effects of THC, the main psychoactive component of cannabis, are sought by pregnant individuals as a way to treat pregnancy-related discomfort typical of the second and third trimesters. To replicate this consumption pattern, Sprague-Dawley dams were treated with 2 mg/kg THC (s.c) from postnatal day (PND) 5 to 20. Experiments were conducted when control- and THC-exposed offspring rats reached adulthood (7 months-old). Rats were implanted in the nucleus accumbens (NAc) with 16-channel electrode arrays used to determine cell firing encoding of rewards and outcome-predictive cues. Potential reward processing impairments introduced by *in utero* THC exposure were probed during fixed and progressive ratio (PR) operant responding, a Go-No-Go (GNG) task, and economic-based demand of food (palatable pellets) and opioid (remifentanyl) rewards. Recorded cells were clustered in order to look at differences in encoding. Specific attention was given to cue and reward response over time. A significant sex difference revealed prenatally-treated males have a higher break-point during PR during natural reward testing, and display increased impulsive-like behavior during GNG testing. In contrast, females prenatally exposed to THC did not show any alteration compared to control animals. Differences in latency and frequency in firing for encoding cells were seen during analysis. These results are in alignment with previous work linking prenatal THC exposure to dysregulated mesolimbic neuronal dynamics underlying sensorimotor gating impairments and exacerbated reward seeking during adulthood. The remarkable sex differences outlined here suggest that males are more vulnerable to the long-term effects of prenatal cannabis exposure.

Disclosures: R. Young-Morrison: A. Employment/Salary (full or part-time):; University of Maryland. **M. Lujan:** A. Employment/Salary (full or part-time):; University of Maryland. **N. Main Afshar:** A. Employment/Salary (full or part-time):; University of Maryland. **J. Cheer:** A. Employment/Salary (full or part-time):; University of Maryland.

Poster

PSTR559. Drugs of Abuse: Reward and Motivation

Location: WCC Halls A-C

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Program #/Poster #: PSTR559.09/OO15

Topic: G.03. Motivation

Support: NIH (DA044980)
NARSAD Young Investigator Award
Research Supplements to Promote Diversity in Health-Related Research

Title: Cocaine self-administration experience alters phasic prefrontal cortical Cocaine self-administration experience alters phasic prefrontal cortical Cocaine self-administration experience alters phasic prefrontal cortical signals of stressor controllabilitysignals of stressor controllabilitysignals of stressor controllability

Authors: *J. B. TORRES, E. WILLIAMS, M. J. MARTIN, C. A. BISHOP, M. P. SADDORIS; Dept. of Psychology & Neurosci., Univ. of Colorado Boulder, Bouder, CO

Abstract: Stress can impair motivated behaviors from reward and drug seeking to avoidance of punishment, though behavioral control over stressful situations can protect against these impairments by engaging prefrontal cortex pathways. That is, animals who have behavioral control over stressful experiences can demonstrate resilience in future settings, while those who lack control over stressful experiences will be more susceptible to future stressors. While this phenomenon has been demonstrated in typical adult rats, less is known about the efficacy of behavioral control in drug-experienced subjects. Our lab and others have shown substantial changes in limbic circuit activity in rats with two weeks of cocaine self-administration and are prone to substantial stress-induced reinstatement after abstinence. This suggests that cocaine self-administration may impair the prefrontal circuits necessary to learn and benefit from behavioral control. To test this, we recorded neurons in the prelimbic (PL) cortex of both male and female rats with either a history of cocaine self-administration (0.5mg/kg/inf; 14d; 2h/d) or controls who self-administered oral water. Following 30 days of abstinence, male and female rats were run on a cued version of an escapable shock (ES) task known to establish behavioral control where wheel turns terminate unavoidable tail shocks (n=100, 0.6-1.0 mA). To assess resilience in both aversive and rewarding settings, we then assessed these animals on a Pavlovian conditioned approach task (both prior to and shortly after ES), followed by a Pavlovian fear conditioning task. While cocaine experience did not affect the ability to learn the wheel-turn escape behavior, prior cocaine experience in males eliminate sign-tracking responses to the cue after ES, but had less effect on GT responses. In females, cocaine did not change how ES influences behavioral responses to fear and reward cues. In contrast, cocaine appeared to prevent more resilient responses to aversive learning following ES experience. Male and female cocaine rats showed greater freezing during shock-predictive cues than their control counterparts, and male cocaine rats (but not females) showed persistently high freezing relative to controls in the post-shock period as well. Finally, single-unit activity in the PL during ES showed that prior cocaine experience altered phasic responses to the shock onset relative to controls in both males and females. Collectively, cocaine experience likely alters brain regions necessary for learned resilience in both motivated and aversive settings in a sex-specific manner.

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Poster

PSTR559. Drugs of Abuse: Reward and Motivation

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Topic: G.03. Motivation

Support: NIH Grant K08DA053441

Title: Validation of a novel, cue-based task to index cognitive-affective reactivity to racial stigma among African American young adults: Implications for substance use outcomes

Authors: *C. M. RISCO, D. BUTLER, K. N. RODRIGUEZ, L. G. HERRERA, S. FIX, E. BERNAT;
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Abstract: The extant literature provides critical observations of the link between self-reported racial discrimination and risk behavior among African Americans (AA). However, the underutilization of cognitive-neuroscience paradigms has limited our understanding of cognitive-affective processes that underlie reactivity and behavior in the immediate context of a stigma event. To fill this gap, we build on the cue reactivity and substance use literature to develop a racial-stigma, cue-focused task to drive event-related designs for assessing (a) cognitive-affective processing of racial stigma events and (b) how racial-stigma stress produces sustained changes in affective and regulatory processes. **Methods.** Within-subject, pre-post design. AA young adults [N=31, M_{age}(SD)=21.75(2.56)] underwent continuous EEG recording while viewing a non-stigma image set (high arousal, unpleasant imagery control), as well as completing behavioral measures of reward/loss feedback responding (Gambling Feedback Task) and impulse control (Go/NoGo). Tasks were administered pre/post viewing a racial-stigma image set. ERP measures included late positive potential (LPP; motivated attention) at CPz to images, and theta and delta during the N2-P3 complex. **Results.** *Stigma Images.* RM-GLM revealed a decrease in LPP amplitude from blocks 1 to 3 (first 3rd of images to last 3rd) for Middle Wave [($F(1, 30)$ = 13.48, $p < .005$), η_p^2 = .31] and Late Wave [($F(1, 30)$ = 19.54, $p < .001$), η_p^2 = .39]—suggesting a regulatory process damping responding (as LPPs to high-intensity images are generally resistant to habituation). *Pre-Post Non-Stigma Images.* Paired samples t-tests indicated an increase in CPz LPP amplitude to negatively-valenced, non-stigma cues pre-post for Early Wave LPP [$t(28)$=-2.82, p=.009], suggesting that exposure to stigma cues potentiate reactivity to other, negatively-valenced cues. *Pre-Post Gambling.* Time-frequency analyses revealed increases in theta (sensitive to loss) [$t(28)$ = -2.27, p = .023, d = .60] and decreases in delta (sensitive to gain) [$t(28)$ = 2.19, p = .028, d = .58], suggesting a shift towards loss. *Pre-Post Go/NoGo*. Pre-post changes in NoGo theta (r = -.43, p = .017) and delta (r = -.36, p = .047) were associated with the down-regulatory process indexed with LPP during the presentation of the stigma images (1st to 3rd blocks). Findings provide a preliminary validation of a cue-based paradigm for indexing changes in cognitive-affective reactivity during and post exposure to racial-stigma cues. The current task protocol brings objective neuroscience measures to processes that mediate the impact of racial stigma on substance use outcomes among AA young adults.

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Poster

PSTR559. Drugs of Abuse: Reward and Motivation

Location: WCC Halls A-C

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Program #/Poster #: PSTR559.11/OO17

Topic: G.03. Motivation

Title: Modafinil combined with citalopram is more effective to induce reinforcing characteristics and withdrawal signs that the combination of modafinil plus atomoxetine in rats

Authors: *J. EMMANUEL[1], J. YEPEZ[2];
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Abstract: Psychostimulants with addictive properties such as cocaine and amphetamines increase the levels of monoamines: dopamine (DA), norepinephrine (NE) and serotonin (5-HT) through interaction with their respective reuptake transporters: DAT, NET, SERT. The involvement of each monoaminergic systems in addictive behavior and the appearance signs of withdrawal is still poorly understood. Modafinil is a weak psychostimulant drug that increases DA levels through its affinity and inhibition of DATs. However, it has low or no affinity for the NET and SERT transporters. Therefore, the stimulation of the monoaminergic systems through pharmacological strategies such as the co-administration of modafinil plus citalopram -CIT- (a specific inhibitor of 5-HT reuptake) or atomoxetine -ATX- (a specific inhibitor of NE reuptake) allows to know the participation of each neurotransmission system in the effects of psychostimulants. Eleven groups of 60-day old male rats were treated chronically (16 days) with MOD at dose of 60mg/kg alone or co-administered with CIT (5mg/kg or 3mg/kg) or ATX (2mg/kg or 4mg/kg). Immediately after pharmacological treatment, anhedonia-like behavior was assessed at 48, 72 and 96h. Active self-administration was assessed in a task called transition bridge and the mixture of MOD+CIT and MOD+ATX was exposed according to the initial treatment. The co-administered groups 30MOD+3CIT and 60MOD+3CIT showed sign of anhedonia with an increase over time meanwhile the co-administered with atomoxetine did not present signs of anhedonia. In the same way the groups co-administered 30MOD+3CIT and 60MOD+3CIT presented a higher active self-consumption of the mixture (MOD+CIT). Although the 60MOD+4ATX group also presented a higher active self-administration of the mixture (MOD+ATX), this was lower than the groups co-administered with citalopram. The co-administration of modafinil plus citalopram induce signs of anhedonia and show higher self-administration in an operant task compared to the co-administration of modafinil plus atomoxetine. This suggests that the serotonergic compared with noradrenergic system seems to have a more important role in the reinforcing properties and signs of withdrawal of psychostimulants when combined with dopaminergic activation

Disclosures: **J. Emmanuel:** None. **J. Yepez:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.01/OO18

Topic: G.04. Emotion

Support: 2020R1A2C200719314

Title: Compassion training in individuals with high aggression facilitates the neural processes of emotion simulation: an fMRI study

Authors: *Y. HWANG, H. YOON, T. KIM, S. KIM;
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Abstract: Compassion entails caring for the well-being of others, and it is closely associated with the motivation to alleviate the suffering of others. A lack of compassion is often considered a risk factor for mental health problems. Previously, our group showed that implicit training to promote compassionate responses to others' distress can increase helping intentions and enhance functional connectivity of the medial orbitofrontal cortex with brain areas involved in emotion simulation. In the current study, we extended the previous research in two significant ways. First, we applied the training task to adults with high aggression. Second, we employed two distinct training approaches targeting empathic concern or personal distress, which are two differential components of empathy. A total of 107 participants with high aggression participated and were randomly assigned to one of the three training groups: concern, distress, or neutral. During the training, all participants read written text scenarios depicting a person in distress and completed a target word that appeared in a fragmented form within the scenario. The target words differed across the groups. In the concern group, the target words denoted empathic concerns (e.g., "worried"), while in the distress group, they denoted personal distress (e.g., "painful"). The neutral group completed emotionally neutral words. Following the training task, all participants performed an empathy rating task inside the brain scanner while viewing short video clips depicting people experiencing emotionally distressing or neutral events. Participants rated empathic distress, empathic care, and helping intentions toward victims depicted in the clips. Preliminary analysis on subjective ratings revealed no group differences. The group x condition (distress vs. neutral) ANOVA on neural responses revealed an activation in the left inferior frontal gyrus (IFG; BA45). The concern group showed increased activation in the IFG while experiencing others' suffering as compared to the distress group. Given the crucial role of the IFG in emotion simulation, our results indicate that implicit training aimed at fostering empathic concern to the suffering of others can facilitate mirroring of others' pain, which can, in turn, promote compassionate responses to others. Further analyses are required to gain better understanding of the specific contribution and mechanisms of implicit training on empathy and compassion responses.

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Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.02/OO19

Topic: G.04. Emotion

Title: Facial trustworthiness judgment is influence by emotion, affect and attractiveness but not age, gender or ethnicity

Authors: *J. YU, H. XU;
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Abstract: Multiple factors affect our judgment of trustworthiness of a face. Previous studies demonstrated that changeable cues from faces, such as emotional expressions, are principal factors affecting trustworthiness evaluation and proposed a shared perceptual basis between facial trustworthiness and emotional expression. Although emotional expressions can be universally recognized, facial trustworthiness evaluation is affected by invariant facial cues, such as face race (the other-race effect), gender, and age. However, these factors were evaluated in separate studies from different participants in each study and lack of a systematic investigation. It remains unclear whether evaluating trustworthiness based on emotional expressions can universally occur across faces with different invariant cues. To systematically investigate the effect of emotional expressions on trustworthiness evaluation, we conducted an online behavioral experiment by Qualtrics. Participants from different ethnicities in Singapore (n = 341: 285 Chinese, 6 Caucasian, 29 Indian, 18 Malay, 3 mixed-ethnicity) judged trustworthiness and other facial traits (attractiveness, affect and dominance) on a scale of 1-100 and indicated the emotion category (neutral, angry, happy, sad, surprised, fearful, and disgusted) and valence (positive or negative) of face stimuli. These faces with seven different emotions were selected from databases of different ethnicities (Chinese, Malay, Indian, and Caucasian) with both male and female faces. Faces of different age groups (old and young adult) were also presented for Chinese faces. Repeated measures ANOVA revealed a significant main effect of emotion on trustworthiness ratings ($F_{(2.82, 861.56)} = 416.09$, $p < 0.001$). Happy faces were judged as more trustworthy and faces with other emotions were perceived as less trustworthy (p's < 0.001) compared to neutral expression. Angry and disgusted faces were perceived as the least trustworthy. In addition, face valence significantly influenced trustworthiness ratings as participants judged positive faces as more trustworthy than negative faces ($t_{(340)} = 26.76$, $p < 0.001$). Repeated measures correlation revealed strong correlation between trustworthiness and attractiveness ($r = 0.54$, $p < 0.001$) but a rather weak correlation between trustworthiness and dominance ($r = -0.1$, $p < 0.001$). These correlations are not influenced by ethnic, gender or age. Our results suggest that facial emotion, valence and attractiveness are important factors of trustworthiness and that these effects on trustworthiness evaluation occur universally for faces of different races, gender, and age.

Disclosures: **J. Yu:** None. **H. Xu:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

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Program #/Poster #: PSTR560.03/OO20

Topic: G.04. Emotion

Title: A bitter game to swallow: An examination of moral and gustatory disgust responses

Authors: *D. M. ROHRER[1], D. B. KATZ[2], R. A. KNIGHT[3], J. N. GUTSELL[3];
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Abstract: Non-human animal (e.g., Katz et al., 2001; Katz et al., 2002) and human studies have shown that disgust arises from activation of insular cortex, a region associated with gustatory (i.e., taste) processing (e.g., Scott et al., 1986a, b; Yaxley et al., 1990; Cerf et al., 1996). The insula also has been found to play a role in moral disgust processing (e.g. Ying et al., 2018). It is unsurprising, therefore, that there is a physiological overlap between moral disgust and its evolutionary foundations in oral disgust (Chapman et al., 2009)—that similar facial motor activity occurs when individuals are given unfair splits of money and when they taste bitter liquids. Eskine et al. (2011) found further evidence for the connection between gustatory and moral disgust by demonstrating that participants given a bitter substance to drink make harsher moral judgments, although Ghelfi et al. (2020) failed to replicate this result. It is thus unclear whether these two types of disgust employ the same neural machinery.We have set out to perform a novel test of this theory, using physiological disgust responses determined by muscle activity. Specifically, we hypothesized that after tasting quinine, participants will have a stronger moral disgust response measured in terms of electromyography (EMG) and in self-report responses when faced with unfair monetary splits. Participants were exposed to either sucrose, water, or quinine prior to playing 2 rounds of receiving a proposed fair or unfair money split. This procedure was repeated 18 times with tastes selected in a pseudo random order (quinine was never given twice consecutively), while EMG was collected from the levator labii (LL), corrugator (COR), masseter (MAS), and zygomatic (ZYG) muscles. Preliminary findings ($n=5$) indicate that tasting quinine caused stronger activity in LL, COR, and MAS than sucrose or water, as predicted. Surprisingly the ZYG activity also increased in response to quinine. Activation of LL and MAS was also observed with unfair monetary splits, consistent with increased self-reported disgust with unfair splits. The lack of a trend toward increases in COR activity suggests that we are measuring disgust and not anger. Finally, our results are in line with Ghelfi et al.'s (2020)—bitter taste pre-exposure did not influence moral disgust. Thus, the current study replicated Chapman et al.'s (2009) findings that moral disgust elicits an EMG response like that evoked by quinine and showed that both moral and gustatory disgust can also be measured via MAS, but did not suggest interactions between the two.

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Poster

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Topic: G.04. Emotion

Support: Russian Science Foundation Grant #22-11-00213, https://rscf.ru/en/project/22-11-00213/.

Title: An electromyographic study of the affective space dimension for complex emotions

Authors: *A. V. SAMSONOVICH, D. V. TIKHOMIROVA, E. A. TOCHINA;
Natl. Res. Nuclear Univ. MEPhI, Moscow, Russian Federation

Abstract: Dimensional models of emotions are popular today, while the true dimension of affective space is a matter of debate. In our recent work (Gadzhiev IM, Knyshenko MP, Dolenko SA, & Samsonovich AV, Cognitive Systems Research, 78:96-105, 2023) we found, using facial electromyography (EMG) and automated facial image analysis, that the part of the affective space represented in facial expressions, when limited to basic emotions, is three-dimensional. Here we confirm this result using EMG recording with an increased number of channels. Furthermore, we extend the study to complex emotions. Surprisingly, we found that three dimensions are not sufficient to accommodate for facial correlates of the extended affective space including basic as well as complex emotions, such as resentment, regret, or shame. In total, 16 college students participated in this study, age 18 to 21, including 2 women and 14 men, all native Russian speakers. Stimuli included pictures of emotional facial expressions labelled with the name of the expressed emotion. Participants were asked to express the specified emotion on their face. 20 different emotions were represented in several pictures each in the stimulus set, including basic and complex emotions. Each participant responded to 40 stimuli, interchanged with rest intervals. EMG recordings were taken from 4 facial muscles: Frontalis (lateral), Corrugator supercilii, Zygomaticus major, and Depressor anguli oris. The signal sampled at 1 KHz was subjected to a wavelet transform and filtering. The resulting power signal was further decomposed into features, that were canonically correlated with the Word2Vec embedding of the emotion labelling word presented in the stimulus. All 4 canonical correlation (CC) coefficients turned significant: r1 = 0.65 ($p<1e-60$), r2 = 0.57 ($p<1e-29$), r3 = 0.52 ($p<1e-13$), r4 = 0.47 ($p<1e-4$). PCA study of the EMG data produced consistent results. The same CC analysis with the same data limited to the 6 basic emotions yielded only 3 significant canonical correlation coefficients: r1 = 0.81 ($p<1e-25$), r2 = 0.69 ($p<1e-9$), r3 = 0.57 ($p<0,002$), r4 =0.48 ($p>0.3$). In conclusion, the present EMG study confirmed our previous result for basic emotions, now with 4 parallel EMG channels, while at the same time indicating that 3 dimensions, and therefore models like VAD or PAD, are not sufficient for representing facial expressions of complex emotions. The lower bound on the affective space dimension for complex emotions found in this study is 4. Possibilities of further validation and rectification of this finding will be discussed.

Disclosures: A.V. Samsonovich: None. **D.V. Tikhomirova:** None. **E.A. Tochina:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.05/OO22

Topic: G.04. Emotion

Support: NIH Grant GM137863

Title: Exploring the negativity bias among individuals with lower empathic ability

Authors: *R. HURTADO, **J. SCHINDLER**, G. GUZMAN, L. APAR, R. ARISTA, S.-M. KANG;
Psychology, California State Univ. Northridge, Northridge, CA

Abstract: The present study aimed to understand how individuals with Low Empathic (LE) ability would process emotional information differently than those with High Empathic (HE) ability by using electroencephalography (EEG). Previous research has shown that negatively valenced emotional information attracts more attention than positively or neutrally valenced information. This sensitivity to negative emotional information is referred to as the negativity bias (Hajcak & Olvet, 2008; Ito, Larsen, Smith, & Cacioppo, 1998). By measuring the Late Positive Potential (LPP) amplitudes, a neurophysiological marker for emotional information, previous work demonstrated that personality characteristics could compromise the emergence of the negativity bias (e.g., Groen et al., 2013; Medina et al., 2016). Based on the previous studies, the current study predicted that people with a difficulty in empathizing with others would exhibit an attenuated negativity bias compared to more empathic individuals. To test this hypothesis, 105 college students (75 female, 30 male) were asked to passively view a total of 135 negative, positive, and neural stimuli taken from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008) in a random order, while their EEG was recorded. The empathic ability of the participants was assessed using the Empathy Quotient (Baron-Cohen & Wheelwright, 2004). The participants were divided into two groups (LE and HE) based on their empathy scores. The results of the current study revealed that there was not only a significant main effect of Empathy group but also a significant interaction effect between Empathy Group and Valence conditions on the average LPP amplitudes of the medial lateral region of the cortex. These outcomes supported the main hypothesis suggesting that the LE group displayed an attenuated negativity bias. The significance of the current findings were discussed in terms of how the attenuated negativity bias might be associated with low empathic individuals' social behaviors.

Disclosures: R. Hurtado: None. **J. Schindler:** None. **G. Guzman:** None. **L. Apar:** None. **R. Arista:** None. **S. Kang:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.06/OO23

Topic: G.04. Emotion

Support: MOST 111-2410-H-A49-049-MY3
MOST 108-2321-B-009-006-MY2

Title: Effects of empathizer feedback on empathy for emotional communication in naturalistic social situations

Authors: *R.-J. HUNG[1], P.-Y. WANG[1], Z.-R. CHEN[1], Y.-L. LIU[1], Y.-S. CHEN[2], L.-F. CHEN[1];
[1]Inst. of Brain Sci., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; [2]Dept. of Computer Sci., Natl. Yang Ming Chiao Tung Univ., Hsinchu, Taiwan

Abstract: Empathy is a multifaceted social process comprising emotional and cognitive components. Due to limitations in experimental designs, few studies investigated empathy in real-life conversations. Our previous research, in which only auditory information was available for participants, found that sadness was less contagious and harder to judge accurately than happiness. Also, empathizer feedback was shown to decrease empathic accuracy but does not affect emotional contagion. Our objective in the current study was to investigate the effects of empathizer feedback on empathy when visual and auditory information was available. Eleven friend dyads (15 females, mean age = 23.18 ± 3.99, age range = 20-27) were recruited for this study. Subjects of each dyad sat face-to-face and took turns being the empathizer (listener) and target (speaker). During the emotional communication task, the target described a happy or sad event, and the empathizer listened attentively with silence ("Unable" to respond) or with brief feedback ("Able" to respond). Emotional contagion (EC) and empathic accuracy (EA) were used as behavioral indicators of empathy; mu suppression was used as a neural indicator of empathy. Our behavioral and neural results showed that there was no significant main effect of feedback and emotion. The behavioral results of post-hoc analysis revealed lower EC scores under the Sad-Unable condition than under the Happy-Unable condition ($P = 0.028$) with no significant difference between the Sad-Able and Happy-Able conditions ($P = 0.053$). The neural results of post-hoc analysis revealed stronger mu suppression under the Sad-Able condition than under the Sad-Unable condition ($P = 0.014$) with no significant difference between the Happy-Able and Happy-Unable conditions ($P = 0.872$). Such results may imply that giving verbal feedback facilitates emotional empathy for sadness by increasing sensorimotor activity when both visual and auditory information is available. Our study unveiled the importance of empathizer feedback in naturalistic social situations.

Disclosures: R. Hung: None. **P. Wang:** None. **Z. Chen:** None. **Y. Liu:** None. **Y. Chen:** None. **L. Chen:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.07/OO24

Topic: H.06. Social Cognition

Support: McDonnell Center for Systems Neuroscience
AFOSR (FA9550-21-1-0088)
NSF (BCS-1945230, IIS-2114644)
NIH (R01MH129426)
Simons Collaboration on the Global Brain

Title: Single-neuron encoding of Theory of Mind in the human medial temporal lobe and medial frontal cortex

Authors: *R. CAO[1], J. DUBOIS[2], A. N. MAMELAK[2], R. ADOLPHS[3], S. WANG[1], U. RUTISHAUSER[2];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Cedars-Sinai Med. Ctr., Los Angeles, CA; [3]Caltech, Pasadena, CA

Abstract: Theory of mind (ToM) is the process of inferring the intentions and emotions of other agents, enabling complex social activities among humans. Understanding the neural basis of ToM is a key question in cognitive neuroscience. Despite numerous neuroimaging studies tackling this question, findings have varied substantially due to the variance in tasks and types of measures. A major debate concerns whether the medial temporal lobe (MTL), including the amygdala and hippocampus, is necessary for ToM, as these areas are directly connected to many components of the putative ToM network, notably including the medial prefrontal cortex (MFC). Additionally, it remains unclear whether ToM recruits a dedicated brain network and how mentalizations across different stimulus types are represented in the brain. In this study, we capitalized on a unique opportunity to simultaneously record single-neuron activity from the MFC and the MTL in neurosurgical patients. We employed the established Why/How task, contrasting social inference (ToM) vs. mere social description (categorization), which has been demonstrated to identify the ToM network in neuroimaging studies. To investigate whether making inferences for different categories and social domains depends on shared neural substrates, we used images of faces, hands, and natural scenes as stimuli. In contrast to prior imaging studies, we found that amygdala and hippocampus also participate in social inference, albeit to a lesser degree than the MFC. Furthermore, neurons tracking domain-general inference were found in the MFC. However, more domain-specific processing for social inference was found in the MTL. Moreover, we found that inference and categorization were represented by two distinct neuronal populations in the MFC and MTL. Our results provide new insights into the neural correlates of ToM at the single-neuron level and highlight the specialized representation of different ToM subtypes.

Disclosures: R. Cao: None. **J. Dubois:** None. **A.N. Mamelak:** None. **R. Adolphs:** None. **S. Wang:** None. **U. Rutishauser:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.08/OO25

Topic: H.06. Social Cognition

Support: Natural Sciences and Engineering Research Council of Canada Discovery Grant to J.C.C.
Canada First Research Excellence Fund "BrainsCAN" grant to Western University

Title: Evaluating how evoked-responses to social interactions are influenced by animacy and visual realism in online environments using functional Near-Infrared Spectroscopy (fNIRS)

Authors: *M. KENT[1,6], E. DELIGIANNIS[2,6], K. P. BABIN[3,6], K. STUBBS[4,6], E. G. DUERDEN[5,6], J. C. CULHAM[3,6];
[2]Neurosci. Program, [3]Dept. of Psychology, [4]BrainsCAN, [5]Fac. of Educ., [1]Western Univ., London, ON, Canada; [6]Western Inst. for Neurosci., London, ON, Canada

Abstract: Given the growing use of online video communication platforms such as Zoom, we used functional near-infrared spectroscopy (fNIRS) to investigate which factors influence brain regions implicated in social cognition during video interactions. First, we investigated whether fNIRS would enable us to extend previous findings from functional magnetic resonance imaging (fMRI) to a social setting, to reveal differences in brain activation for interactions with a live vs. pre-recorded partner[1]. We also investigated whether brain activation depended on visual realism, that is whether the partner was rendered as a real human face (via video) or as a cartoon human-like avatar (Apple Memoji). We hypothesized that if social processing depended only upon the attribution of behavior to an actual human, activation would be affected by animacy (live vs. pre-recorded) alone; whereas, if social processing depended upon visual realism, activation would be affected by both animacy and visual realism. Participants (n = 25; 13 males, 12 females; average age = 22 yrs) engaged in a conversation with an experimenter who appeared either live via video call or as a pre-recorded video and who was either shown with direct video stream or rendered as an avatar. Gaze patterns and brain activitation were studied using a NIRScout system (NIRx medical systems, Berlin, Germany) and EyeLink 1000 eye tracker (SR Research, Toronto, Canada) while participants interacted with an experimenter in a social paradigm[1]. A customized fNIRS montage provided coverage of the regions of interest, including bilateral temporoparietal junction (TPJ), posterior superior temporal sulcus (pSTS) and prefrontal cortex (PFC), regions commonly associated with "Theory of Mind". Each run (8-9 minutes) consisted of 16 trials presented in a jittered event-related design. Two localizers for the "Theory of Mind" network were used. Preprocessing and analysis of the fNIRS data were conducted using Brain AnalyzIR Toolbox. Results suggest that there are neural differences in right temporoparietal and prefrontal brain regions when comparing human and avatar interactions ($p<0.05$), suggesting neural processes are affected by the visual realism of the face. It remains unclear whether the animacy of the interaction significantly influences neural processes. These methods allow us to examine how the brain processes different types of social interactions, which may play an important role

as the virtual world becomes more prominent. [1]Rice, K., & Redcay, E. (2016). Interaction matters: A perceived social partner alters the neural processing of human speech. *NeuroImage*, *129*, 480-488.

Disclosures: **M. Kent:** None. **E. Deligiannis:** None. **K.P. Babin:** None. **K. Stubbs:** None. **E.G. Duerden:** None. **J.C. Culham:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.09/PP1

Topic: H.02. Perception and Imagery

Support: NIH F32 MH117933 (to AMD)
Simons Center for the Social Brain at MIT Postdoctoral Fellowship (to AMD)
Simons Center for the Social Brain at MIT Targeted Project Funds (to JDEG)

Title: Expectation modulates repetition suppression of faces and speech

Authors: S. NISHITH[1], A. CARDINAUX[1,2], C. LI[3], T. K. PERRACHIONE[4], J. D. GABRIELI[1,2], *A. D'MELLO[5,7,6];
[1]McGovern Inst. for Brain Res., [2]Dept. of Brain and Cognitive Sci., [3]Hock E. Tan and K. Lisa Yang Ctr. for Autism Res. at Massachusetts Inst. of Technol., MIT, Cambridge, MA; [4]Dept. of Speech, Language, and Hearing Sci., Boston Univ., Boston, MA; [5]Dept. of Psychiatry, [6]Peter O'Donnell Jr. Brain Inst., UT Southwestern Med. Ctr., Dallas, TX; [7]Dept. of Psychology, UT Dallas, Richardson, TX

Abstract: Repeated exposure to a stimulus reduces activation in brain regions sensitive to that stimulus. This “repetition suppression” (RS) phenomenon has been associated with learning and memory, and is reduced in neurodevelopmental conditions such as autism and dyslexia. While RS may reflect bottom-up perceptual processes due to adaptation, RS magnitude is greater when repetition is expected (“expectation suppression”), suggesting a role for top-down signals in minimizing neural responses related to perceptual prediction error. Here, we measured RS for two kinds of high-level stimuli - visual faces and auditory speech - under two conditions that manipulated participants’ expectation that stimuli would repeat. We assessed whether RS to each type of stimulus was modulated by expectation of repetition, and where this expectation suppression occurred in the brain. We measured BOLD response changes using fMRI in 22 neurotypical adults (11M; mean age=31.9±8.6 yrs). In separate runs, participants attended to face photographs or audio recordings of spoken words while detecting rare deviant stimuli (upside-down faces; time-reversed words). Trials consisted of sequential stimulus pairs presented for 250ms (interstimulus interval, ISI=250ms) or 700ms (ISI=200ms) each, for faces and speech

respectively. On some trials, the same stimulus was presented twice; on others, two different stimuli were presented. Repetition probability was manipulated throughout the run: in high repetition-probability blocks, stimuli repeated on 75% of trials; in low repetition-probability blocks, stimuli repeated on 25% of trials. This design modulates participants' top-down expectation of repetition to reveal "expectation suppression" effects, operationalized as an interaction between repetition probability (high vs. low) and repetition suppression (non-repeating vs. repeating). We extracted RS values from each block type from within participants' own face and speech processing networks, obtained from functional localizers. Repeated-measures ANOVAs revealed that several regions showed RS including the fusiform face area (FFA) for faces, and left temporoparietal regions for speech. RS was modulated by expectation in most regions, with reduced RS in low repetition-probability blocks (FFA p=0.051; L MTG/STG p=0.01). These findings replicate prior work showing that expectation modulates RS in the FFA, and newly show that RS in language-sensitive regions is also sensitive to expectation. Importantly, not all stimulus-sensitive regions were sensitive to expectation, suggesting that top-down signals affect perceptual processing differently across the brain.

Disclosures: S. Nishith: None. **A. Cardinaux:** None. **C. Li:** None. **T.K. Perrachione:** None. **J.D. Gabrieli:** None. **A. D'Mello:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.10/PP2

Topic: H.02. Perception and Imagery

Title: Effects of the putative pheromone androstadienone on resume evaluation

Authors: *T. JECHURA[1], E. BURNS[2];
[2]Psychological Sci., [1]Albion Col., Albion, MI

Abstract: The present study experimentally investigated the effects of the putative human pheromone androstadienone (AND) on the evaluation of a proposed applicant via paper resume. Considering the emotional, perceptional, and processing effects that AND has on individuals, it was predicted that exposure to AND while evaluating a resume would positively impact the ratings of the applicant's job qualification, hiring desirability, and inferred personality traits, particularly in women. Participants (n=52) were given a fictional resume and a job description. Half the participants were exposed to AND, whereas the control group was only exposed to the acetone vehicle. Participants completed questionnaires measuring their perception of the applicant's job qualifications, personality traits, and hiring potential. They were then asked to determine whether the applicant should be hired for the position. Results showed that there was a significant general effect of AND on perceived extraversion (p=.040), perceived openness (p=.013), and desirability to hire (p=.006) as well as a trend towards significance in job qualification scores (p=.052) and in perceived conscientiousness ratings (p= .069). In women,

there was a significant effect of AND on perceived agreeableness ratings (p=.015). The present study demonstrates that AND affects processing of information and perception of others.

Disclosures: **T. Jechura:** None. **E. Burns:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.11/PP3

Topic: G.04. Emotion

Support: AFOSR (FA9550-21-1-0088)
NSF (BCS-1945230, IIS-2114644)
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Japan Society for the Promotion of Science Grant-in-Aid for Early-Career Scientists (No. 22K15626)

Title: Functional connectivity between the amygdala and prefrontal cortex underlies processing of emotion ambiguity

Authors: *S. SUN[1], H. YU[2], R. YU[3], S. WANG[4];
[1]Tohoku Univ., Sendai, Japan; [2]Univ. of California, Santa Barbara, Univ. of California, Santa Barbara, Santa Barbara, CA; [3]Mgmt., Hong Kong Baptist Univ., Hong Kong, Hong Kong; [4]Washington Univ. in St. Louis, Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Processing facial expressions of emotion draws on a distributed brain network. In particular, judging ambiguous facial emotions involves coordination between multiple brain areas. Here, we applied multimodal functional connectivity analysis to achieve network-level understanding of the neural mechanisms underlying perceptual ambiguity in facial expressions. We found directional effective connectivity between the amygdala, dorsomedial prefrontal cortex (dmPFC), and ventromedial PFC, supporting both bottom-up affective processes for ambiguity representation/perception and top-down cognitive processes for ambiguity resolution/decision. Direct recordings from the human neurosurgical patients showed that the responses of amygdala and dmPFC neurons were modulated by the level of emotion ambiguity, and amygdala neurons responded earlier than dmPFC neurons, reflecting the bottom-up process for ambiguity processing. We further found parietal-frontal coherence and delta-alpha cross-frequency coupling involved in encoding emotion ambiguity. We replicated the EEG coherence result using independent experiments and further showed modulation of the coherence. EEG source connectivity revealed that the dmPFC top-down regulated the activities in other brain regions. Lastly, we showed altered behavioral responses in neuropsychiatric patients who may

have dysfunctions in amygdala-PFC functional connectivity. Together, using multimodal experimental and analytical approaches, we have delineated a neural network that underlies processing of emotion ambiguity.

Disclosures: **S. Sun:** None. **H. Yu:** None. **R. Yu:** None. **S. Wang:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.12/PP4

Topic: G.04. Emotion

Title: Neural dynamics of the influence of bodily resonances on emotional prosody perception

Authors: *G. SELOSSE[1], D. BENIS[2], D. GRANDJEAN[3], L. CERAVOLO[4];
[1]Univ. of Geneva, Geneva, Switzerland; [2]Neurosci. of Emotion and Affective Dynamics Lab., Univ. de Geneve, Geneve, Switzerland; [3]Neurosci. of Emotion and Affective Dynamics Lab., Geneva, Switzerland; [4]Psychology, Swiss Ctr. For Affective Sci., Geneve, Switzerland

Abstract: Emotional prosody is defined as suprasegmental and segmental changes in voice and related acoustic parameters that can inform the listener about the emotional state of the speaker. Despite a large corpus of literature in psychological and brain mechanisms in emotional prosody perception, the perspective of embodied cognition in these mechanisms have been largely neglected. Here we investigated the influence of induced bodily vibrations in the categorization of ambiguous emotional vocalizations in an event-related potential study (*N*=24). The factorial design included Vocal emotion [anger and fear] and Vibration [anger, fear, and none] as factors. Emotional voices were morphed between a fearful expression with the speaker's identity-matching angry expression, creating blends of emotions in each voice. Emotional congruent and incongruent vibrations were delivered on the skin through transducers placed close to the vocal cords. The main hypothesis was that induced bodily vibrations would constitute a potential interoceptive feedback that would influence the perception of emotions, especially for more ambiguous voices. Behavioural results showed that vibration skewed participants emotional ratings by accentuating responses congruent with the vibration. ERP results indicated that N100 and P200 components subtending the early processing of emotional prosody were significantly modulated by vibrations in the congruent setting which could be considered as a facilitation effect for early emotional prosody processing. A significant modulation of the late positive component was also observed in the incongruent setting, suggesting an error processing mechanism. These results suggest that vibrations would play a significant role in vocal emotion perception through embodied mechanisms at the behavioral and neural levels.

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Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.13/PP5

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Inter-brain synchronization and sense of unity with others cheering together: An EEG hyperscanning study

Authors: *M. SHIMADA, M. AZUMA, S. SHIMADA;
Meiji Univ., Kawasaki-shi, Japan

Abstract: At sports events, for example, people often gather to cheer. However, inter-brain synchronization between people cheering together is still unclear. Previous research has shown that brain activity is synchronized in pairs watching a video together (Hasson & Frith, 2016). In addition, the observer's brain activity is influenced by the performer's outcome (Shimada et al., 2016). In this study, we investigate the synchronization of brain activity when pairs cheer individually or together, including the influence of the performer's outcome. Twelve pairs of healthy subjects (mean age 22.2 ± 1.4 years) participated in the experiment. Two experimental conditions were conducted: a condition in which the subjects cheered individually, with a wall between them (with-wall condition), and a condition in which the subjects cheered together, without a wall between them (no-wall condition). Subjects were required to cheer a Kendama (a Japanese game) performer presented in the video, and their EEG was measured using the hyperscanning method. The video included two types of outcomes: success and failure. The Phase Synchronization Index (PSI) was calculated as an index of synchronization. The questionnaire about the sense of unity among the participant, their partner, and the performer was conducted at the end of each condition. The results showed that the score of the questionnaire was significantly higher in the no-wall condition than in the with-wall condition. A 2 (wall: with, no) x 2 (outcome: success, failure) ANOVA on PSI showed no significant interactions between any of the combinations of the channels. To investigate the relationship between the sense of unity and the synchronization of brain activity, a correlation analysis of the difference (with-wall condition - no-wall condition) between the scores of the questionnaire and PSI was performed in the channel combinations that showed higher PSI values in the no-wall condition than in the with-wall condition. The results showed significant positive correlations with scores in the following combinations of the channels: occipital lobe region (Oz) and right middle region (C4) in the alpha band, inferior parietal region (P6) and frontal lobe region (Fz), occipital lobe region (Oz) and temporal region (T7) in the beta wave band. Oz is related to visual information processing, and C4 is the area related to the innervation of the hand. P6, Fz, and T7 are involved in inferring the psychological state of others. These results suggest that cheering with others enhances the sense of unity, and the sense is related to the synchronization of brain activity between the area related to visual information processing and empathy.

Disclosures: M. Shimada: None. **M. Azuma:** None. **S. Shimada:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.14/PP6

Topic: H.06. Social Cognition

Title: The effects of virtual presence on physical and autonomic responses to social situations in autism: a virtual reality game-based approach

Authors: *T. SIMMONS[1], J. SNIDER[3], L. CHUKOSKIE[2];
[2]Physical Therapy, Movement, and Rehabil. Sci., [1]Northeastern Univ., Boston, MA; [3]Univ. of California San Diego, San Diego, CA

Abstract: Fluid human social interaction demands a complex exchange of verbal and non-verbal signals and is a discriminating feature for many individuals with autism. We propose to characterize differences in social interaction related to lower level movement and physiological arousal responses. Our experiment uses a fully immersive virtual reality (VR) video game to characterize differences in gaze behavior, gross motor movement, and physiological arousal in an autistic population. In comparisons between autistic and non-autistic individuals, these measures have previously shown distinct differences during social interactions and other highly dynamic situations. We seek to advance this literature by characterizing these signals during a game with respect to the presence or absence of another virtual player. We created a VR pattern completion game that can be played in three conditions: individually, cooperatively, and competitively with minimal changes in game complexity. We examined movement and physiological arousal measures between groups and across conditions. We observed differences in gaze duration between groups and conditions for specific areas of interest (AOI) in the virtual environment. We also observed differences across group and condition in our movement measures, which examined response sequences to dynamic stimuli. Finally, we found that autistic individuals showed an exaggerated arousal response, indexed by pupil size, following game-based movements during social conditions. These findings demonstrate the effect of a virtual presence during game play, and establish a foundation for examining game-based social interaction in different populations. Future work will focus on expanding our understanding of these effects by manipulating the dynamics of social and non-social aspects of the virtual environment.

Disclosures: **T. Simmons:** None. **J. Snider:** None. **L. Chukoskie:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.15/PP7

Topic: H.06. Social Cognition

Title: Is there a brain area dedicated to socially guided spatial attention?

Authors: *M. GÖRNER, P. W. DICKE, P. THIER;
Hertie Inst. For Clin. Brain Res. / AG Thier, Tuebingen, Germany

Abstract: Apart from language, our gaze is arguably the most important means of communication. Where we look lets others know what we are interested in and allows them to join our focus of attention. In several studies our group investigated the neuronal basis of gaze following behavior in humans and macaques and described a Gaze Following Patch (GFP) in the posterior temporal cortex as being of central importance for this function. To our knowledge, this makes it the most promising candidate as a substrate of Baron-Cohen's Eye-Direction-Detector (EDD), an integral part of his influential Mindreading System. With the latter, Baron-Cohen proposed a network of domain-specific neurocognitive modules that are necessary to establish a Theory of Mind - the attribution of mental states to others. The tenet of domain-specificity requires that the EDD processes only and exclusively eye-like stimuli with their typical contrast and movement properties. In the present human fMRI study, which we preregistered at OSF, we aimed to critically test if the GFP fulfills this criterion. Specifically, we asked if it is equivalent to or different from the visual motion processing areas (MT+) located in the same part of the brain because gaze cues usually - and in our previous experiments - involve visual motion due to saccadic changes of gaze position. To critically test the possible congruence of the GFP and MT+ we compared the BOLD responses within the GFP evoked by gaze-following and cubes-following. Rapid rotation caused the vertices of the cubes to point in specific directions, and participants had to use this information, similar to the line of sight of a portrait, to select a target among the distractors. We also compared the BOLD responses within the GFP to responses within an MT+ ROI defined using a standard motion localizer paradigm. Resorting to a Bayesian analysis approach, we collect data until the criterion of strong evidence (Bayes Factor >= 10) is met for all hypotheses. The interim results suggest that the GFP cannot be distinguished from areas involved in general visual motion processing, arguing against the existence of domain specificity as posited by Baron-Cohen's Mindreading System.

Disclosures: **M. Görner:** None. **P.W. Dicke:** None. **P. Thier:** None.

Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.16/PP8

Topic: H.06. Social Cognition

Support: DFG Grant KU 3972/2-1
Emerging Talents Initiative Grant 2021-2_Phil_01_Kulke

Title: Brain oscillates; eyes do not move: Inhibiting gaze in social situations

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Abstract: Human behaviour strongly depends on the social context we are in. A person watching a video can freely look wherever they like without social repercussions. However, the case differs in live social situations (e.g., a waiting room) where gaze additionally signals a desire to interact with others. If an interaction is not desired, people therefore avoid staring at others (civil inattention). This avoidance of gaze has been demonstrated with behavioural measures, which cannot rule out the alternative explanation that participants lack interest in the other person. The current preregistered (https://osf.io/837wm, https://osf.io/e2kr) set of studies used simultaneous gaze recording and electroencephalography to compare eye movements and neural responses during a "waiting room situation", allowing the computation of alpha power as a measure of covert attention. Participants were asked to wait in a room in which either a live confederate was present (live social situation) or a video of the same confederate was presented on a computer screen (non-social video situation). Their gaze and alpha power were measured as indicators of overt and covert attention. Infants in the first year of life (N = 20), preschoolers between 3 and 5 years (N = 20), and adults (N = 20) were tested to map the development of social inhibition of gaze. All participants looked significantly longer at the video of the confederate than at the live confederate (infants: $t(19) = -2.26$, $p = .036$, $d = 0.505$, children: $t(19) = -4.54$, $p < .001$, $d = 1.02$, adults: $t(19) = -4.0568$, $p < .001$, $d = .907$). However, participants' alpha power did not significantly differ between the live social and the non-social video situation, with Bayesian analyses providing evidence for a null effect ($BF = 0.26$). This finding suggests that participants are comparably attentive in the live and video situations as indicated by alpha power but inhibit their gaze in the live social situation, in line with implicit social rules. Interestingly, already infants in the first year of life showed inhibition of gaze in live social situations. However, the difference in gaze between the video and the live confederate increased until preschool age when adult levels were reached. The combination of EEG and eye-movement measures allowed us to suggest for the first time that decreased looking at live strangers compared to videos is not related to a lack of interest, as alpha power was comparably high, but to inhibition of gaze despite an interest in the other person. This suggests that infants are already sensitive to the social context and avoid gazing at strangers, although EEG alpha power indicates attentiveness towards them.

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Poster

PSTR560. Human Emotion: Social Perception and Evaluation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR560.17/PP9

Topic: H.06. Social Cognition

Support: IBS-R015-D1
NRF-2019M3E5D2A01060299
NRF-2019R1A2C1085566

Title: Surprise Elicits Neural Event Segmentation in Naturalistic Social Contexts

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Abstract: Unexpected behaviors of others trigger updates in our belief of their traits, which in return enable us to make more accurate predictions regarding their future actions. While previous studies have demonstrated the role of surprise signals in facilitating learning and discretizing continuous experiences, which involve abrupt changes in neural states, the neural mechanism underlying surprise-based learning in naturalistic social cognition remains poorly understood. This study aimed to identify the behavioral and neural signatures associated with surprise, which elicits belief updates, during the observation of naturalistic social interactions.
We hypothesized that neural activity patterns, reflecting the dynamic representation of character traits, would undergo modifications following the observation of the surprising actions, leading to distinct neural event boundaries. To examine this hypothesis, we collected surprise ratings for social interactions depicted in a movie featuring a two-person conversation. Subsequently, a separate group of participants assessed the perceived personality of each character along six trait dimensions after each scene. Additionally, we obtained fMRI data from an independent group of participants who watched the movie inside the scanner without a specific task. To identify the time-points at which shifts in neural states occurred, we utilized the GSBS (Greedy State Boundary Search; Geerligs et al., 2021) algorithm, a data-driven method for detecting neural state boundaries. By calculating the proportion of participants exhibiting neural event boundaries at each time point within periods centered around the peaks of surprise ratings, we determined the likelihood of neural changes aligning with moments of surprise.
Our results revealed a significant correlation between surprise ratings and changes in the representation of character traits. Furthermore, we found that neural event boundaries followed peaks in surprise ratings, particularly within brain regions involved in representing social information, such as the angular gyrus (AG), posterior cingulate cortex (PCC), and dorsolateral prefrontal cortex (DLPFC). These findings suggest that surprise signals triggered by the social actions of others induce shifts in neural states in the social brain regions, reflecting updated beliefs about others.

Disclosures: **J. Ro:** None. **Y. Seo:** None. **L.J. Chang:** None. **W. Shim:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.01/PP10

Topic: G.09. Drugs of Abuse and Addiction

Support: AA025713
AA020024
AA020023

Title: Adolescent binge ethanol exposure and neuroimmune activation contribute to developmental acquisition of alcohol tolerance

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Abstract: Binge drinking is common during adolescence, and epidemiological studies suggest that AUD is strongly associated with an adolescent age of drinking onset. Alcohol tolerance (i.e., an acquired reduction in acute alcohol responsivity) is a universally recognized key symptom of alcohol use disorder (AUD), but the developmental mechanisms underlying acquisition of tolerance are poorly understood. Using the preclinical adolescent intermittent ethanol (AIE) model of human adolescent binge drinking, our laboratories and others find AIE induces neuropathology that persists well into adulthood, including lasting neuroimmune induction, disruption of neurotransmitter systems, and increased adult ethanol self-administration. To begin to investigate developmental mechanisms underlying acquisition of low ethanol responsivity (i.e., tolerance), we developed an ethanol response battery (ERB) to provide measures of intoxication, hypothermia, plasma biomarkers, motor coordination, and balance across cumulative ethanol doses (i.e., 0.0 g/kg, 0.5 g/kg, 1.0 g/kg, 2.0 g/kg, and 3.0 g/kg). In Experiment 1, ERB assessment of alcohol-naïve male and female adolescent (P40) and adult (P85) Wistar rats revealed diminished ethanol responsivity in adolescents relative to adults. In Experiment 2, ERB assessment of adult (P80) Wistar rats following AIE treatment (5.0 g/kg, i.g., 2-days on/2-days off from P25-P54) revealed decreased ethanol responsivity, relative to CONs. In Experiment 3, ERB assessment of adult (P70) ethanol-naïve female Wistar rats following a single adolescent (P40) dose of the TLR4 agonist lipopolysaccharide (1.0 mg/kg, i.p.) revealed a reduction in ethanol responsivity, relative to CONs. In Experiment 4, we assessed whether treatment with the HMGB1 antagonist glycyrrhizic acid post-AIE would reverse the AIE-induced low ethanol responsivity adult female Wistar rats. Glycyrrhizic acid treatment reversed the AIE induction of low alcohol responsivity to CON levels. Taken together, these data reveal that (1) adolescents exhibit lower ethanol responsivity than adults; (2) AIE treatment induces lasting low ethanol responsivity (i.e., tolerance) in adulthood; (3) proinflammatory neuroimmune activation, which is a consistent finding associated with adolescent alcohol exposure, contributes to low ethanol responsivity in adulthood, and (4) treatment with the HMGB1 inhibitor glycyrrhizic acid reverses AIE-induced low ethanol responsivity in adulthood.

Disclosures: R. Vetreno: None. **F.T. Crews:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.02/PP11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA 055105

Title: Ontogeny of catechol-o-methyltransferase expression in the rat prefrontal cortex: effects of methamphetamine exposure

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Abstract: Methamphetamine (METH) is a widely abused stimulant drug that has been demonstrated to greatly increase dopamine release in numerous brain regions. The effects of METH on dopamine signaling contributes to many of its reinforcing effects, and dysregulation of the dopaminergic system can lead to long-lasting neurobiological and behavioral consequences. Catechol-o-methyltransferase (COMT) is an enzyme that is involved in the breakdown of catecholamines, and its role in dopamine clearance is thought to be especially important in the prefrontal cortex (PFC) where dopamine transporter expression is relatively scarce. This study utilized a rat model to characterize the ontogeny of COMT protein expression in the PFC across adolescence, which is a developmental stage that has been shown to involve significant reorganization of dopaminergic innervation in the PFC. Adolescence is also when the initiation of drug use often occurs, and research in both animal and human studies has suggested that early life substance use can lead to more severe consequences than are seen with adult use. In Experiment 1, drug-naïve male and female Sprague-Dawley rats were sacrificed on postnatal day (P) 29, 39, 49 or 69. Expression of COMT within the PFC was then analyzed via Western blot analysis to determine the ontogeny across peri- adolescence. In Experiment 2, rats were injected from P40-48 or P70-78 with saline or METH (twice daily, escalating from 0.5-5 mg/kg) and sacrificed on P49 or P79. Our results from Exp. 1 show that COMT expression increases across adolescence in a sex-dependent manner. Analysis of METH effects on COMT expression is ongoing. These preliminary findings suggest that COMT may be a substrate through which METH induces lasting changes in dopamine signaling, and that adolescents may be particularly vulnerable to these effects.

Disclosures: L.K. Carrica: None. **J.M. Gulley:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.03/PP12

Topic: G.09. Drugs of Abuse and Addiction

Title: Age difference in response to psychostimulant-opioid combination in rats

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Abstract: Combining psychostimulants and opioids is common among individuals who abuse drugs. The present research assessed age-related drug vulnerabilities and compared responsiveness of young and old rats to co-administration of a psychostimulant and an opioid. Male Wistar rats (3 months, n=12; 18 months, n=12) received drug treatments: saline; amphetamine (0.5 mg/kg), an indirect D1 agonist; morphine (1.25 mg/kg), a direct mu agonist; amphetamine+morphine; or amphetamine+morphine+SCH23390 (0.05 mg/kg), a D1 antagonist. Each animal's activity was monitored in an open field for 24 hours post-injection. An acute state was defined as an increase in activity during the first 4 hours post-injection; and an acute withdrawal was defined as a decrease in activity during 13-24 hours post-injection. When given alone, amphetamine and morphine increased acute activity in both young and old rats, with a greater increase after amphetamine compared to morphine. However, neither drug produced acute withdrawal. Co-administration of amphetamine and morphine produced distinctive age differences during both the acute and the withdrawal states. In young rats, morphine briefly diminished amphetamine-induced hyperactivity during the acute state, and the combination did not produce acute withdrawal. In old rats, morphine substantially augmented amphetamine-induced hyperactivity during the acute state, and the combination did produce acute withdrawal. SCH23390 given 15 minutes after the amphetamine+morphine combination blocked acute hyperactivity in both young and old rats and prevented acute withdrawal in old rats. Our data indicate that young and old animals show similar responses to dopamine manipulations, but different responses to an added opioid. Such age-specific responses to psychostimulant-opioid combination provide further evidence that the dopamine and/or opioid systems change with aging and also suggest that the age difference may reflect a change in the opioid system. As the population ages, identifying aging-related changes and unique drug vulnerabilities in older individuals will be important for developing differential treatments.

Disclosures: I. White: None. **W. White:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

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Program #/Poster #: PSTR561.04/PP13

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute of Mental Health R01MH098348 (D.C.K. and S.M.)

Title: Adolescent substance use and neural reactivity to stress

Authors: *S. DAMANI[1], D. GREY[1], J. PURCELL[1], S. MRUG[1], M. SCHUSTER[2], M. ELLIOT[3], S. TORTOLERO EMERY[4], D. KNIGHT[1];
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Abstract: Adolescence is a maturational period during which recreational substance use often begins and emotional processes undergo important development. Adolescent substance use may have long-term ramifications on the brain function that underlies emotional processes, like stress reactivity. The present study investigated the relationship between adolescent substance use (i.e., alcohol, tobacco, and cannabis) and neural reactivity to acute psychosocial stress in young adulthood. Stress reactivity is mediated by a neural network that consists of the prefrontal cortex (PFC), inferior parietal lobule, hippocampus, and amygdala. We hypothesized that stress-induced activity within these brain regions would vary with substance use. Substance use was assessed, and latent growth curve modeling (LGCM) was used to estimate trajectories of substance use across ages 11, 13, 16, & 19 years in participants (N = 1594) from the Healthy Passages study. A subset of these participants (n = 301; 20 years of age) completed a psychosocial stress task (Montreal Imaging Stress Task) during functional magnetic resonance imaging. Linear mixed-effects models compared stress-evoked brain activity and behavioral stress reactivity to cumulative substance use during adolescence. Tobacco use varied negatively with stress rating and skin conductance response (SCR), and cannabis use varied negatively with SCR. No relationship was observed between alcohol use and behavioral measures. Dorsomedial PFC, hippocampal, and amygdala reactivity to stress varied negatively with cumulative alcohol use. PFC (ventromedial and dorsolateral) and hippocampal activity varied negatively with cumulative tobacco and cannabis use. Separate analyses used multivariate modeling to compare stress-evoked brain activity to each participant's trajectory of use based on the LGCM (early use, change, and acceleration) for each substance. Early alcohol use was associated with stress-related ventromedial PFC activity and change in alcohol use was associated with dorsolateral and ventromedial PFC activity. Early tobacco use was associated with PFC (dorsolateral, ventrolateral, and dorsomedial) and hippocampal activity, while acceleration of tobacco use was associated with dorsolateral PFC activity. Early cannabis use was associated with PFC (dorsolateral, ventrolateral, and dorsomedial) activity and acceleration of cannabis use was associated with dorsolateral and dorsomedial PFC activity. These findings demonstrate that stress-related PFC, hippocampal, and amygdala activity varies with adolescent substance use and may have long-term ramifications upon emotional function.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.05/PP14

Topic: G.09. Drugs of Abuse and Addiction

Support: The Jean Phillips Shibley Endowment (T.J.G.)
Penn State University (T.J.G.)
Minciencias Fulbright Colombia (C.N.),
Republic of Türkiye Ministry of National Education Scholarship (H.S.Ü.)

Title: Genetic background and sex modulate adult nicotine sensitivity and brain functional connectivity after adolescent exposure in mice

Authors: *C. NOVOA[1,2], S. ÜNSAL[3,6,1], N. ZHANG[3,4,5,1], T. J. GOULD[2,4,1];
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Abstract: Early substance use predicts a higher risk for severe substance use disorders later in life. Adolescents are a particularly sensitive group due to notable turning points in brain maturation and the propensity to adopt risky behaviors characteristic of this age. Despite generally increased susceptibility during this stage of development, biological and environmental factors moderate the risk for substance use disorders and result in individual differences that are not yet well understood. Elucidating the nature of individual differences in risk for substance use disorders may help concentrate efforts for more efficient prevention and treatment. Here we studied the contributions of genetic background and sex to the long-term effects of adolescent nicotine exposure on adult anxiety-like behavior, nicotine sensitivity, and functional brain connectivity. We treated male and female adolescent mice (PND 37-49) of the strains C57BL/6J and DBA/2J with nicotine for 12 days via subcutaneous osmotic minipumps (free base 24 mg/kg/day). Four weeks after the termination of nicotine exposure, we evaluated these mice for anxiety-like behavior (PND 79), nicotine-induced hypolocomotion (PND 86-88), and functional brain connectivity through resting-state functional MRI (PND 96). We observed that adolescent nicotine exposure resulted in enduring adaptations of anxiety-like behavior depending on sex and strain. Adult DBA/2J mice showed a larger hypolocomotor response than C57BL/6J to acute nicotine (s.c. 0.81 mg/kg), and mice pre-exposed to nicotine recovered faster from the effects of nicotine on locomotor activity. Preliminary evaluation of functional MRI data suggests that DBA/2J mice have higher functional connectivity between the frontal cortex and hippocampus than C57BL/6J mice, and nicotine-treated mice in both strains had lower functional connectivity between the frontal cortex and hippocampus compared to saline-treated mice. These results indicate that nicotine exposure during adolescence has long-lasting effects on nicotine sensitivity, brain function, and affective processes that may contribute to an increased risk of substance use in adulthood. Moreover, these results show that biological factors such as sex and genetic background play an important role in moderating the effects of drug exposure and are essential for understanding individual differences in susceptibility to drug misuse. Further analysis of functional MRI data will provide insight into the adaptations of neural circuitry that occur with adolescent drug exposure, which serve as a putative mechanism for enduring risks derived from adolescent exposures.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.06/PP15

Topic: G.09. Drugs of Abuse and Addiction

Support: AA027915
Miami University

Title: The effects of lead exposure during adolescence on fentanyl drinking behavior in mice

Authors: *C. M. THACH, R. A. ZEGARELLI, K. D. REAM, T. W. PERRY, A. K. RADKE; Psychology, Miami Univ., Oxford, OH

Abstract: Distinct comorbidities between attention deficit/hyperactivity disorder (ADHD) and substance use disorders (SUDs) call for investigations of potential causative factors. Lead (Pb), a neurotoxin that competes with calcium at the synapse, is an environmental factor that can cause cognitive and learning disorders, such as ADHD. Previous preclinical research has found that Pb affects relapse, but not consumption, in mice self-administering alcohol. The current study investigated whether adolescent exposure to Pb influences oral fentanyl consumption in male and female mice by using Drinking in the Dark, a model of consumption in which mice self-administer for 2-hours during their active phase of the light/dark cycle. It was predicted that Pb exposure would increase risk of displaying fentanyl addictive behaviors, operationalized as higher consumption and continued consumption despite negative consequences (i.e., aversion-resistance). Mice were divided into Pb and control groups and were balanced by sex. Treatment took place from PND21 (wean date) to PND42, which is adolescence. The Pb group received 30ppm Pb acetate, which would reflect levels of Pb found in environments of inner cities, while the control group received 2.7mL acetic acid mixed into water. After the mice reached PND60 (adulthood), they entered the drinking paradigm, where they were given the choice of reverse osmosis water or 10μg/mL fentanyl acetate for 10 sessions. Aversion resistance was tested by adding increasing concentrations of quinine (0μM, 100μM, 250μM, 500μM), a bitter tasting solute, to the 10μg/mL fentanyl acetate on sessions 11-14. Rates of consumption for fentanyl and the fentanyl + quinine solutions were significantly different between sexes, with females consuming more than males. All groups showed aversion resistance, failing to reduce intake at any concentration of quinine. The Pb groups tended to drink less than control groups, although Pb treatment affected rates of fentanyl consumption differently between males and females. Although these results are different than initially predicted, they call for future investigation of the effects of Pb on addictive behavior, with a particular focus on potential sex-dependent effects. Future studies should also investigate the impact of Pb exposure in additional models of addictive behavior, such as relapse and withdrawal.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.07/PP16

Topic: G.09. Drugs of Abuse and Addiction

Support: T32AA026577

Title: Reward sensitivity is increased by adolescent morphine treatment and persistently decreased by withdrawal

Authors: *J. D. ROITMAN[1], D. MCANDREW[2], M. LOH[3], R. DONKA[4];
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Abstract: Adolescent substance abuse drives significant changes that persist through adulthood in reward processing, motivated behavior, and decision making. Adolescent opioid use is a significant risk factor for later substance use disorders, however the mechanism of this increased susceptibility is unknown. Opioids are highly reinforcing and have been shown to directly modulate reward pathway activity in the brain. To determine the effects of adolescent opioid treatment and withdrawal on reward sensitivity, we used a rate-frequency intracranial self-stimulation (ICSS) paradigm.Rats were implanted on PN28 with stimulating electrodes targeting the medial forebrain bundle (-2.5 AP; +/- 1.5 ML; -7.5 DV from dura). ICSS sessions were conducted twice daily (7:30 AM and 5:30 PM) for ~30 minutes per session. Once the minimal reinforcing stimulation amplitude was determined for each subject, they were trained to stable performance on an adapted rate-frequency task that used fewer, more discriminable frequencies to enable more rapid training. Rats were randomly assigned to morphine (5 mg/kg, i.p.) or saline control (0.5 ml/kg, i.p.) treatment groups. Injections were administered 20 minutes prior to the morning rate-frequency ICSS session from PN 45 to 51. Twice-daily sessions continued following treatment until PN60 to measure withdrawal effects.Reward sensitivity was stable across baseline, injection, and post-injection rate frequency sessions in control rats. Daily morphine administration resulted in a significant increase in reward sensitivity relative to baseline in morning sessions for each of the 7 treatment days. However, we observed a significant decrease in reward sensitivity in the afternoon sessions on those days. During the entire 9-day withdrawal period, rats showed a persistent decrease in reward sensitivity relative to baseline, with no recovery trend. This contrasts with our measurements in adult rats which typically recover to baseline within 3 days post morphine treatment. Adolescent exposure has significant long-term effects on reward sensitivity, indicating an interaction between opioid administration and development. Further research will examine potential mechanisms for persistent effects on reward sensitivity following adolescent morphine exposure.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Program #/Poster #: PSTR561.08/PP17

Topic: G.09. Drugs of Abuse and Addiction

Support: This work was supported by a grant from the NIDA (K01DA043615) to M.A.P.

Title: Distinct Subgroups within Adolescents with Family History of Drug Use: Insights from the ABCD Study using K-means Clustering

Authors: *A. RAMAKRISHNAN[1,2], R. SHAIK[2], S. HASS[2], S. FRANGOU[2,4], I. IVANOV[2,3], P. MUHAMMAD[2,3];
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Abstract:
Objectives: Family history of alcohol use is a risk factor for the development of alcohol use in adolescents. However, there are other environmental exposures that could modulate the risk loading of the family history of alcohol use. This study aims to explore distinct subgroups among a cohort of adolescents with a family history of drug use from the ABCD study, based on sociodemographic and environmental factors.
Methods: Baseline data from the ABCD study of 7222 adolescents (ages: 9 - 10 years old) with a family history of drug use were analyzed. K-means clustering, an unsupervised machine learning algorithm, was used to identify distinct subgroups based on 22 variables encompassing demographic factors, parent drug use, school engagements, and peer involvement. The optimal number of clusters was determined using silhouette analysis. One-way ANOVA and pairwise comparisons were used to identify sub-group's characteristics.
Results: Four distinct clusters emerged among adolescents with a family history of drug use: Cluster 1 (n=2531) represented adolescents with higher levels of school engagement ($p = 0.0001$), and positive peer interactions ($p<0.034$). Cluster 2 (n=2124) comprised of parents with higher income ($p<0.021$). Cluster 3 (n=1485) comprised adolescents with higher parental education ($p =0.034$). Finally, cluster 4 (n=1082) displayed limited participation in school and peer engagements ($p = 0.001$), child victimization ($p =0.000$), and long-term bullying ($p=0.007$). Analyses for group differences in neurocognitive assessments and neuronal density are underway.
Conclusions: This study utilized K-means clustering to identify distinct subgroups among adolescents with a family history of drug use. The findings highlight the heterogeneity within and between these clusters based on sociodemographic factors. These insights can inform targeted interventions and support programs tailored to specific subgroups. Further research

should focus on exploring the impacts of these interaction patterns on various aspects of adolescent development.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Program #/Poster #: PSTR561.09/PP18

Topic: G.09. Drugs of Abuse and Addiction

Support: University of Minnesota's Internal Cross Divisional Grant 2022

Title: Characterization of internalizing and externalizing behavior in children with prenatal methamphetamine exposure using a novel database created from electronic health records

Authors: *T. A. RAWSTERN[1], J. LEHMAN[1], E. FECZKO[1], J. LOLENG[2], S. ANDERSON[2], Y. RAE[1], R. J. HERMOSILLO[3], L. MOORE[3], A. RANDOLPH[1];
[1]Pediatrics, [2]Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Minnesota, Portland, OR

Abstract: The effects of methamphetamine (MA) use go far beyond the user, with approximately 0.7% to 4.8% of children in the U.S. being exposed to MA prenatally. Interventions for MA use are currently limited to behavioral interventions, leaving this population at severe risk of continued use during pregnancy. Prenatal methamphetamine exposure (PME) is a serious public health issue. Unlike alcohol, the developmental consequences of PME are not well characterized, leaving these individuals vulnerable to inadequate support including medical, school, and governmental resources. There is a social and clinical need to understand the effects and symptomology for those prenatally exposed to MA to ensure quality treatment and equitable access to resources. In response, a retrospective cross-sectional database was created using the electronic health records of patients seen at the University of Minnesota's Adoption Medicine Clinic, a pediatric clinic with a patient population largely composed of individuals with prenatal substance exposures. This novel database was used to investigate the internalizing and externalizing behavioral profile of children prenatally exposed to MA. Using patients with data from the standardized Achenbach's Child Behavior Checklist (CBCL) assessment, multiple linear regression models were developed (controlling for age, gender, race/ethnicity, and adverse childhood experiences) to assess the association between PME and observable behavioral outcomes. Of 267 patients with CBCL data (5-18 years old), 74 patients were identified as prenatally exposed to MA based on maternal self-report, caregiver report, and/or toxicology results. The CBCL is composed of a variety of scaled and standard composite scores. Each of the scores included on the CBCL were examined to determine if the met the definition of internalizing, inner-directing behavior (e.g. anxiety), or externaling beahavior, negative outer-directing behavior (e.g. aggressive behavior). PME was not significantly

associated with any internalizing behavior clinical scale. However, the Emotional Reactivity (p: 0.0317) and Rule Breaking (p: 0.01015) externalizing behavior scales were associated with prenatal exposure to MA. These results suggest PME plays a role in behavioral development of exposed children, impacting the relationship between an individual and their surrounding environment. To better understand the overall impact of PME on behavioral and neuronal development, further investigation is needed. These insights lay the groundwork to develop a global diagnostic profile for PME- allowing impacted individuals to receive proper treatment and resources.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.10/PP19

Topic: G.09. Drugs of Abuse and Addiction

Support: 5K99DA049908
4R00DA049908
5R21DA049253

Title: Prefrontal cortical microglial transcriptional patterns relate to behavioral changes after prenatal morphine exposure

Authors: ***B. L. SMITH**[1], J. L. BOLLINGER[2], S. WOODBURN[2], T. A. GUZMAN[2], A. H. BRENDLE[2], A. G. MAKELA[1], E. S. WOHLEB[2], T. REYES[2];
[1]Psychological Sci., Northern Kentucky Univ., Highland Heights, KY; [2]Pharmacol. & Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: We have recently found that prenatal morphine (MO) exposure induces sex-dependent executive function deficits in adult offspring using the 5-choice serial reaction time task (5CSTT) in mice. Immunohistology in the prefrontal cortex (PFC) revealed that prenatal MO decreased CD68 levels in IBA1+ cells in male offspring, yet this was increased in females. These results suggest that shifts in microglia phenotype may contribute to executive function deficits after prenatal MO exposure. To further characterize microglial responses in the PFC, whole transcriptome analyses were performed following prenatal MO exposure. We predicted that prenatal MO exposure would cause sex-specific gene expression changes in PFC microglia, and these would likely relate to executive function outcomes in the 5CSRTT. Female C57/BL6 mice were given MO via the drinking water (0.3 mg/ml with 0.2% saccharin; n = 17) or saccharin only (n = 16) one week prior to mating with DBA males. Maternal exposure continued throughout gestation and lactation until offspring were weaned on postnatal day 21. Executive function was

assessed in offspring from postnatal weeks 13-20 using the 5CSRTT (n = 9-13/sex/group). Microglia were then sorted from dissected PFC for RNA-Seq using fluorescence activated cell sorting. In the 5CSRTT, male MO-exposed offspring had reduced accuracy and female MO-exposed offspring had increased inattentive behavior. Prenatal MO exposure upregulated 58 genes and downregulated 31 genes in male microglia (adjusted $p < 0.05$). Though a similar number of genes were altered in female microglia (51 upregulated, 70 downregulated), only 3 differentially expressed genes overlapped in both sexes: Irf2bpl, Kctd21, and Smim3. Hierarchical gene x behavior clustering analysis of significantly differentially expressed genes by normalized behavioral performance revealed few gene expression patterns that related to behavior across MO and SCH offspring combined. However, performing the same hierarchical clustering within MO offspring and SCH offspring independently yielded genes that related to behavior in MO but not SCH offspring. Genes that significantly correlated to behavior in MO offspring were Sco2 in males and Otub2 in females. Prenatal MO exposure alters the microglia transcriptome in adult offspring in a largely sex-dependent manner. Microglial gene expression relates to behavioral function in MO offspring.

Disclosures: **B.L. Smith:** None. **J.L. Bollinger:** None. **S. Woodburn:** None. **T.A. Guzman:** None. **A.H. Brendle:** None. **A.G. Makela:** None. **E.S. Wohleb:** None. **T. Reyes:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.11/PP20

Topic: G.09. Drugs of Abuse and Addiction

Support: CSU AAUP

Title: Impact of early prenatal maternal marijuana use on brain development and behavior in the rat

Authors: *R. JEFFREY[1,2], K. BORDNER[3];
[2]Biol., [3]Psychology, [1]Southern Connecticut State Univ., New Haven, CT

Abstract: Maternal rates of marijuana use, especially during the first trimester, are on the rise, yet we know little about the long-term consequences of brief marijuana exposure during the early prenatal period. Recent changes in federal regulations leading to the decriminalization of marijuana possession, coupled with the growing potency of the drug and rising use signal that we are at the cusp of an emerging public health crisis. Our labs aim to assess brain and behavioral changes across development that are associated with marijuana exposure in the earliest stages of pregnancy. The long-term objective of our work is to shed light on the effects of marijuana use by mothers during early pregnancy on their offspring.
Previous work in our labs examined the effects of prenatal WIN55-212,2, a potent CB1 receptor agonist, on brain and behavior of the developing rodent. Preliminary results revealed significant

main effects of prenatal WIN administration on body-weight and anxiety-like behavior. Motivated by these results we are now administering moderate doses of delta-9-tetrahydrocannabinol (THC) at either at the beginning of pregnancy or during the last days of gestation in the rat. Offspring are then evaluated on behaviors related to unconditioned anxiety, social interactions and social communication. Preliminary results reveal differences in birth weight, social behaviors, and anxiety-like behaviors in animals exposed to THC during the earliest days of gestation compared to controls.
This research is ongoing and provides valuable information to health care providers when consulting with their human patients about possible effects of marijuana use during pregnancy, as well as opening doors for further investigations into the use of marijuana on development and health of the child.

Disclosures: R. Jeffrey: None. **K. Bordner:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.12/PP21

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027773

Title: Adolescent ethanol exposure increases neuronal excitability via downregulation of glutamate transport in the paraventricular nucleus of the thalamus, contributing to adult anxiety susceptibility

Authors: *P. BIGGS[1], H. KIM[1], A. BENNETT[1], S. KANG[2];
[2]Dept. of Pharmacol. & Toxicology, [1]Med. Col. of Georgia at Augusta Univ., augusta, GA

Abstract: Repeated ethanol exposure during adolescence increases risk for displaying anxiogenic phenotype in adulthood, but the underlying mechanisms are not fully understood. The paraventricular nucleus of thalamus (PVT) has been considered a hub brain area controlling brain anxiety network. Indeed, recent anatomical and functional studies suggest that the PVT presents a variety of neural signals according to the early-life experience and the activities are significantly correlated with anxiety-like behavior. It remains unknown how the repeated ethanol exposure during adolescence affects the coordinated activities of astrocytes and neurons of the PVT in adulthood, and consequent behavioral adaptation. To investigate this, we compared the cellular activities in the PVT and behavioral consequences of mice withdrawn from adolescent repeated ethanol exposure (AAE) and ethanol naïve counterparts (CON), using electrophysiological, biochemical, chemogenetic, transgenic, and behavioral approaches. Briefly, mice were exposed to air or vaporized ethanol in a vapor inhalation chamber for three weeks from P28 to P49. Each daily cycle consisted of ethanol vapor for 16 h followed by 8 h of abstinence in their home cage. This was repeated each day for 4 consecutive days, followed by 3

days of abstinence. The cellular activities and animal anxiety-like behavior were evaluated after 3 weeks withdrawal from the AAE paradigm. We observed that the firing and ΔFosB immediate early gene expression of the PVT neurons were increased in the CIE group compared to those of the ethanol-naïve CON group. Behavioral evaluation suggests increased anxiety-like behavior in CIE mice with unimpaired locomotion shown by decreased time spent within the center of the open field test. The anxiety-like behavior was alleviated through a chemogenetic approach utilizing the hM4Di DREADD and its ligand J60 to selectively silence neuronal activity in the PVT. Western blot analysis showed that GLT-1 (an astrocyte glutamate transporter, as known as EAAT2, slc1a2) expression levels in the PVT were significantly reduced in the CIE group compared to those of the CON group. The selective upregulation of GLT-1 in the PVT astrocytes via the expression of GFAP-promoter driven Cre recombinase in the PVT of GLT-1 Ai9 mice alleviated the anxiety-like behavior. These findings highlight the significant role of PVT astrocytic GLT-1 in the anxiogenic phenotype in adulthood induced by withdrawal from adolescent repeated ethanol exposure, suggesting that GLT-1 in the PVT could serve as a therapeutic target for alcohol use disorder and comorbid emotional disorders.

Disclosures: **P. Biggs:** None. **H. Kim:** None. **A. Bennett:** None. **S. Kang:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.13/PP22

Topic: G.09. Drugs of Abuse and Addiction

Title: Acquisition of fentanyl seeking in adolescence following maternal separation is sex and hormone dependent

Authors: *F. ABOALROB[1], Z. KNAUSS[2], D. MUELLER[3];
[1]Kent State Univ. - Biol. Sci., Stow, OH; [3]Biol. science, [2]Kent State Univ. - Biol. Sci., Kent, OH

Abstract: The opioid epidemic is a major health crisis in the U.S. resulting in 107,622 deaths in 2022. Most recently, 9.2 million people began the non-medical use of prescription opioids including 19% of adolescents. The susceptibility to substance use disorders is influenced by a number of factors, including traumatic childhood experiences such as parental neglect. Early Childhood Neglect (ECN) accounts for up to 75.3% of all child maltreatment cases in the US annually and coincides with susceptibility to opioid use disorder (OUD); survivors are twice as likely to be prescribed opioids and 4.5 times more likely to develop OUD. Thus, we assessed the effects of maternal separation (MS), a potent form of ECN, on seeking behavior during adolescence and adulthood using a rat model of fentanyl-induced conditioned place preference (CPP). Sprague Dawley rat pups (n=65; male=23, female=42) were cross fostered at birth and received 3-hour daily MS on P2-P18 or were allowed to receive full maternal care. Rats were assigned to adolescent (P32) or adult (≥P50) place conditioning in a three-chamber apparatus for eight days under one of four conditions: 1) control - saline (1 ml/kg, s.c.), 2) control – fentanyl (5

ug/kg, s.c.), 3) MS – saline, or 4) MS – fentanyl. Extinction testing was conducted 24-hours post-conditioning for three days during adolescence and for eight days and then weekly in adulthood until day 91 or until extinction criteria were met. We found that MS enhanced the magnitude and persistence of fentanyl seeking in adolescent and adult males as compared to control non-MS rats ($p<0.05$). In contrast, MS impaired the formation of fentanyl seeking in adolescent and adult female rats as compared to control non-MS rats ($p<0.05$). Thus, MS induced a significant but sex-dependent alteration in the expression of fentanyl seeking during adolescence and adulthood. To determine the role of sex hormones in the observed sex differences, we assessed whether masculinizing females with 1mg/kg testosterone propionate on P1 would result in male-like responses to fentanyl in adolescence and adulthood. Indeed, masculinized females showed enhanced fentanyl seeking following MS during adolescence compared to control females ($P<0.05$) and matched the responses of males that had MS. These trends continued into adulthood. Importantly, MS suppressed exploratory behavior and locomotion in females suggesting MS exacerbated anxiety. These findings are consistent with human studies on the effects of ECN on the propensity and persistence of OUD, permitting future studies on the neurobiological mechanisms by which MS alters fentanyl seeking sex-dependently later in life.

Disclosures: **F. Aboalrob:** None. **Z. Knauss:** None. **D. Mueller:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

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Program #/Poster #: PSTR561.14/Web Only

Topic: G.09. Drugs of Abuse and Addiction

Support: CONACYT Fellowship 738736

Title: Exposure to toluene modifies steroid hormone levels in female pregnant and non-pregnant mice

Authors: *V. CERBANTEZ, G. RODRIGUEZ-MANZO, S. CRUZ;
Pharmacobiology, CINVESTAV SUR, Mexico City, Mexico

Abstract: Toluene is a solvent widely used in industrial and household activities because it is the main compound in many products like thinner, paints, inks, and glues. These products can be inhaled to experience their psychoactive effects, with concentrations that can reach several thousands of ppm, for brief periods, repeatedly. Under toluene intoxication, people can experience incoordination, dizziness, slurred speech, and hallucinations among other symptoms due to its effect as a central nervous system depressor. Solvent misuse during pregnancy can result in fetal solvent syndrome, possibly due to interrupting endocrine homeostasis during development. However, the neuroendocrinological effects affecting both mother and child are poorly characterized. This study aims to carry out a hormonal screening to identify alterations in steroid hormones in pregnant and non-pregnant female mice exposed to 8,000 ppm, twice a day

for five days per week (7-10 days of exposition), to elucidate possible neuroendocrine produced by toluene inhalation that might result in fetal developmental alterations. Our results show that toluene exposure before gestational day six significantly decreases the percentage of successful pregnancies. In addition, non-pregnant female mice exposed to toluene have higher testosterone and corticosterone serum levels than air-exposed animals, and pregnant mice treated with toluene have higher levels of corticosterone than non-pregnant mice. These changes could be one of the homeostasis disruptions impacting growth and cognitive development in mice exposed to toluene prenatally.

Disclosures: V. Cerbantez: None. **G. Rodriguez-Manzo:** None. **S. Cruz:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.15

Topic: G.09. Drugs of Abuse and Addiction

Title: Behavioral effects of nicotine and ethanol exposure in adolescent rodents

Authors: M. A. ANDREWS[1], A. BALDWIN[1], H. WEBBER[1], *G. FERNANDEZ[2];
[1]Psychology, [2]Christopher Newport Univ., Newport News, VA

Abstract: Concurrent rates of alcohol and nicotine use are rising, especially given the increasing popularity of electronic nicotine delivery systems. Previous literature indicates that drug exposure during early developmental stages can have detrimental effects on learning and memory, as well as emotional regulation during adulthood. Our current study examines the behavioral effects of nicotine and ethanol exposure in adolescent rats on drug reward and anxiety-like behavior. Starting on post natal day 28, male and female Sprague Dawley rats were exposed to either a subcutaneous injection of 0.4 mg/kg nicotine at a dose of 1 ml/kg and an intraoral gastric gavage of 20% ethanol at a dose of 5 g/kg, or combined saline injections and water gavage. All rats received a total of 12 exposures on an intermittent, 2 day on/ 2 day off schedule. Repeated exposure to nicotine and ethanol did not have a significant effect on the percent of time spent in the open arm of an elevated plus maze in male or female rodents. Similarly, adolescent nicotine and ethanol exposure did not differentially affect reward related behavior using a single trial nicotine conditioned place preference protocol, although all animals spent significantly more time in the nicotine paired chamber. While our studies did not yield significant interactions, future studies will examine development related effects by including an adult exposure group, and compare spine density changes in the rodent prefrontal cortex.

Disclosures: M.A. Andrews: None. **A. Baldwin:** None. **H. Webber:** None. **G. Fernandez:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

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Program #/Poster #: PSTR561.16/PP23

Topic: G.09. Drugs of Abuse and Addiction

Title: The Effects of Repeated Ketamine Administration on Behavior, Cognition, and Corticosterone Levels in Adolescent Female Mice

Authors: J. ACEVEDO[1], A. WELTER[2], ***J. SIEGEL**[3];
[1]The Lundquist Inst. for Biomed. Innovation at Harbor-UCLA Med. Ctr., Torrance, CA; [2]Univ. of Minnesota, Twin Cities, Minneapolis, MN; [3]Oregon State Univ., Corvallis, OR

Abstract: Ketamine is emerging as an effective, rapid acting antidepressant, and has been approved for the treatment of treatment-resistant depression. The majority of the preclinical research on ketamine has been in males, despite data showing females are diagnosed with Major Depressive Disorder at higher rates. Furthermore, the preclinical literature on behavioral and cognitive effects of ketamine shows conflicting results and the majority of research has focused on effects in adults, with limited data in adolescent models. We examined the effects of repeated sub-anesthetic ketamine exposure on locomotor activity, anxiety-like behavior, novel object recognition memory, depression-like behavior, and plasma corticosterone levels in adolescent female mice. Mice were exposed to 15 mg/kg ketamine for 10 consecutive days, and behavior was measured in the open field test, novel object recognition test, and Porsolt forced swim test. Plasma corticosterone levels were measured following behavioral testing. There was no effect of ketamine on locomotor activity, anxiety-like behavior, novel object memory, or plasma corticosterone levels. Ketamine increased the number of immobile episodes, but not the percent time immobile, in the Porsolt forced swim test, indicating a potential increase in depression-like behaviors. These results suggest that sub-anesthetic ketamine exposure in adolescent female mice does not reduce anxiety- or depression-like behaviors as is often seen in adults and males.

Disclosures: J. Acevedo: None. **A. Welter:** None. **J. Siegel:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Program #/Poster #: PSTR561.17/PP24

Topic: G.09. Drugs of Abuse and Addiction

Support: Department of Anesthesiology, the Lieberman Research Endowment
NIH Grant R21DA046284

Title: Sedation with Midazolam in the NICU: A Double-Edge Sword

Authors: *N. NGUYEN[1,2], A. FLORES[2], J. YI[2], P. ATHOTA[2], D. MEYER[2], R. BHAKAT[2], S. YELAMANCHILI[2], G. PENDYALA[2];
[2]Dept. of Anesthesiol., [1]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: In the US in 2021, approximately 10% of newborns were born preterm (<37 weeks of gestation), placing them at risk for various health issues such as low birth weight, respiratory depression, and heart problems due to their underdeveloped bodies. To improve their chances of survival, preterm infants often undergo extensive surgeries and mechanical ventilation, which can cause stress and agitation. To manage these conditions, sedative agents are frequently administered. However, studies have shown a link between childhood exposure to anesthesia/sedatives and cognitive impairments. Midazolam (MDZ) is a commonly used benzodiazepine sedative in the Neonatal Intensive Care Unit (NICU) and the effects associated with its long-term use in neonates and subsequent outcomes at different stages of development i.e., early childhood, early adolescence, late adolescence, and early adulthood remain poorly understood. With this knowledge gap in mind, our study examined the long-term effects of MDZ exposure on neurodevelopment using a preclinical rodent model. We established a dose-escalation regimen from postnatal day (P) 3 pups until P21 to comprehensively characterize how early-life exposure to MDZ impacts neurodevelopment outcomes at different tiers ─ phenotypic, molecular, and behavioral levels. Our data demonstrated that repetitive exposure to MDZ during the neonatal period negatively affects the overall physique attributes in early childhood. Notably, while the expression levels of proinflammatory cytokines were not significant during early childhood, increased levels in their expression were seen in adulthood. In addition, we observed alterations in neurochemistry in adulthood. Lastly, we observed trends of increased anxiety-like behavior, nociception, and reduced social interaction effort during early adolescence as compared to other stages. In summary, our study for the first time provides a comprehensive characterization of the repetitive effects of MDZ in neonates can impact the outcomes at different stages of life. These data provide an excellent springboard to further delineate mechanisms that could contribute to overcoming neurodevelopmental complications associated with long-term MDZ use in neonates.

Disclosures: **N. Nguyen:** None. **A. Flores:** None. **J. Yi:** None. **P. Athota:** None. **D. Meyer:** None. **R. Bhakat:** None. **S. Yelamanchili:** None. **G. Pendyala:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Program #/Poster #: PSTR561.18/PP25

Topic: G.09. Drugs of Abuse and Addiction

Support: UMN Internal Grant - Pediatric Cross-Divisional

Title: Executive function measures and prenatal methamphetamine exposure: a retrospective study of children and adolescents using electronic health records

Authors: *J. LEHMAN[1], E. FECZKO[2], R. HERMOSILLO[3], T. RAWSTERN[4], J. LOLENG[4], S. ANDERSON[4], Y. OUM[4], L. MOORE[3], A. RANDOLPH[2];
[1]Office of Academic Clin. Affairs, [2]Pediatrics, Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Minnesota, Portland, OR; [4]MIDB, Univ. of Minnesota, Minneapolis, MN

Abstract: The intrauterine environment plays a significant role in neurodevelopment. Prior research shows associations between prenatal exposure to recreational substances and adverse outcomes in cognitive and behavioral development. The deleterious impacts of prenatal alcohol exposure have been well established, however, the developmental consequences of other exposures including prenatal methamphetamine (MA) exposure (PME) have not been as extensively investigated or recognized. Considering the rise of MA use in the US, with nearly 400,000 women estimated to have used MA in 2021, a pressing demand exists to elucidate the impact of PME on downstream neurocognitive functioning. To this end, we carried out a retrospective cross-sectional study investigating associations between PME and measures of executive functioning (EF) using data collected from the electronic health records of patients seen at the University of Minnesota's Adoption Medicine Clinic (AMC)—a pediatric clinic whose patient population includes children with prenatal substance exposures. Our sample included 214 children (4-18 years) with neuropsychological assessment data from the Behavior Rating Inventory of Executive Function, Second Edition (BRIEF-2). Clinical scales from the BRIEF-2 assess a child's difficulty with behaviors related to domains of EF. Prenatal exposures to different substances were confirmed by AMC clinicians using caregiver testimonies, maternal toxicology reports, and/or legal records, and were examined as binary variables (Exposed/ Unexposed). Associations between prenatal exposures and BRIEF-2 scores were analyzed via multiple linear regression, controlling for age, gender, race/ethnicity, caregiver education, geographic region, and adverse childhood experiences. Results indicated that PME (n=48) was significantly associated with elevated scores on the Working Memory (β=3.372, ***p***=0.043) and Plan/Organize (β=3.211, ***p***=0.049) clinical scales according to BRIEF-2 standards. Trends were also observed with the Task-Monitor scale (β=3.139, ***p***=0.057) and the Cognitive Regulation Index (β=3.118, ***p***=0.051). Collectively, these results suggest PME may be associated with increased struggles in a child's ability to plan, carry out, and complete tasks, having major implications on a child's academic performance and overall functioning. Further investigation of PME's neurodevelopmental role is necessary to better understand the needs of MA-exposed children and develop a psychopathological profile of the exposure.

Disclosures: **J. Lehman:** None. **E. Feczko:** None. **R. Hermosillo:** None. **T. Rawstern:** None. **J. Loleng:** None. **S. Anderson:** None. **Y. Oum:** None. **L. Moore:** None. **A. Randolph:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Program #/Poster #: PSTR561.19/PP26

Topic: G.09. Drugs of Abuse and Addiction

Support: RO1DA046794

Title: Alprazolam (Xanax) exposure during adolescence exacerbates spontaneous morphine withdrawal

Authors: *A. M. CARDONA-ACOSTA[1], N. MEISSER[2], C. A. BOLAÑOS-GUZMÁN[3]; [1]Psychological and Brain Sci., [2]Texas A&M Univ., College Station, TX; [3]Texas A&M Univ., college station, TX

Abstract: Reports show that patients seeking treatment for opioid use disorder and/or withdrawal are commonly also benzodiazepine co-dependent (De Wet et al., 2004; Mahoney et al., 2021). Alprazolam (Xanax; ALP) is a potent, short-acting benzodiazepine that is widely prescribed for the treatment of anxiety disorders. However, its use and abuse has increased in recent years, fueling the opioid drug epidemic (Jann et al., 2014). Concomitant ingestion of ALP and opioids has also been reported in the adolescent population, resulting in a heightened risk for developing substance use disorders (SUDs) (Clark et al., 1998; Mccabe et al., 2012). Surprisingly little is known about ALP-opioid interactions and the potential negative effects that come with their co-ingestion during this critical period of development. Therefore, this study was designed to investigate the effects of ALP exposure during adolescence on spontaneous morphine (MOR) withdrawal. Adolescent C57BL/6J male mice (postnatal day [PD] 35) were pretreated with either vehicle (VEH) or ALP (0.5 mg/kg) once daily from PD35-49. The mice were then treated twice daily with either saline (SAL) or escalating doses of MOR for 6 consecutive says. On day 7, the mice received a challenge dose of MOR (20 mg/kg) and spontaneous withdrawal signs were observed 2, 4, 8, and 24 hours after the last MOR injection. Mice pretreated with ALP exhibited significant weight loss during MOR treatment. Twenty-four hours after discontinuation of MOR treatment, ALP pretreated mice showed significant weight loss when compared to the VEH-MOR-treated controls. Moreover, ALP pretreated mice exhibited a significant increase in total withdrawal signs (i.e., jumping, chewing/licking, paw tremors, headshakes) when compared to the VEH-MOR-treated controls. Overall, our findings have critical implications for the perpetuation of SUD's during adolescence as potentiated withdrawal symptoms can drive the continuation of drug use.

Disclosures: A.M. Cardona-Acosta: None. **N. Meisser:** None. **C.A. Bolaños-Guzmán:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.20/PP27

Topic: G.09. Drugs of Abuse and Addiction

Title: Identifying Cocaine-Induced RNA Modifications using Mass Spectrometry Analysis

Authors: *S. SEYEDNEJAD[1], L. DENG[2], M. J. LEHANE[1], D. FABRIS[2], G. C. SARTOR[1];
[1]Pharmaceut. Sci., [2]Dept. of Chem., Univ. of Connecticut, Storrs, CT

Abstract: Repeated cocaine use causes persistent neuroadaptive changes that are modulated, in part, by epigenetic factors. While much research has focused on the roles of post-translational modifications (PTMs) on histones and non-histone proteins in substance use disorder (SUD) models, RNA modifications (i.e., epitranscriptomic alterations) have received relatively little attention. There are over 150 types of RNA post-transcriptional modifications (rPTM), which diversify the functionality of RNAs and potentially contribute to pathophysiologic conditions. Recent efforts using antibody-based approaches have implicated epitranscriptomic changes, like methylation of adenosine residues in SUD models. However, antibody-based methods are indirect, susceptible to non-specific binding, and often restricted to a single type of modification. Alternatively, mass spectrometry (MS)-based approaches possess the ability to identify RNA modifications directly and comprehensively, but such methods have not been used to study rPTMs in SUD models. Here, for the first time, we used high-resolution MS via a Thermo Scientific LTQ-Orbitrap Velos instrument to characterize rPTMs induced by acute and repeated cocaine exposure in the nucleus accumbens of male and female Sprague Dawley rats. In the acute group, rats received a single injection of cocaine (15 mg/kg i.p) or saline (n = 16), while in the repeated group, rats received daily cocaine (15 mg/kg i.p) or saline injections for 10 days (n = 16). The nucleus accumbens and other brain regions were collected 24 h after the last injection. MS analysis identified over 20 different types of rPTMs, and multiple rPTMs were found to be significantly altered by cocaine exposure ($P < 0.05$). Ongoing experiments using tandem mass spectrometry are being employed to differentiate possible isomeric species detected during rPTM analysis. Future studies examining cocaine-induced rPTMs in different brain regions, cell types, and subcellular compartments will reveal novel epitranscriptomic mechanisms involved in SUD, which may ultimately lead to new and effective therapeutic avenues for this devastating disease.

Disclosures: S. Seyednejad: None. **L. Deng:** None. **M.J. Lehane:** None. **D. Fabris:** None. **G.C. Sartor:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Program #/Poster #: PSTR561.21/PP28

Topic: G.09. Drugs of Abuse and Addiction

Title: A role for (2R,6R)-Hydroxynorketamine in opioid withdrawal

Authors: *M. LEHANE[1], G. C. SARTOR[2], C. R. DRINKUTH[2];
[2]Pharmaceut. Sci., [1]Univ. of Connecticut, Storrs, CT

Abstract: A role for (2R,6R)-Hydroxynorketamine in opioid withdrawal Lehane MJ, Drinkuth CR, Sartor GC Department of Pharmaceutical Sciences, University of Connecticut
The ongoing opioid crisis remains a major public health issue. To combat opioid use, relapse, and overdose mortalities, new and effective treatments are urgently needed. Recently, researchers have turned to dissociative and psychedelic drugs to treat multiple psychiatric disorders, including substance use disorders. For example, ketamine, a dissociative anesthetic, has been shown to be a rapid-acting antidepressant, and new data has identified ketamine as a potential treatment for opioid use disorder (OUD). However, due to its dissociative properties and abuse potential, the use of ketamine for the treatment of OUD may be limited. (2R,6R)-hydroxynorketamine (HNK), a ketamine metabolite, has also exhibited therapeutic effects similar to ketamine in preclinical models of depression, stress, and pain, but unlike ketamine, HNK lacks abuse potential, making it a potentially safer treatment option. Because HNK has displayed efficacy across multiple preclinical models of psychiatric disorders, we sought to examine the effects of HNK in animal models of OUD. First, we investigated the role of HNK in oxycodone relapse-like behavior. Using conditioned place preference (CPP) procedure, we found that HNK (30 mg/kg) attenuated reinstatement of oxycodone CPP in male and female mice without altering locomotor activity. Next, we tested the effects of HNK on opioid withdrawal behavior. In male and female mice, opioid dependence was induced using an escalating dose regimen of oxycodone over 8 days. Mice were then injected with saline or HNK (10 or 30 mg/kg) 24 h after the last oxycodone exposure. The next day, withdrawal symptoms were measured during naloxone-precipitated withdrawal. Compared to the saline-treated group, HNK alleviated somatic symptoms of opioid withdrawal (paw tremors, writhing, and grooming) and global withdrawal scores. In ongoing experiments, brain c-fos expression is being measured in oxycodone-dependent mice treated with vehicle or HNK to identify potential brain regions associated with the therapeutic effect of HNK. Together, these initial experiments demonstrate the efficacy of HNK in animal models of OUD and open the door for future experiments to investigate the behavioral and molecular effects of HNK in advanced models of OUD.

Disclosures: M. Lehane: None. **G.C. Sartor:** None. **C.R. Drinkuth:** None.

Poster

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Program #/Poster #: PSTR561.22/QQ1

Topic: A.09. Adolescent Development

Support: P50AA017823
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T32GM108563
U19NS128613
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T32NS115667

Title: Adolescent binge-like alcohol consumption in mice promotes sex-specific changes in adult somatostatin neuropeptide signaling and exploratory behaviors

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Abstract: Somatostatin (SST) neurons regulate cognitive and exploratory behaviors and are dysregulated by both acute and chronic alcohol use. Evidence from numerous laboratories, including ours, suggests that adult alcohol exposure causes reductions in inhibitory GABA neuron function (including SST neurons) and consequent disinhibition of excitatory pyramidal neurons - particularly within cortical regions. These changes are likely to be involved in the heightened risk of psychiatric disorders seen with chronic alcohol use. Our previous data suggest that adolescent alcohol consumption uniquely alters adult SST signaling in the prelimbic cortex. Here, we examined the behavioral consequences associated with SST dysregulation after adolescent binge-like drinking. Male and female C57BL/6J mice (n=10/sex/treatment) consumed alcohol or water in a drinking-in-the-dark (DID) paradigm throughout adolescence (PND 29-54) and underwent behavioral testing (in open field, elevated plus maze, sucrose preference tests) 24 hours or 30 days after cessation of DID. Generalized linear modeling was used to examine the influence of sex, total alcohol consumed, and last alcohol binge on behavioral outcomes while accounting for cohort and litter effects. 24 hours after DID, alcohol consumed throughout adolescence and during the last binge had opposing effects on exploratory behavior in the elevated plus maze, where total alcohol increased and final binge alcohol decreased latency to enter an open arm. In contrast, 30 days after cessation of DID, sex significantly interacted with total alcohol consumed throughout adolescence, where latency to enter an open arm was decreased in males and increased in females in alcohol-exposed groups. A similar effect of sex and adolescent alcohol was also observed for the number of entries into an open arm, which again was increased in males and decreased in females due to adolescent alcohol exposure. Taken together, this suggests that a single alcohol binge during adolescence can suppress exploratory behavior in the short term. However, males may be more susceptible to increases, while females may experience reductions in exploratory behaviors at protracted timepoints. These findings suggest that adolescent alcohol exposure causes persistent sex-specific changes in exploratory and risk-taking behavior, likely via disruption of prelimbic SST signaling. Future work will characterize changes in prelimbic SST release after adolescent alcohol and explore SST as a therapeutic target for treatment of alcohol-induced damage.

Disclosures: L. Seemiller: None. **A. Sicher:** None. **M. Hossain:** None. **P.J. Drew:** None. **N. Zhang:** None. **N.A. Crowley:** None.

Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.23/QQ2

Topic: G.09. Drugs of Abuse and Addiction

Support: DOST2020-04-A1-265

Title: Sex differences in conditioned place preference following chronic toluene inhalation in adolescent rats

Authors: A. C. CARAMPEL[1], J. N. BACAR[2], J. B. ASIS[1], J. C. MUNAR[2], S. K. KO[2], K. A. LADINES[3], C. C. GREGORIO[3], G. J. QUIRK[2], *R. CENA-NAVARRO[2];
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Abstract: Abuse of inhaled solvents (toluene) is a significant public health issue worldwide and is especially prevalent among Filipino street children. Both male and female adolescents use inhalants, but prior animal studies have focused on males only. Indeed, toluene-induced effects on conditioned place preference (CPP) and anxiety-like behavior have not been reported in female rats. Accordingly, adolescent male (n=38) and female (n=42) Sprague Dawley rats were chronically exposed to either clean air, 1500 ppm, or 3000 ppm of toluene for 6 days, and were evaluated for drug-seeking behavior during abstinence with the CPP task. Rats exposed to 3000 ppm of toluene (but not 1500 ppm) showed significant CPP on days 1 and 8 (D1, D8) of abstinence, as evidenced by more time spent in the drug-associated chamber compared to controls (ANOVA main effect of treatment: p=0.0011; control vs. 3000 on D1: p=0.0014; control vs. 3000 on D8, p=0.0093). This significant CPP in toluene-exposed rats was no longer observed on day 22 (D22) of abstinence (control vs. 3000 on D22: p=0.1989). There was no significant effect of sex in the 3-way ANOVA (p=0.4069), but when males and females were analyzed separately, females showed significant CPP on both D8 and D22 of abstinence (main effect: p=0.0046; control vs. 3000 on D8, p=0.0027; control vs. 3000 on D22: p=0.0359), whereas males did not reach significance at either timepoint (main effect: p=0.1977). This apparent sex-difference in CPP was not due to differences in toluene intake or metabolism, as headspace gas chromatography showed similar blood levels of toluene in adolescents of both sexes following inhalation on the first and last day of toluene exposure (ANOVA main effect of sex: p=0.6573). Chronic toluene exposure did not increase anxiety-like behavior in the elevated plus maze (EPM) or open field tests, however, we observed a significant sex difference in control rats, with females showing significantly less anxiety in the EPM than males (p=0.0015; proportion of time in open arms of males=0.1855; Proportion of time in open arms of females=0.4261). Thus, following chronic exposure to toluene, female adolescent rats exhibit sustained CPP to a greater extent than males. Female adolescent inhalant users constitute an underreported and understudied group, and warrant further investigation to develop sex-specific treatments for inhalant use disorder.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

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Program #/Poster #: PSTR561.24/QQ3

Topic: G.09. Drugs of Abuse and Addiction

Support: DOST2020-04-A1-265

Title: Cognitive and social behaviors during abstinence from chronic toluene inhalation in adolescent rats

Authors: *J. ASIS[1], J. C. MUNAR[2], A. C. CARAMPEL[1], J. BACAR[2], C. C. GREGORIO[3], G. J. QUIRK[2], R. CENA-NAVARRO[2];
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Abstract: Abuse of inhaled solvents (toluene) is a significant public health issue worldwide and is especially prevalent among Filipino street children. In addition to compulsive drug-seeking, chronic inhalant use can also induce long-term cognitive and social deficits despite prolonged abstinence. An important animal model of inhalant use is chronic exposure of rats to inhaled toluene vapor, however, most prior studies of cognitive and social deficits only examined acute exposure to toluene. The existing chronic exposure studies used very high doses or tested only during early withdrawal. In our study, we assessed the effects of low-dose chronic toluene inhalation on memory, social behavior, and behavioral flexibility during prolonged abstinence. We exposed adolescent male (n=38) and female (n=42) Sprague Dawley rats to clean air, or toluene vapors (1500 ppm or 3000 ppm) for 6 days over a 12-day period. Significant conditioned place preference was observed for the 3000 ppm dose, and was stronger in females than in males (see Carampel et al., this meeting). We found intact novel object memory in all three treatment groups when tested at day 7 of abstinence in both males and females, and there was no impairment by toluene in either sex (One-way ANOVA: Males $p=0.7743$, Females $p=0.6727$). We next tested sociability on Day 11 of abstinence by assessing rats' preference for a live demonstrator rat vs. an inanimate object. All groups showed a significant preference for the rat (all p's<0.001), with no significant impairment by toluene in either males or females (One-way ANOVA: Males $p= 0.2632$, Females $p= 0.4720$). We assessed social novelty the following day, by comparing the preference for the same demonstrator rat vs. a novel same-sex rat. Toluene significantly reduced preference for the novel rat at the 1500 ppm dose (Post-hoc Tukey's test: $p=0.0245$), with a trend at the 3000 ppm dose ($p=0.1070$), with no apparent difference between the sexes. Experiments are ongoing to assess the effects of toluene exposure on appetitive reversal learning. Thus, chronic toluene exposure in adolescent rats leaves memory and sociability intact during abstinence, but impairs rats' preference for social novelty. The lack of effect by toluene on novel object memory suggests that the deficit in social novelty is not due to a loss of 24-hour memory, but an altered response to relevant social cues. These results suggest

that adolescent inhalant users may avoid new social interactions after stopping drug use, and may benefit from social interventions to substitute for interactions with drug-seeking peers.

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Poster

PSTR561. Developmental Effects of Drugs and Alcohol

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR561.25/QQ4

Topic: A.09. Adolescent Development

Support: 1RF1MH121267-01
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U01 DA053625-01
1R01NS105477
P01HD067244
U54NS11717
R01LM014017
DA08259
HL136520
UM1AI164599
U41HG007234
HHMI

Title: Single-cell long-read mRNA isoform regulation is pervasive across mammalian brain regions, cell types, and development.

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Abstract: Single-cell long-read mRNA isoform regulation is pervasive across mammalian brain regions, cell types, and development.
RNA isoforms influence cell identity and function. Until recently, technological limitations prevented a genome-wide appraisal of isoform influence on cell identity in various parts of the brain. Our prior work developed single-cell isoform sequencing for fresh[1] and frozen[2]

tissues, spatial isoform sequencing[3] and accurate long-read interpretations[4]. Here, I present results under review[5] mapping isoforms accross multiple mouse brain regions, cell subtypes, and developmental timepoints from postnatal day 14 (P14) to adult (P56). For 75% of genes, full-length isoform expression varies along >=1 axis (brain-region, cell-subtype or age), underscoring the pervasiveness of isoform regulation across multiple scales. As expected, splicing varies strongly between cell types. However, certain gene classes including neurotransmitter release and reuptake as well as synapse turnover, harbor significant variability in the same cell type across anatomical regions, suggesting differences in network activity may influence cell-type identity. Glial brain-region specificity in isoform expression includes strong poly(A)-site regulation, whereas neurons have stronger TSS regulation. Furthermore, developmental patterns of cell-type specific splicing are especially pronounced in the P21-to-P28 transition. The same cell type traced across development shows more isoform variability than across adult anatomical regions, indicating a coordinated modulation of functional programs dictating neural development. As most cell-type specific exons in P56 mouse hippocampus behave similarly in newly generated data from human hippocampi, these principles may be extrapolated to human brain. However, human brains have evolved additional cell-type specificity in splicing, suggesting gain-of-function isoforms. Taken together, we present a detailed single-cell atlas of full-length brain isoform regulation across development and anatomical regions, providing a previously unappreciated degree of isoform variability across multiple scales of the brain.
1.Gupta*,Collier* et al, Nature Biotechnology, 2018; 2.Hardwick*,Hu*,Joglekar* et al, Nature Biotechnology, 2022; 3.Joglekar et al, Nature Communications, 2021; 4.Prjibelski*,Mikheenko* et al, Nature Biotechnology, 2023; 5.Joglekar et al, biorxiv, 2023

Disclosures: H.U. Tilgner: None. **A. Joglekar:** None. **W. Hu:** None. **B. Zhang:** A. Employment/Salary (full or part-time):; bzhang@spatialgenomics.com. **O. Narykov:** None. **M. Diekhans:** None. **J. Balacco:** None. **J. Marocco:** None. **L. Ndhlovu:** None. **T.A. Milner:** None. **O. Fedrigo:** None. **E.D. Jarvis:** None. **G. Sheynkman:** None. **D. Korkin:** None. **M. Ross:** None.

Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.01/QQ5

Topic: G.09. Drugs of Abuse and Addiction

Support: Research funded by Philip Morris International

Title: Nicotine self-administration in rats is altered by nicotine infusion dose and the MAO inhibitor harmane

Authors: *E. LEVIN, C. WELLS, M. STOUT;
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Abstract: Nicotine has been found to be the principal chemical driver of tobacco addiction. Like any drug nicotine's biological effects, including its self-administration, are related to dose. It has been conjectured that lowering the concentration of nicotine in tobacco could reach a dose that is below that which would promote addiction. Alternatively, lowering nicotine concentration in tobacco could cause compensatory increases in use to overcome the lower dose per intake. With the current study, we tested nicotine self-administration over an order of magnitude range of doses per infusion in a rat model to test for possible compensatory increases in self-administration vs. lower self-administration. Adult female Sprague-Dawley rats were given 1-h sessions in the classic IV operant self-administration paradigm. The rats were given the opportunity to self-administer nicotine at doses of 3, 10 or 30 µg/kg/infusion. As has been seen before, the benchmark 30 µg/kg/infusion dose resulted in reliable self-administration. In rats given the lower 10 µg/kg/infusion dose, there was a compensatory increase in the number of self-administration infusions, such that the total amount of nicotine self-administration was only slightly lower as was seen with the benchmark 30 µg/kg/infusion dose. In contrast, rats given an even lower dose of 3 µg/kg/infusion, showed no compensatory increase in nicotine self-administration infusions. The total amount of self-administration dropped off significantly relative to the higher infusion doses. There seems to be a biphasic dose-response curve with nicotine in which slightly lowering the infusion dose causes compensatory increases in self-administration, whereas more substantial dose reductions do not. Tobacco contains a variety of neuroactive chemicals in addition to nicotine. Some chemicals like harmane inhibit monoamine oxidase, that could impact nicotine self-administration, since monoamine oxidase catabolizes dopamine, which is key for the reinforcing effects of nicotine. The current study examined the interactions of harmane on the range of nicotine doses available for self-administration. Administration of harmane was found to increase nicotine self-administration regardless of the unit nicotine infusion dose, providing information that other compounds in tobacco can influence nicotine self-administration in rats. This study in rats showed that lowering the infusion dose of nicotine modestly results in a compensatory increase in responding for nicotine, but that a greater nicotine dose reduction does not cause increased responding for nicotine resulting in a very low total level of nicotine self-administration.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.02/QQ6

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant P50DA037844

Title: Dose-dependent effects of a single psilocybin administration on nicotine motivation in male and female rats

Authors: *E. A. RAKOWSKI[1], E. HOLMES[2], B. M. THOMPSON[3], C. P. KING[4], P. J. MEYER[5];
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Abstract: Recent clinical studies suggest that hallucinogenic compounds are potential therapeutic adjuncts for psychological disorders including substance use disorder. For example, heavy smokers have reported significantly longer periods of abstinence after a single administration of the hallucinogenic tryptamine psilocybin, the psychoactive ingredient found in the mushroom genus *Psilocybe*. Our study aimed to assess whether this effect of psilocybin could be replicated in animal model of nicotine motivation. Male (n=27) and female (n=29) Sprague-Dawley rats were subjected to 12 days of nicotine self-administration (0.3 mg/kg/infusion) on a fixed-ratio (FR) schedule that increased from FR1 to FR5. Rats then received an intraperitoneal dose of psilocybin (1 or 4 mg/kg or saline vehicle). They were then left undisturbed for two days before undergoing two progressive-ratio tests a week apart. We found a significant main effect of psilocybin treatment on breakpoint, active responses, and infusions earned (p's<.05), where rats that received the 4 mg/kg dose had higher scores on all three measures than the 1 mg/kg and control groups. The effect of psilocybin treatment on inactive responses also had a trend toward statistical significance (p=.07), where rats that received the 1 mg/kg treatment had higher average responding. Future directions include investigating the effect of multiple psilocybin treatments over time on drug use and exploring other drug classes besides nicotine to determine specific effects on certain types of drugs of abuse.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.03/QQ7

Topic: G.09. Drugs of Abuse and Addiction

Title: Chronic Progesterone Treatment Reduces Nicotine Consumption and Alters Accumbens Microglial Morphology in a Sex-Specific Fashion

Authors: *P. KENDRICK[1], P. ABNEY[1], E. BONDY[1], E. MAHER[1], S. KHATRI[1], H. A. BIMONTE-NELSON[2], C. D. GIPSON[1];
[1]Pharmacol. and Nutritional Sci., Univ. of Kentucky, Lexington, KY; [2]Arizona State Univ., Tempe, AZ

Abstract: Nicotine use is a substantial burden to public health. Progesterone (P4) has been examined clinically as a smoking cessation agent and shows promise for women but not men. We previously found that neuroimmune signaling within the nucleus accumbens core (NAcore) is driven by nicotine seeking and consumption in a sex-specific fashion, whereby female rats are more susceptible to nicotine-induced neuroimmune consequences as compared to males. To date, there are no studies evaluating neuroimmune mechanisms by which P4 may yield sex-specific efficacy in reducing nicotine use. Male and female Long Evans rats underwent nicotine (0.06 mg/kg/infusion) or saline self-administration (SA) for 10 sessions, followed by 15 sessions with P4 (1.75 mg/kg in 0.1 mL sesame oil, SC) or vehicle treatments 2 h prior to SA sessions. NAcore microglial morphological analysis was then conducted on Iba1-positive cells via 3DMorph. Females were vaginally swabbed each day, and uterine horns were collected at sacrifice to verify hormone treatments. Daily P4 treatments following acquisition of nicotine SA decreased consumption in female but not male rats. Chronic, systemic P4 also increased NAcore microglial reactivity following nicotine SA as compared to saline and vehicle-treated rats, but this was specific to females and decreased ramification index (*t*-test, $p<0.05$)). We further found an increase in the total number of microglia present n females, indicating that P4 may prevent microgliosis after nicotine SA. We further found that uterine horn weights were significantly decreased following P4 treatments, which decreased further in the nicotine SA group. Together, inhibiting nicotine-induced chronic NAcore microglia reactivity may underlie the sex-specific efficacy of P4. Our results may justify examination of other possible therapeutics that have anti-inflammatory properties for smoking cessation, which may yield higher efficacy across both biological sexes. Next, we will evaluate the role of NAcore astrocytic P4 receptors in P4-neuroimmune interactions following nicotine SA using a pAAV-GFAP-PR-GFP shRNA knockdown strategy.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.04/QQ8

Topic: G.09. Drugs of Abuse and Addiction

Support: Intramural Research Project of the NIH, NIDA

Title: Childhood trauma and emotional awareness impact neural correlates of chronic nicotine use

Authors: *A. QUAM, K. BIERNACKI, T. J. ROSS, B. SALMERON, A. C. JANES;
NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD

Abstract: There is an undeniable link between childhood trauma and substance use. However, the neurobiological underpinnings of this association are less clear. To clarify the neurobiological links between childhood trauma and nicotine dependence, we focused on alexithymia, which is defined by difficulty identifying and describing feelings and is a transdiagnostic trait associated with both childhood trauma and nicotine dependence. 102 individuals who use nicotine chronically (47% female) and 102 matched healthy controls (46% female) completed the childhood trauma questionnaire (CTQ) and Toronto Alexithymia Scale (TAS-20) and underwent a 16-min resting state fMRI scan on which whole-brain Coactivation Pattern (CAPs) analysis was completed using 8 predefined brain states. Total time spent in state and frequency of transitions into states were compared between groups and analyzed with relation to CTQ and TAS-20 scores using a mixed-effects ANOVA and linear regression models respectively. A significant group by state interaction was noted ($F = 9.12$, $p < 0.01$), where individuals who use nicotine chronically spent more time in and transitioned more frequently to the frontoinsular-default mode network (DMN) ($p<0.001$).There was also an alexithymia by group by state interaction in relation to total time spent in the frontoinsular-DMN state ($F = 4.42$, $p = 0.037$); for individuals who use nicotine chronically, greater alexithymia was associated less time in the frontoinsular-DMN state ($r = -0.26$, $p = 0.007$). Overall, CTQ and alexithymia scores were positively correlated ($r = 0.21$, $p = 0.003$). Moreover, alexithymia mediated the relationship between CTQ scores and time spent in the frontoinsular-DMN state in individuals who use nicotine chronically ($p = 0.014$), but not healthy controls ($p = 0.64$). In a follow-up moderated mediation, there was a significant difference between the group's mediations ($p = 0.024$). In those with chronic nicotine use, our data shows that greater alexithymia relates to less time spent in the frontoinsular-DMN. Others have shown that, greater time spent in this same network relates to higher levels of rumination, which is repetitive thinking about negative emotional states. Taken together, these data suggest that a moderate amount of time spent in the frontoinsular-DMN state may be ideal as too much or too little time spent in this state is associated with either unawareness of, or a hyper focus on, emotional states. The current findings emphasize the importance of considering both emotional and developmental factors in understanding nicotine addiction and its neural correlates.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

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Program #/Poster #: PSTR562.05/QQ9

Topic: G.09. Drugs of Abuse and Addiction

Support: Virginia Youth Tobacco Programs (VYTP)
Virginia Foundation for Healthy Youth
GMU Foundation

Title: The expression of multiple genes in the mesolimbic dopamine pathway exhibit sex-dependent left-right asymmetries that are altered by a single nicotine injection in adolescent mice

Authors: *K. FRYXELL[1], R. L. MURPHY[2], A. D. HUDSON[3];
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Abstract: Dopamine signaling during adolescence is of key importance in drug abuse and psychological disorders. We analyzed the expression of mRNAs and proteins encoding dopamine receptors, as well as mRNAs of two other genes implicated in dopamine metabolism. Because the endocytosis of dopamine receptors is triggered by dopamine signaling, and because a fraction of the endocytosed receptors are degraded, we hypothesized that compensatory transcription of dopamine receptor genes would be stimulated by dopamine signaling. Adolescent male and female mice were injected with a single dose of 0.5 mg/kg nicotine or saline. We found that dopamine receptor mRNAs were often higher on the right side of the brain at baseline, particularly in females, and were increased by a single nicotine dose, which also reduced or eliminated the left-right asymmetry. In contrast, *Cd81* mRNA was strongly up-regulated by nicotine in the female right ventral striatum and right ventral tegmentum, while *Th* mRNA was up-regulated by nicotine only in the ventral tegmentum (on both sides, in both sexes). At the protein level, dopamine receptor proteins showed far less variation between left and right sides of the brain, and between mice injected with nicotine vs. saline, which is consistent with our hypothesis. Our hypothesis was also supported by measurements of the fraction of dopamine receptor proteins exposed on the cell surface, which showed that the fraction of D1 dopamine receptors exposed on the cell surface was lower (implying greater signaling-driven endocytosis) in the female right ventral striatum, where D1 mRNA levels were also elevated at baseline.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.06/QQ10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant
Smoking Research Foundation

Title: Mouse model of operant oral nicotine self-administration

Authors: *A. D. ARMENTA VEGA[1], S. IKEMOTO[2], Y. ARIMA[3];
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Abstract: Nicotine is an active ingredient that leads to addiction to tobacco products. Nicotine binds to the nicotinic acetylcholine receptors (nAChRs) of the brain and has many downstream effects, one of them being positive reinforcement that leads to habit formation and addiction to nicotine. However, it is not well understood how nicotine produces such effects, and there is no established behavioral procedure to examine the reinforcing effects of nicotine in mice, a model species for neural investigation. Our goal is to examine if oral nicotine self-administration can work as an effective model of nicotine reinforcement. Firstly, we found that mice orally consume nicotine solutions. Using a free choice procedure in which mice had access to nicotine solutions and water daily for 24 hours, mice readily consumed nicotine solutions. As the concentration of the nicotine solutions increased, mice consumed more nicotine per day despite consuming less volume of the nicotine solutions. We also found sex differences: female mice consumed more nicotine than males. Additionally, we were able to train mice to operantly respond for oral administration of nicotine. Mice were placed in operant chambers where they had access to a nicotine solution upon pressing on a lever. They learned to respond for oral nicotine with a fixed ratio schedule of 4. However, once mice learned to operantly respond for oral nicotine, behavioral responses were not sensitive to manipulations including differential concentrations of nicotine solution and pharmacological challenges with nicotinic receptor agonists and antagonists. These observations suggest that behavior reinforced by oral nicotine is highly habitual. Our operant self-administration of oral nicotine procedure has potential to be an effective model for investigating neural mechanisms of the reinforcing and habitual effects of nicotine.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

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Program #/Poster #: PSTR562.07/QQ11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH U18 DA052416 to EG, CDF and BB

Title: Role of GPR3 in Nicotine Withdrawal in Mice

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Abstract: Avoidance of withdrawal symptoms, such as cravings, anxiety, trouble concentrating, weight gain and irritability are a large reason for continued use of nicotine products after quitting attempts. The medial habenula (MHb) is a brain region located in the diencephalon, just ventrolateral to the third ventricle, and is critical in modulating nicotine withdrawal symptoms. G-protein coupled receptor 3 (GPR3), an orphan Gαs coupled receptor, is highly expressed in the MHb. While we have previously found that a GPR3 agonist alters nicotine intake, it is unknown if it has any effect on withdrawal symptoms. Here, we began by validating a mouse model of nicotine withdrawal through daily exposure to electronic cigarette vapor. After 8 days of exposure, mice showed heightened levels of somatic withdrawal behaviors, which was evident 24 hrs after the last exposure. Following validation of our withdrawal model, we tested the effects of the GPR3 agonist on withdrawal behaviors, and we found altered somatic symptoms of withdrawal. Further studies are currently validating the selectivity of the effects with mice lacking the GPR3 receptor. Overall, these data support GPR3 as a novel target for nicotine cessation therapeutic development. Supported by the National Institute on Drug Abuse (NIH U18 DA052416 to EG, CDF and BB)

Disclosures: **A.S. Mogul:** None. **E. Gay:** None. **B.E. Blough:** None. **C.D. Fowler:** None.

Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.08/QQ12

Topic: G.09. Drugs of Abuse and Addiction

Support: TRDRP T32DT5202
TRDRP T32IR4866
TRDRP T31IR1767

Title: Lynx2 Proteins Expressed in the Cortex Affect Nicotine Seeking Following Nicointe Abstinence

Authors: *M. R. BAUTISTA, V. LALLAI, J. P. FOWLER, C. D. FOWLER;
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Abstract: Nicotine dependence continues to affect millions of individuals in the United States and results in severe adverse health consequences. Thus, nicotine dependence remains to be the leading cause of preventable death and disease. Very few individuals are successful in nicotine cessation each year,thereby highlighting the mild efficacy of currently available therapeutics targeting nicotine cessation. Of interest, endogenous allosteric modulators acting on the nicotinic acetylcholine receptor (nAChR) have emerged as a novel target of interest for drug development. Lynx2 is a negative allosteric modulator of the nAChR and have been shown to significantly decrease nAChR activity in the presence of an agonist, impacting downstream nAChR-mediated behaviors. In this study, we assessed for nicotine seeking behavior after abstinence in subjects

treated with viral mediated knockdown of lynx2 or a scrambled vector in the cortex. Our data suggest that knockdown of lynx2 in the cortex altered nicotine-relapse related behaviors in comparison to mice treated with the scrambled vector. Together, these findings demonstrate that lynx2 proteins expressed in the cortex play a significant role in nicotine-relapse related behaviors.This work is supported by funding from the Tobacco-Related Disease Research Program (TRDRP T32DT5202 to MB, TRDRP T32IR4866 CDF, and TRDRP T31IR1767 CDF)

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

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Program #/Poster #: PSTR562.09/QQ13

Topic: G.09. Drugs of Abuse and Addiction

Support: FRM SPF20200501192
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Labex MemoLife

Title: Dopamine release in Claustrum is involved in nicotine-induced changes in social decision making.

Authors: *É. VICQ[1,2], T. LE BORGNE[2], A. MOUROT[2], P. FAURE[2], C. SOLIÉ[2];
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Abstract: Nicotine, when administered chronically, disrupts the activity of numerous neuronal structures, including the dopamine (DA) reward circuit that originates in the ventral tegmental area (VTA). VTA DA neurons project to both limbic and cortical regions, including an overlooked structure called the claustrum (CLA), which has recently been associated with context reinforcement and decision-making. VTA DA neurons are important for processes such as reinforcement learning, motivation and social interactions. However, it is unclear whether DA release in the CLA affects social decision-making, and how chronic nicotine influences this pathway. To address these questions, we first assessed the social behavior of mice, exposed to chronic nicotine compared to those not exposed, using the 3-chamber task. We found that nicotine-treated mice show greater preference for a novel conspecific over a familiar one, and this preference was sustained over time. In contrast, in control animals, the preference for the new mouse diminished more rapidly. Next, we used retrograde tracing methods to confirm the presence of VTA DA terminals in the CLA. Subsequently, we recorded DA release in the CLA of mice expressing the GRAB-DA sensor. We found that both an intraperitoneal injection of nicotine and free social interactions led to an increase in DA release in the CLA, which indicates the involvement of the CLA in social interactions and reaction to nicotine. We further explored how chronic nicotine exposure affected DA release during both free social interaction and in the 3-chamber task. We observed a similar increase in DA release in the CLA during the free

interaction with a conspecific in the two groups. However, animals under chronic nicotine exhibited sustained DA release in the CLA throughout the interaction with the novel conspecific in the 3-chamber task, whereas untreated mice did not, consistent with our behavioral observations. These results suggest that CLA DA is crucial in social decision-making under chronic nicotine. Finally, to causally link CLA DA release with the observed behavior, we are currently using optogenetics to mimic the effect of chronic nicotine on the VTA-CLA pathway. Our results suggest that the VTA-CLA pathway is involved in the adaptations in social decision-making observed after chronic exposure to nicotine.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: Canadian Institutes of Health Research
Natural Sciences and Engineering Research Council

Title: Identification of novel sex-dependent neural biomarkers underlying adolescent nicotine exposure risks for mood and anxiety disorders

Authors: *T. NG[1], M. SARIKAHYA[2], R. M. HUDSON[2], H. J. SZKUDLAREK[2], E. PÉREZ-VALENZUELA[2], T. UZUNESER[2], **S. R. LAVIOLETTE**[2];
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Abstract: Smoking-related diseases remain the top global cause of preventable mortality. Recent increases in teen vaping represents a new smoking epidemic and is highly concerning given the vulnerability of the adolescent brain. Specifically, brain regions including the prefrontal cortex (PFC) and mesolimbic pathway, including the ventral tegmental area (VTA) and nucleus accumbens (NAc) are implicated in both nicotine dependence and pathological phenotypes linked to mood and anxiety disorders. Clinical studies report that nicotine dependence is causally linked to increased risks for mood and anxiety disorders. Thus, females experience a higher prevalence of mood and anxiety disorders and greater challenges in smoking cessation therapies, suggesting a potential sex-specific response to nicotine exposure and mood/anxiety disorder risks. However, the majority of pre-clinical studies only utilized male animal models, and sex differences in response to adolescent nicotine exposure are currently poorly characterized. Thus, to investigate sex differences in mood and anxiety-related outcomes in response to adolescent nicotine exposure, adolescent male and female Sprague Dawley rats received either nicotine (0.4 mg/kg) or saline injections s.q., 3x daily for 10 post-natal days (PND 35-44), followed by an

integrated combination of behavioural testing, *in-vivo* electrophysiology and Western Blot analyses in adulthood. Our results demonstrate that chronic adolescent nicotine exposure induces significant and long-lasting anxiety/depressive-like behaviours, disrupted neuronal activity patterns and molecular signaling pathways targets in nicotine-treated male rats, but no significant effects in female cohorts. Specifically, nicotine-exposed males exhibited altered levels of α7 acetylcholine nicotinic receptor (nAChR), β2 nAChR, glutamate decarboxylase 65 (GAD65), dopamine (DA) 1 receptor (D1R), D2R, and brain-derived neurotrophic factor (BDNF) in the PFC and NAc. Remarkably, nicotine-exposed females either showed no significant differences or opposite trends in these biomarkers, suggesting possible neuroprotective compensatory mechanisms operating in the adolescent female brain. Follow up studies in male brains using matrix-assisted-laser deionization imaging (MALDI) revealed profound alterations in DA, gamma-aminobutyric acid (GABA) and glutamate signals in PFC and NAc. Our findings reveal multiple novel differences between the adolescent male vs. female brain and suggest important new biomarkers for understanding differential sex-dependent risks for male vs. female adolescent smokers.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

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Euro-HPC EHPC-REG-2021R0074

Title: Toward a Wearable Continuous Nicotine Monitor (CNM) for Human Biofluids

Authors: *A. NICHOLS[1], C. B. MAROTTA[2], H. LUKAS[3], N. HALOI[4], S. HUANG[1], E. J. FINE[1], N. FRIESENHAHN[2], B. N. COHEN[1], D. A. DOUGHERTY[2], W. GAO[3], S. L. MAYO[1], R. J. HOWARD[4], E. R. LINDAHL[5], H. A. LESTER[1];
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Sci., Caltech, Pasadena, CA; [4]Dept. of Applied Physics, [5]Dept. of Biochem. and Biophysics, KTH Royal Inst. of Technol., Stockholm, Sweden

Abstract: The time course of nicotine in the brain helps to determine (a) how much a person smokes or vapes, and (b) how to help a person quit. The initial time course ("bolus") of nicotine produced by a vaping device determines how effectively that device helps a person to switch completely from harmful combustible tobacco. Yet existing ways to measure the pharmacokinetics of nicotine are tedious, invasive, and expensive. We report progress toward developing a wearable continuous nicotine monitor (CNM) that will sample human biofluids (sweat, interstitial fluid) to resolve the personal pharmacokinetics of nicotine users. To this end, we developed the latest genetically encoded **i**ntensity-based **Nicotine**-**S**e**n**sing **F**luorescent **R**eporter for nicotine: iNicSnFR12 ($\Delta F_{max}/F_0$ = 11, EC_{50} = 8.7 μM, S-slope = 2.6 μM^{-1}) via computational protein design, molecular dynamics, site-saturated mutagenesis, and site-directed mutagenesis. iNicSnFR12 detects nicotine in PBS plus diluted human and rat serum at concentrations within the relevant nicotine concentration for the biofluids of human smokers and vapers (linear from 70 to 200 nM, with corrections for endogenous choline concentrations). To convert the fluorescent readout of iNicSnFR12 to an amperometric one, we introduced (a) cysteine residues at locations in iNicSnFR12 that undergo the largest movements during the conformational change upon nicotine binding and (b) a gold -binding sequence. We coupled a maleimide derivative of redox-reactive methylene blue to the introduced cysteines and performed square wave voltammetry and cyclic voltammetry on porous gold electrodes coated with this iNicSnFR12 variant. To date, we have been able to detect nicotine at sub-μM concentrations. The amperometric signals provide a molecular basis for the development of a wearable CNM that could gather, in real time, personal pharmacokinetic data of smokers and vapers. Data sets obtained with the proposed device will result in real steps towards the reduction of tobacco-related disease, with potential extensions of the technology to other therapeutic and abused drugs. Support: NIH GM-123582 and DA043829, California TRDRP 27FT-0022, 27IP-0057, Wallenberg Foundation, Swedish Research Council (2019-02433, 2021-05806), Swedish e-Science Research Centre, BioExcel Center of Excellence (EU-823830), Swedish National Infrastructure for Computing, Euro-HPC (EHPC-REG-2021R0074).

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.12/QQ17

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of nucleus accumbens inhibition on the expression of stress-induced behavioral sensitization

Authors: K. STEELE, D. ROEVER, T. ST. VINCENT, *J. CORTRIGHT;
Univ. of Wisconsin - River Falls, River Falls, WI

Abstract: Drug addiction is a major public health and serious economic concern in the United States costing taxpayers billions of dollars annually. Clinical research indicates that individuals exposed to stress are more vulnerable to drug addiction. In fact, exposure to stress leads to an increase in the number of cigarettes smoked and is associated with nicotine craving and relapse. Recently, we have shown that exposure to variable stress produces an enhanced, or sensitized, response to nicotine in an animal model of addiction. Behavioral sensitization has been proposed as an animal model that may reflect plasticity in the mesocorticolimbic dopamine (DA) system underlying drug addiction. Intermittent exposure to nicotine induces behavioral sensitization, as evidenced by an enhanced locomotor response to a subsequent injection of the drug. Further, repeated exposure to nicotine produces neuroadaptations in the mesocorticolimbic system, such as sensitized DA overflow in the nucleus accumbens (NAcc), the site of the expression of behavioral sensitization. Similarly, exposure to variable stress also induces enhanced sensitivity to drug-induced locomotion, or crosssensitization between stress and drug. More specifically, variable stress exposure promotes locomotor sensitization to nicotine. The mesocorticolimbic DA system also displays responses to stressors, such as an enhanced DA response. Thus, it is logical that the site of the expression of behavioral sensitization in response to nicotine is also the NAcc. Therefore, activity in this system might cause drug-induced locomotor sensitization and alterations in motivated responses for drug. Thus, the proposed experiments assessed the consequences of NAcc inactivation on the expression of stress-induced behavioral sensitization in response to a subsequent nicotine challenge. Locomotor activity was measured to determine the effect of NAcc inactivation on the expression of this phenomenon. These experiments make use of an animal model of the development of drug addiction that has significant overlap with the human condition. To date, inactivation of the NAcc attenuates the expression of stress-induced sensitization following a challenge injection of nicotine. Therefore, in addition to the further elucidation of the neural underpinnings of the development and manifestation of addiction, the results obtained have important implications for the development of effective and lasting smoking cessation interventions in humans.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

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Program #/Poster #: PSTR562.13/QQ18

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R36DA050000

Title: Investigating the effects of mental health, perceived health risk, and demographics on at-home electronic cigarette self-administration for adult cigarette smokers

Authors: S. LANG[1], D. HATSUKAMI[2], *M. GADES[3];
[1]Neurosci., Univ. of St. Thomas, Saint Paul, MN; [2]Univ. of Minnesota, Minneapolis, MN; [3]Univ. of St. Thomas Neurosci. Program, Saint Paul, MN

Abstract: Background: Cigarettes are the leading cause of preventable death in the United States. E-cigarettes (ECs) may provide a unique harm reduction possibility for nicotine users unable or unwilling to quit cigarettes, as they contain up to 95% fewer tobacco-related carcinogens and toxicants than cigarettes. Laboratory studies indicate that increasing EC abuse potential-or the likelihood that someone will use them for their central nervous system effects-increases EC uptake and facilitates substitution with cigarettes. Previous studies, however, lack real-world use, which is an important measure of abuse potential and can be measured through at-home self-administration. This preliminary study, therefore, recorded at-home self-administration of cigarettes and ECs and assessed whether participant characteristics and EC product characteristics predicted use. Methods: This single-blinded study included 23 adult (21+) cigarette smokers (F=6, M=17) who do not currently use ECs. Participants chose to use one of four EC (Juul) pods with different % nicotine salt by weight and flavor (3% Tobacco, 3% Menthol, 5% Tobacco, 5% Menthol) after 3 days of sampling all pods. They were sent daily texts with a link to a questionnaire to record daily at-home use of cigarettes and the chosen EC for 10 days. The participants also completed mental health, perceived health risk, and demographic questionnaires. Multiple regression analyses examined whether perceived health risk, perceived addiction risk, depression and anxiety, and age were related to at-home self administration of ECs (average puffs taken per day and average EC use sessions per day), cigarettes (average cigarettes per day), and flavor choice (menthol vs. tobacco). Results: Depression and anxiety scores as well as perceived health and addiction risks of ECs in comparison to combustible cigarettes did not significantly predict any of our outcome variables. However, younger participants took significantly more EC puffs per day and used it for more sessions per day than older participants ($p=0.018$ and $p=0.029$, respectively). Additionally, younger participants were significantly more likely to choose menthol pods over tobacco pods ($p=0.027$). Conclusion: Perceived health ratings and mental health scores did not significantly predict EC and traditional cigarette use in this sample, however younger participants chose menthol and used ECs more than older participants. These results suggest that there may be age or generational differences in how ECs are viewed and used, which may have an impact on abuse potential of ECs, but a larger study is needed to confirm these results.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

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Title: Beta2 nAChR Activation on VTA DA Neurons Is Sufficient for Nicotine Reinforcement in Rats

Authors: *N. WALKER[1], Y. YAN[1], M. A. TAPIA[1], B. R. TUCKER[1], L. N. THOMAS[1], B. E. GEORGE[1], A. M. WEST[1], C. B. MAROTTA[2], H. A. LESTER[3], D. A. DOUGHERTY[2], K. M. HOLLERAN[1], S. R. JONES[1], R. M. DRENAN[1];
[1]Physiol. & Pharmacol., Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Div. of Chem. and Chem. Engin., [3]Biol., Caltech, Pasadena, CA

Abstract: Mesolimbic nicotinic acetylcholine receptor (nAChRs) activation is necessary for nicotine reinforcement behavior, but it is unknown whether selective activation of nAChRs in the dopamine (DA) reward pathway is sufficient to support nicotine reinforcement. In this study, we tested the hypothesis that activation of β2-containing (β2*) nAChRs on VTA neurons is sufficient for intravenous nicotine self-administration (SA). We expressed β2 nAChR subunits with enhanced sensitivity to nicotine (referred to as β2Leu9′Ser) in the VTA of male Sprague Dawley (SD) rats, enabling very low concentrations of nicotine to selectively activate β2* nAChRs on transduced neurons. Rats expressing β2Leu9′Ser subunits acquired nicotine SA at 1.5 μg/kg/infusion, a dose too low to support acquisition in control rats. Saline substitution extinguished responding for 1.5 μg/kg/inf, verifying that this dose was reinforcing. β2Leu9′Ser nAChRs also supported acquisition at the typical training dose in rats (30 μg/kg/inf) and reducing the dose to 1.5 μg/kg/inf caused a significant increase in the rate of nicotine SA. Viral expression of β2Leu9′Ser subunits only in VTA DA neurons (via TH-Cre rats) also enabled acquisition of nicotine SA at 1.5 μg/kg/inf, and saline substitution significantly attenuated responding. Next, we examined electrically-evoked DA release in slices from β2Leu9′Ser rats with a history of nicotine SA. Single-pulse evoked DA release and DA uptake rate were reduced in β2Leu9′Ser NAc slices, but relative increases in DA following a train of stimuli were preserved. These results are the first to report that β2* nAChR activation on VTA neurons is sufficient for nicotine reinforcement in rats.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: National Research Foundation (NRF) of Korea
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Brain Korea 21 FOUR Program

Title: Computational markers for successful prognosis in Nicotine Use Disorder:A longitudinal EMA study in real-world clinical settings

Authors: *J.-H. LEE[1], J. YANG[1], S. LEE[1], H. KIM[1], M. A. PITT[4], J. I. MYUNG[4], H. PARK[5], H. JOH[2], W.-Y. AHN[1,3];
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Abstract: The efficacy of treatment for Nicotine Use Disorder (NUD) remains notably limited, and the accurate prediction of successful cessation of smoking presents an ongoing challenge. Previous studies showed that several psychological variables such as craving or depression are primary predictors of smoking, but individual differences that prompt smoking decisions and successful cessation remain unclear. To address this gap, we conducted a longitudinal study using ecological momentary assessment (EMA) via smartphone application and decision-making tasks. Treatment-seeking smokers (N=87) engaged in a smoking cessation clinic that was assisted with medication (i.e., bupropion, varenicline, and nicotine replacement therapy) for 5-6 weeks. Participants provided daily self-reports on psychological variables (i.e., anxiety, craving level for smoking, depression, mood, and stress) and completed two decision-making tasks powered by adaptive design optimization (ADO). With ADO, which is a way to optimize experimental design with information theory and Bayesian data analysis, we could substantially reduce the number of trials required for each task and increase the reliability and precision of individual measures. The two tasks, choice under risk and ambiguity (CRA) and delay discounting (DD) task, produced individual parameters of risk-taking, ambiguity-aversion, and impulsivity (i.e., discounting rate of delayed reward) based on computational models.A time-lagged prediction analysis demonstrated that the computational marker of ambiguity aversion was negatively predictive of daily consumption of cigarettes on the following day (R^2=0.60). Craving and depression levels were significantly predictive of a larger amount of smoking on the following day. In line with this, a logistic regression analysis revealed that individuals' ambiguity aversion assessed during the early phase of the clinic positively predicted prospective success in reduced amount of smoking (β=1.17, OR=3.22, AUC score: 0.750). The main effects of the two analyses survived after accounting for covariates such as medication intake and variance in daily subjective reports such as craving.The current results suggest that the application of computational modeling, specifically through decision-making tasks, offers insights into the identification of predictors of smoking behaviors and treatment for patients with NUD. We expect that these findings could potentially pave the way for the development of

individually-tailored interventions and enhance the prognostic capabilities of nicotine-cessation clinics.

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Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: DePauw University
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Title: Nicotine Response Behavior in Larval Zebrafish

Authors: *H. SCHNEIDER, E. YOSHINAGA, M. ZAHID, L. ARAYA, T. NGUYEN, S. PORTER, A. FAGAN, B. URBAN, E. NEUBAUER, I. DENARDO;
DePauw Univ., Greencastle, IN

Abstract: Tobacco-use is one of the main causes of preventable diseases. Nicotine, the addictive chemical in cigarette smoke, activates nicotinic acetylcholine receptors which also have been shown to play a critical role in nicotine avoidance behavior. The alpha3 and alpha5 subunits of the pentameric acetylcholine receptor appear to reduce nicotine self-administration at high nicotine concentrations in mice. The alpha3 subunit is also a expressed in the peripheral nervous system where is forms the alpha3-beta4 (ganglionic) acetylcholine receptor. We use pharmacological and gene-modification methods to explore the role of nicotinic acetylcholine receptors in zebrafish. The high fecundity, relative short generation time and genetic tools present a major advantage for studying the genomics of nicotine seeking and avoidance behavior. While we have used a behavioral choice test to identify nicotine-seekers and avoiders, additional tests have been developed to better characterize the role of alpha3-beta4 (ganglionic) acetylcholine receptors also in the peripheral nervous system. A set of stimuli that we explored include tapping (mechanosensitive stimulus), light (photosensitive stimulus), and temperature (temperature sensitivity) in brief 5 to 10 minute testing paradigms. All acute behavioral tests have utilized the Daniovision system and EthovisionXT software (Noldus). The test show that mutations of the zebrafish chrna3 gene result in reduced movement activity and reduced responses to tapping. Changes to light stimulation and temperature seems to be unchanged compared to wild-type larval zebrafish. Treatment of larval zebrafish with the nicotinic acetylcholine receptor agonists SR16584 and varenicline have similar outcomes. The comparison of responses in acute tests and behavioral choice experiments for nicotine-seeking and avoidance will provide more insight the role of the alpha3 nicotinic acetylcholine receptor subunit in the

central and peripheral nervous system. Future studies will address the role of alpha5 and beta4 nicotinic acetylcholine receptors.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Targeting IPN α-conotoxin-sensitive nAChRs for alleviation of nicotine withdrawal

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[1]Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA; [2]Univ. Utah, Salt Lake Cty, UT

Abstract: Tobacco smoking is the leading cause of preventable death in developed countries. Relief from nicotine withdrawal (WD) is a primary driver of relapse and continued use and relapse of tobacco products in the long term. Currently available cessation methods effectively alleviate WD but side effects of these therapies reduce compliance. β2* nicotinic acetylcholine receptors (nAChRs; *demonstrates assembly with other subunits) support nicotine WD and reinforcement. Medial habenula (MHb) inputs to the interpeduncular nucleus (IPN) are enriched with nAChRs, including β2*, and are well-known to support nicotine WD behavior. Conotoxin-sensitive nAChRs α6β2* and α3β2* represent a subclass of MHb-IPN nAChRs, which have a more selective expression profile than other nAChRs within this pathway, and which have not been explored for their selective role in nicotine WD. Surgically implanted guide cannulas directed towards the IPN of adult Long Evans female rats utilized selective α-conotoxin antagonists (α-CTX) to determine IPN α6β2* and α3β2* contributions to WD behavior. Subsets of nicotine-naïve and chronic nicotine-exposed rats (0.25 mg/ml oral nicotine in 2% saccharin solution) were given once/week IPN infusions of either α-CTX H9A (α6β2*), PeIA (α3β2*) or MII (α6β2* and α3β2*) using a Latin Square design for dosing (0, 5 and 10 pmol). Nicotine WD testing took place 18-24 hr following removal of nicotine solution in an open field chamber. Following these weekly IPN WD studies, rats were given 0 and 10 pmol neuroanatomical control infusions of each α-CTX and again tested for somatic WD. Upon completion of behavioral studies, rats were perfused and their brains harvested. During nicotine WD, rats showed significantly increased somatic signs as compared to when they were nicotine-naïve, where rats show very few somatic signs ($p = 0.007$). α-CTX administration had no effect on somatic signs

when rats were nicotine-naïve (*p's* > 0.05), indicating that α-CTX had no effect on this behavior in the absence of nicotine. Neuroanatomimcal control studies also failed to show any significant effects of any of the α-CTXs on somatic WD (*p's* > 0.1). IPN infusions of α-CTXs, MII ($F_{2,24}$ = 3.25, p = 0.056) and H9A ($F_{2,32}$ = 2.55, p = 0.094) in nicotine WD rats showed a trend of decreased somatic signs. There was no change in somatic signs with PeIA infusions ($F_{2,28}$ = 0.61, p = 0.55). These preliminary data suggest that nicotine WD is alleviated by selective inhibition of the α6β2*, but not the α3β2* nAChRs in the IPN.

Disclosures: E.S. Geisler: None. **R. Balasubramanian:** None. **J. McIntosh:** None. **P. Whiteaker:** None. **D.H. Brunzell:** None.

Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.18/QQ22

Topic: G.09. Drugs of Abuse and Addiction

Support: Agence nationale de la recherche (ANR) grant

Title: The interpeduncular nucleus acts as a brake on the rewarding effects of nicotine

Authors: *M. CISCATO[1], J. JEHL[1,2], É. VICQ[1,2], J. FRANGIER[3], N. GUYON[1], S. MONDOLONI[2], C. NGUYEN[2], J.-P. HARDELIN[1], F. MARTI[1,2], P.-J. CORRINGER[3], P. FAURE[1,2], A. MOUROT[2,1];
[1]ESPCI ParisTech, Paris, France; [2]Sorbonne Université, Neurosci. Paris Seine, Paris, France; [3]Inst. Pasteur, Univ. de Paris, Paris, France

Abstract: The rewarding effect of nicotine consumption is attributed to the activation of dopaminergic neurons in the ventral tegmental area (VTA). However, when mice are exposed to high concentrations of nicotine, their consumption is reduced, reportedly due to the activation of the interpeduncular nucleus (IPN). However, the response of the IPN to low doses of nicotine and its influence on the rewarding effect of the drug is unknown. To address this issue, we developed a suicide antagonist that specifically targets nicotinic acetylcholine receptor (nAChR) containing the β4 subunit, which is the major subtype found in the IPN. We combined this precise pharmacological tool in designer knock-in mice with in vivo electrophysiology to show that even low doses of nicotine act on IPN neurons, to either activate or inhibit distinct neuronal populations. We found that nAChRs containing the β4 subunit were involved only in the IPN's activation response. Furthermore, inhibition of the IPN's response to nicotine led to an increased sensitivity of the VTA to the drug and enhanced its rewarding effects in a conditioned place preference paradigm. These findings indicate that the IPN acts as a regulatory brake on the VTA, even at low doses, and exerts control over the motivational properties of the drug.

Disclosures: **M. Ciscato:** None. **J. Jehl:** None. **É. Vicq:** None. **J. Frangier:** None. **N. Guyon:** None. **S. Mondoloni:** None. **C. Nguyen:** None. **J. Hardelin:** None. **F. Marti:** None. **P. Corringer:** None. **P. Faure:** None. **A. Mourot:** None.

Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.19/QQ23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA044242

Title: Methoxsalen inhibits the acquisition of nicotine self-administration: attenuation by cotinine replacement in rats

Authors: *Z. DING, E. NESLUND, D. SUN, X. TAN;
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Abstract: Cigarette smoking remains the leading preventable cause of morbidity and mortality. Nicotine is the primary reinforcing ingredient in cigarettes sustaining addiction. Cotinine is the major metabolite of nicotine that produces a myriad of neurobehavioral effects. Cotinine supports self-administration and rats with a history of intravenous self-administration of cotinine exhibit relapse-like drug-seeking behavior, suggesting cotinine may also be reinforcing. To date, a potential contribution of cotinine to nicotine reinforcement remains unknown. Nicotine metabolism is mainly catalyzed by hepatic CYP2B1 enzyme in the rat and methoxsalen is a potent CYP2B1 inhibitor. The study tests the hypothesis that methoxsalen inbibits nicotine metabolism and self-administration, and that cotinine replacement attenuates the inhibitory effects of methoxsalen. Acute methoxsalen decreased plasma cotinine levels and increased nicotine levels following subcutaneous nicotine injection. Repeated methoxsalen reduced the acquisition of nicotine self-administration, leading to fewer nicotine infusions, disruption of lever differentiation, smaller total nicotine intake, and lower plasma cotinine levels. On the other hand, methoxsalen did not alter nicotine self-administration during the maintenance phase despite great reduction of plasma cotinine levels. Cotinine replacement by mixing cotinine with nicotine for self-administration dose-dependently increased plasma cotinine levels, counteracted effects of methoxsalen, and enhanced the acquisition of self-administration. Neither basal nor nicotine-induced locomotor activity was altered by methoxsalen. These results indicate that methoxsalen depressed cotinine formation from nicotine and the acquisition of nicotine self-administration, and that replacement of plasma cotinine attenuated the inhibitory effects of methoxsalen, suggesting that cotinine may contribute to the development of nicotine reinforcement.

Disclosures: **Z. Ding:** None. **E. Neslund:** None. **D. Sun:** None. **X. Tan:** None.

Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.20/QQ24

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA052119
NIH Grant DA021274
NIH Grant GM127251

Title: Effects of repeated nicotine vapor exposure and cessation on intracranial self-stimulation brain reward thresholds in adult male and female rats

Authors: *V. GARCIA[1], A. M. SWEENEY[1], L. MAYNEZ-ANCHONDO[1], K. NEGISHI[2], L. E. O'DELL[1], A. M. KHAN[2], I. A. MENDEZ[3];
[1]Psychology, [2]Biol. Sci., Univ. of Texas at El Paso, El Paso, TX; [3]Pharmaceut. Sci., Univ. of Texas at El Paso Sch. of Pharm., El Paso, TX

Abstract: In recent years, electronic cigarette use has increased substantially, raising concern about the effect of vaping on public health. Importantly, studies have found that women use two-fold higher concentrations of nicotine in e-cigarettes and display greater symptoms of nicotine dependence than men. Research has started to elucidate the effects of repeated exposure to nicotine vapor on the brain and behavior; however, its effects on brain reward function remain unclear. Thus, this study aims to assess the effects of chronic nicotine vapor exposure and cessation on intracranial self-stimulation (ICSS) of the mesolimbic brain reward circuitry, in a sex dependent manner. Adult male and female rats were tested for ICSS across 14 days of exposure to 0 mg/mL vapor vehicle control (50/50 vegetable glycerin/propylene glycol) or 24 mg/mL nicotine vapor and for 14 days following cessation of repeated vapor exposure. Results with male rats revealed no effect of nicotine vapor exposure on ICSS stimulation thresholds; however, thresholds were higher in nicotine vapor exposed rats during the 14 days following cessation of repeated nicotine vapor exposure, when compared to the ICSS thresholds of vehicle control rats. The study with females did reveal a decrease in ICSS threshold during the last week of nicotine vapor exposure in rats being exposed to nicotine vapor, relative to vehicle controls. The effects of cessation of nicotine vapor exposure on ICSS thresholds during withdrawal are currently being determined; however, preliminary results from day 1 and 2 suggest a similar increase in ICSS thresholds to that seen in male rats. These data suggest that repeated nicotine vapor exposure decreases ICSS brain reward thresholds in female, but not male rats. Findings also reveal that cessation from repeated nicotine vapor exposure causes increases in brain reward thresholds in both male and female rats, similar to that seen in other drugs of abuse. Further studies are needed to fully comprehend the effects that nicotine vapor exposure has on the brain and behavior. Furthermore, the inclusion of female rats in pre-clinical studies assessing the health of effects of vaping will be necessary for explaining sex differences observed between male and female electronic cigarette users.

Disclosures: **V. Garcia:** None. **A.M. Sweeney:** None. **L. Maynez-Anchondo:** None. **K. Negishi:** None. **L.E. O'Dell:** None. **A.M. Khan:** None. **I.A. Mendez:** None.

Poster

PSTR562. Nicotine: Cognitive, Behavioral, and Physiological Effects

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR562.21/QQ25

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant SC2DA052119

Title: Effects of adolescent nicotine vapor exposure on behavioral and cellular measures of nicotine withdrawal in adulthood

Authors: *L. MAYNEZ-ANCHONDO[1], M. A. URBINA[2], O. ROHRER[1], P. GINER[2], V. GARCIA[2], A. M. KHAN[3], I. A. MENDEZ[4];
[2]Psychology, [3]Biol., [1]The Univ. of Texas At El Paso, El Paso, TX; [4]Pharmaceut. Sci., The Univ. of Texas At El Paso Sch. of Pharm., El Paso, TX

Abstract: The lack of research on the negative health effects of e-cigarettes is concerning, as e-cigarette use increased dramatically in adolescents, from 1.5% in 2011 to over 20% in 2022. Our pre-clinical rat model of e-cigarette use has revealed that adolescent and young adult nicotine vapor exposure increases nicotine vapor self-administration 5 months later, in adulthood. Here we present the long-term effects of adolescent nicotine vapor exposure on behavioral and cellular measures of withdrawal, in these same rats. Male adolescent Sprague-Dawley rats (n=24, PD=58) were divided into four, 10-day nicotine vapor exposure groups: No Exposure, Adolescent Exposure, Young Adult Exposure and both Adolescent and Young Adult Exposure. Nine months after adolescent exposure they were tested for nicotine vapor self-administration across 10 consecutive days (PD=341). During adulthood (PD=507) all rats received 5 additional days of nicotine vapor self-administration sessions. Immediately after the last session, on the fifth day, they were induced into precipitated withdrawal by systemic injection of the nicotinic receptor antagonist, mecamylamine. Behavioral measures of withdrawal were assessed, including physical signs, elevated-plus maze arm preference, and light-dark transition runway preference. Animals were sacrificed 90 minutes after injections of mecamylamine and induction of Fos protein expression was examined in brain regions implicated in nicotine reward and withdrawal. No differences were seen between groups during withdrawal in the physical signs or elevated plus maze measures. Rats with adolescent-only vapor exposure spent less time in the lit side of the light-dark runway compared to other groups. Preliminary observations of Fos immunoreactivity suggest that naive rats exposed to nicotine vapor during adulthood only, may have higher regional activation during withdrawal in the interpeduncular nucleus and the nucleus accumbens. Our findings demonstrate that adolescent exposure to nicotine vapor causes long-term increases in motivation to self-administer nicotine vapor and anxiety-like behavior during

withdrawal. Future experiments will continue to investigate the effects of nicotine vapor on cells and circuits implicated in nicotine use disorder.

Disclosures: L. Maynez-Anchondo: None. **M.A. Urbina:** None. **O. Rohrer:** None. **P. Giner:** None. **V. Garcia:** None. **A.M. Khan:** None. **I.A. Mendez:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.01/QQ26

Topic: H.01. Attention

Support: Vassar College Startup Funds

Title: Assessing the role of astrocytes in sustained attention using 2% citric acid to motivate performance for a water reward in rats

Authors: *C. A. MENGDEN[1], D. K. RAI-GERSAPPE[2], D. KANGRIWALA[2], K. RATTRAY[2], L. A. NEWMAN[1];
[1]Psychological Sci., [2]Neurosci. and Behavior Program, Vassar Col., Poughkeepsie, NY

Abstract: Appetitive operant conditioning commonly uses food or water to reward correct responses, however motivation for these rewards requires food or water restriction. Both food and water restriction require vigilant monitoring and are classified as USDA Category E for pain and distress. Our lab focuses on the role of astrocytes in cognition, requiring appetitive behavioral tasks. Astrocytes are heavily involved in glucose regulation and metabolism, glutamate and GABA recycling, and water regulation through aquaporin channels, motivating us to find ways for food and water to be freely available when examining astrocytes in cognition tasks. Recent studies have shown that adding a low amount of citric acid to *ad libitum* water supply may be an alternative to motivate behavior for a non-citric acid water reward in simple reward tasks. Our study examines if *ad libitum* 2% citric acid water will motivate behavior for water on a difficult to learn sustained attention task. Male and female Long Evans rats were trained on a sustained attention task requiring shaping to acquire the final task in which trials are presented at variable intervals (12 +/- 3 s) and rats must detect the presence of a light signal (25, 100, or 500 ms) by pressing one lever for a signal and another lever for a nonsignal. In both males and females, there was no significant difference in time to learn any stage of the task, indicating sufficient motivation from the less palatable citric acid water as compared to a standard water restriction paradigm. Vaginal smears were taken for female rats to assess whether performance changed based on estrogen levels at each estrous cycle phase. Male and female rats given the 2% citric acid water had slightly higher weights than water restricted rats, indicating that the citric acid water may mitigate weight loss from water restriction. In order to assess functional changes in astrocytes we examined glutamine synthetase, a marker of astrocytic

glutamate recycling. Our results suggest that citric acid water may provide a viable alternative to water restriction for appetitive operant conditioning on cognition tasks.

Disclosures: C.A. Mengden: None. **D.K. Rai-Gersappe:** None. **D. Kangriwala:** None. **K. Rattray:** None. **L.A. Newman:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.02/QQ27

Topic: H.01. Attention

Support: NIMH R56MH126233

Title: Measuring task engagement in a mouse continuous performance test using pose estimation and visual field analysis

Authors: *Y. LI[1], T. VAN KRALINGEN[1], K. MARTINOWICH[1,2,3], G. V. CARR[1,4]; [1]Lieber Inst. for Brain Develop., Baltimore, MD; [2]The Solomon H. Snyder Dept. of Neurosci., [3]Dept. of Psychiatry and Behavioral Sci., [4]Dept. of Pharmacol. and Mol. Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: As one of the core features of many neuropsychiatric disorders, attention deficits negatively impact functional outcome and quality of life for patients. Continuous performance tests (CPTs) have been widely used in clinical settings to assess attentional function. CPTs have been translated to mouse and rat models as the rodent continuous performance test (rCPT). In this study, we combined traditional psychometric analysis of CPT performance with markerless pose estimation and visual field analysis (VFA) to objectively measure task engagement across rCPT sessions. Additionally, we have tested extended session length to quantify time-on-task vigilance decrements. Our results show the rCPT session length from 45 to 90 minutes induces significant decreases in performance over time. Interestingly task engagement does not decrease over time, suggesting that the decrease in performance represents a vigilance decrement qualitatively similar to what is reported in the clinical literature. Furthermore, we evaluated the effects of the FDA-approved ADHD medication amphetamine on rCPT performance. We found that amphetamine significantly improves rCPT performance by decreasing false alarms and increasing overall task engagement. Together, these findings provide validation for rCPT modifications that increase the translational value of the task.

Disclosures: Y. Li: None. **T. van Kralingen:** None. **K. Martinowich:** None. **G.V. Carr:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

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Program #/Poster #: PSTR563.03/QQ28

Topic: H.01. Attention

Support: NARSAD Young Investigator Grant 28812
NIH grant R01EY005911

Title: Specific timing of the causal contribution of locus coeruleus norepinephrine neuronal activity to perceptual sensitivity associated with visual spatial attention in monkeys

Authors: *S. GHOSH, J. H. MAUNSELL;
Univ. of Chicago, Chicago, IL

Abstract: Previous work from our lab has shown that the locus coeruleus (LC), a primary source of the neuromodulator norepinephrine (NE), plays a spatially selective functional role in controlling of visual spatial attention by improving monkeys' behavioral sensitivity (*d'*) to the contralateral half of the visual field (*Ghosh and Maunsell, 2022*). However, it remains unknown how the behavioral impact of LC spiking varies moment to moment over the natural time course for sensory perception, which for many behaviors involves only a few hundreds of milliseconds. To test this, we trained two rhesus monkeys to perform a demanding visual orientation change-detection task where attention was equally distributed between two spatial locations in opposite hemifields. Monkeys reported an orientation change of a test stimulus relative to a sample stimulus by making a saccade to the appropriate saccade-target. We selectively expressed excitatory opsins (ChR2) in LC-NE neurons unilaterally and activated them with weak binary white-noise optogenetic stimulation (intensity, 10 mW; bin size, 25 ms) throughout the trial. The temporal contribution of the spiking of LC-NE neurons to perceptual detection of the orientation change was measured by reverse correlation. White-noise optogenetic stimulation powers were aligned to the visual stimulus onset for correct detection (and incorrect detection with opposite sign). Trial averaged optogenetic powers revealed the temporal weighting of LC-NE neuronal spiking to correctly detect a change in the visual stimulus either contralateral or ipsilateral to the stimulated LC (the neuronal-behavioral kernel). We found that the kernel for contralateral performance peaks before visual stimulus onset (session average, monkey S, 73 ms, 3320 trials; monkey P, 89 ms, 1040 trials; above 95% confidence interval (CI)). This suggests that LC spiking at this time is the most potent for modulating visual sensory processing for correctly detecting an orientation change during the attention task. In contrast, for correct detection of the ipsilateral stimulus orientation change, LC neuronal-behavioral kernel had no detectable effect (<95% CI). This suggests that LC activity was irrelevant to perceptual detection performance on the ipsilateral visual stimulus. Together, these results demonstrate that the spatially selective LC-NE contribution before the onset of the visual stimulus presumably because the axonal delays in relaying signals to the ipsilateral visual areas to put neurons in the correct neuronal state. Spiking that happens earlier or later in the LC had no measurable impact on visual perceptual performance.

Disclosures: S. Ghosh: None. **J.H. Maunsell:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.04/RR1

Topic: H.01. Attention

Support: NIH Grant 4R00MH117271-03

Title: In vivo imaging of neuromodulatory control over cognitive flexibility in the prefrontal cortex

Authors: *M. A. PREIBISZ-KAMAT[1], J. RYBCZYK[1], A. MITCHELL[1], T. J. SPELLMAN[2]; [1]Univ. of Connecticut Hlth. Ctr., Farmington, CT; [2]Neurosci., Univ. of Connecticut, Farmington, Farmington, CT

Abstract: Impairments in attentional set-shifting, a form of cognitive flexibility, are seen in a range of psychiatric diseases and neurodegenerative disorders. Previous work by our lab and others has implicated the prefrontal cortex (PFC) in modulating set-shifting. However, the cell types involved, the roles of each in supporting set-shifting, and which types are responsible for deficits remains largely unknown. Evidence has shown that the release of the neurotransmitters acetylcholine (ACh), dopamine (DA), and noradrenaline (NA) in the PFC may contribute to set-shifting.To identify the specific roles of prefrontal ACh, DA, and NA release during set-shifting, we adopted a Cre-recombinase (Cre) targeting strategy to modulate activity in neuromodulator-specific cells while imaging pan-neuronal activity across the PFC in mice performing an attentional set-shifting task. In mice expressing Cre in choline acetyltransferase positive (ChAT+) neurons, the excitatory opsin ChRimson was expressed in cells of the basal forebrain (BF, N=4 animals) or the PFC (N=4 animals), and in mice expressing tyrosine hydroxylase (TH), ChRimson was expressed in the ventral tegmental area (VTA, N=4 animals) or the locus coeruleus (LC, N=4 animals). In the same animals, the fluorescent calcium indicator GCaMP8f was expressed in neurons of the PFC under a chronically implanted imaging prism. All mice underwent training on successive stages of a previously validated attentional set-shifting paradigm, and spontaneous activity, task-associated activity, and responses to closed-loop optogenetic stimuli were recorded. Robust task-critical reward-related signaling was observed, with cells encoding reward omissions significantly outnumbering those encoding reward delivery. While calcium activity encoded all task-critical variables, this behavioral variable coding was found to exhibit diverse modulation to catecholamine and cholinergic release, recapitulating earlier studies using pharmacological and/or behavioral measures. Pan-neuronal activity in the posterior parietal cortex (PPC, N=4 animals) was imaged during the same task, and while this activity was found to comprehensively encode the same task-related variables, this coding was more transient than that found in PFC and lacked the persistent activity critical for maintaining task-critical variables across trials.Together, these findings lend further support for a critical role for DA, NA, and ACh in attention-critical neural coding across large populations of PFC neurons. Future research directions are discussed.

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Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.05/RR2

Topic: H.01. Attention

Support: PHS Grant R01DA045063

Title: Neuroimmune states contribute to cholinergic dysfunction in sign-tracking rats

Authors: ***H. CARMON**[1], E. HALEY[3], V. PARIKH[3], N. TRONSON[2], M. SARTER[2]; [2]Dept. of Psychology and Neurosci., [1]Univ. of Michigan, Ann Arbor, MI; [3]Dept. of Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: Sign-trackers (ST) and goal-trackers (GT) are fundamentally distinct phenotypes with differences in behavioral strategies, neuroimmune states, and dopaminergic and cholinergic systems function. Specifically, ST rats attribute incentive salience toward reward cues, which manifests as an approach to and contact with Pavlovian cues, and for vulnerability to addiction-like behavior. In contrast, GTs do not attribute incentive salience toward reward cues, and instead approach the food receptacle or “goal”. STs also exhibit poor attentional performance relative to GTs, mediated by attenuated cholinergic activity and a failure of intracellular choline transporters (CHTs) to translocate into the synaptosomal plasma membrane. Here we investigated poly-ubiquitination, the post-translational modification responsible for disrupted CHT trafficking, with the hypothesis that elevated cytokine signaling in STs contributes to increased ubiquitinated CHTs (ub-CHTs). We previously demonstrated that intracellular CHTs, but not plasma membrane CHTs, are highly ubiquitinated in male and female sign-tracking rats when compared with GTs. Activation of the innate immune system by systemic administration of lipopolysaccharide (LPS) increased ubiquitination levels of cortical and striatal CHTs in GTs, but not STs. This suggests that at baseline ub-CHTs are already at maximum levels in STs and therefore unresponsive to an additional immune challenge. STs and GTs also showed differences in neuroimmune states. At baseline, prior to an immune challenge, cytokine levels in cortex and striatum are elevated in STs compared to GTs. In the cortex, LPS increased levels of the chemokines CCL2 and CXCL10 in both STs and GTs, demonstrating a robust neuroimmune response in both phenotypes. However, for all other cytokines measured, only GTs showed increased levels after LPS. Therefore, ST and GTs also differ in neuroimmune processes, including cytokine levels, microglia activity, and neuronal states, both at baseline and stimulated states. This finding opens the possibility that individual differences in neuroimmune states contribute to cholinergic function, behavioral phenotypes, and vulnerability to addiction.

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Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

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Program #/Poster #: PSTR563.06/RR3

Topic: H.01. Attention

Support: NIH/NIDCD 04845 (LMR)
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Schmitt Program for Integrative Neuroscience

Title: Contextual modulation in primate ventrolateral prefrontal neurons during audiovisual task-switching

Authors: ***M. F. FUCHS**, Y. CAI, K. K. SHARMA, M. D. DILTZ, L. M. ROMANSKI; Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Communication is largely a multisensory phenomenon. During audiovisual communication, attention may focus on one modality or switch between modalities in order to extract time-varying information accurately. Previous studies investigating feature selective attention indicate that neuronal activity is often increased to attended features of a stimulus, thereby enhancing reliability. Furthermore, behavioral studies of modality selective attention indicate that subjects respond quicker and more accurately when a target appears in the expected modality. Prior studies suggest that attention-induced changes in sensory cortex, may reflect top-down control by executive networks including the prefrontal cortex. To examine modality-specific attentional modulation in the primate prefrontal cortex, we compared responses to a compound naturalistic audiovisual stimulus under different contexts. We trained macaque monkeys to perform an audiovisual nonmatch-to-sample task (NMTS) where a face-vocalization movie was presented, and repeated, until either the face or the vocalization component changed, and was detected with a button press. This audiovisual NMTS task was run in a randomized context where either the face or vocalization mismatched from trial to trial; or in a single modality block-design where each trial in a block was always a change in the vocalization component or always a change in the face component of the audiovisual movie. Recordings were made in one subject with a 64 channel implanted micro-array (Microprobes) in the ventrolateral prefrontal cortex (VLPFC). Analysis of neural activity during the decision period of the task indicated a significant difference in the mean firing rate of 24/48 cells (paired T-test, $p < 0.05$) during the single-modality block-design, compared to responses during the randomized context, indicating attentional modulation by modality context. In 78% cells, mean firing rate was increased during the predictable single modality block compared to the randomized context. In this well-learned task, there was no significant difference in performance accuracy for the randomized trials versus the single modality blocks of auditory- or visual-change trials (paired T-

test, $p = 0.36$ auditory trials, $p = 0.48$ visual trials). However, increased difficulty with a longer delay period and novel stimuli may elicit performance differences in random versus predictable contexts. Further analyses focused on information theoretic measures and time-course of neuronal responses will be done to assess modality specific contributions to audiovisual discrimination and processing.

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Poster

PSTR563. Circuits of Attention Across Species

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Title: Characterizing Population Dynamics of Prefrontal Cortex which Govern the Modulation of Visual Processing

Authors: *J. C. GONZALEZ-AMORETTI, A. C. SNYDER;
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Abstract: The prefrontal cortex (PFC) plays a crucial role in visual attention by modulating neural activity in visual processing regions. Specific PFC sub-regions, such as the frontal eye fields (FEF) and the ventral prearcuate region (VPA; area 46v), are implicated in top-down attentional modulation. FEF is associated with spatial attention, while VPA is linked to feature-based attention. However, the underlying neurocomputational mechanisms of this modulatory control remain unclear. Despite the lack of direct projections to visual processing regions, inactivation of VPA reportedly reduces feature-selectivity in V4 and FEF, with which it shares reciprocal projections. Therefore, if modulation of feature-selective visual responses arises in VPA, it is likely mediated by an intermediate region like FEF. Given these reports, it is hypothesized that top-down attentional signals emerge through the neuronal dynamics of interareal PFC networks, with FEF integrating signals from VPA to modulate visual cortical responses. This signal integration may be accompanied by transformations carried out by distinct specialized subpopulations to facilitate shifts between feature and spatial attention signals. This project aims to uncover population dynamics in FEF and VPA that implement the transformation between feature and spatial signals, facilitating allocation of attention. Utilizing natural image

visual search tasks that engage feature-based and spatial attention, combined with neural recordings, can uncover mechanisms underlying the integration and transformation of attentional signals within the population dynamics of FEF and VPA. Behavioral analyses have revealed set-size effects on performance suggestive of covert attentional strategies preceding overt target selection. A spatiofeatural index metric was developed to characterize selective properties of neurons in FEF and VPA. An array placed in the LPFC of a macaque performing an active fixation version of the task, revealed sets of neurons in FEF and VPA that were spatial-, feature- or mixed-selective. Neurons closer to FEF show greater sensitivity to the spatial placement of a stimulus, while those near VPA exhibit stronger response to object identity. Other units among these clusters demonstrated mixed-selective properties. NHPs are undergoing further training to elucidate how these subpopulations interact during visual search. Using population-level analyses, the temporal evolution of information across different subpopulations in FEF and VPA can be quantified. These findings will contribute evidence of the systematic mechanisms underlying top-down attention in the PFC.

Disclosures: J.C. Gonzalez-Amoretti: None. **A.C. Snyder:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.08/RR5

Topic: H.01. Attention

Support: Brain and Behavior Research Foundation Young Investigator Award
NIMH Grant R01MH105592

Title: Chemogenetic stimulation of the locus coeruleus regulates cell type-specific expression of Apoe in the mouse frontal cortex

Authors: *L. RAMOS[1], G. E. CRAIG[2], S. R. ESSIG[1], N. J. EAGLES[3], A. E. JAFFE[3], K. MARTINOWICH[3], H. L. HALLOCK[1];
[1]Neurosci., Lafayette Col., Easton, PA; [2]Neurosci., Emory Univ., Atlanta, GA; [3]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Apolipoprotein-E (*APOE*) is a gene that encodes for a protein involved in lipid transportation in the central nervous system. *APOE* allele variants are commonly associated with differences in Alzheimer's disease (AD) progression and severity, but are also strongly correlated with attentional performance in healthy humans. Despite this knowledge, how *APOE* expression affects brain function during attention remains unclear. Using chemogenetic stimulation and bulk RNA-sequencing, we find that the mouse frontal cortex (FC) is enriched for *Apoe* transcripts following DREADD-mediated depolarization of the locus coeruleus (LC). Deficits in attentional function are associated with altered activity in the FC and LC in patients with disorders such as schizophrenia, attention deficit hyperactivity disorder (ADHD), and major

depressive disorder (MDD), suggesting that *APOE* is involved in attention by virtue of its expression in these brain areas. To investigate cell type-specific expression of *Apoe* in the FC, we used a dual-virus approach to express either the excitatory DREADD receptor hM3Dq in LC neurons with projections to the FC (12 mice total - 6 male/6 female), or a control reporter in LC neurons with projections to the FC (12 mice total - 6 male/6 female). We injected all mice with clozapine-N-oxide (CNO) to activate the DREADD receptor, took slices of the FC, and used single-molecule *in situ* hybridization (RNAscope) to look at a) *Apoe* expression in *Rbfox3*-expressing cells (neurons) vs. *Gfap*-expressing cells (astrocytes), b) *Apoe* expression in *Slc17a7*-expressing cells (glutamatergic neurons) vs. *Gad1*-expressing cells (GABAergic neurons), and c) *Apoe* expression in *Pvalb*-containing cells (parvalbumin interneurons) and *Sst*-containing cells (somatostatin interneurons). We found that increases in *Apoe* expression in the FC following depolarization of LC inputs is neuron-specific, and heavily tied to GABAergic neurons. The results of these experiments yield insights into how *Apoe* expression affects function in cortical microcircuits that are important for attention-guided behavior.

Disclosures: **L. Ramos:** None. **G.E. Craig:** None. **S.R. Essig:** None. **N.J. Eagles:** None. **A.E. Jaffe:** None. **K. Martinowich:** None. **H.L. Hallock:** None.

Poster

PSTR563. Circuits of Attention Across Species

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.09/RR6

Topic: H.01. Attention

Support: NIH Grant F31NS122428

Title: Using a fluorescent neuromodulator reporter to predict cortical acetylcholine levels from arousal state

Authors: *E. NEYHART[1], N. ZHOU[2], R. G. LAW[1], C. SMITH[2], Z. H. MRIDHA[2], M. J. MCGINLEY[2], J. REIMER[2];
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Abstract: When an animal is awake, the cortex goes through spontaneous state changes on a fast timescale which are defined by neural signatures such as more depolarized and less variable membrane potential, as well as behavioral markers such as locomotion and pupil dilation. Although we still don't know exactly how these neural features of state arise, we do know that acetylcholine (ACh) can influence cortical state. However, we still don't know precisely when and where ACh is available to influence the cortex during spontaneous fast state changes. Activity of cortex-projecting areas of the cholinergic basal forebrain (CBF) has previously been associated with locomotion and with pupil size, and although we also have direct evidence that ACh levels are changing in accordance with both of these behavioral markers, a detailed analysis of ACh's release and clearance kinetics in vivo in relation to these variables and to activity of the

CBF has yet to be done. Furthermore, the question of whether cortical ACh levels can be predicted from behavioral markers of state remains open. We took advantage of recent advances in genetically encoded fluorescent sensor technology to address these questions and determined the in vivo rise and clearance time of ACh levels in the cortex, as well as the effective clearance rate of acetylcholinesterase. We recapitulated findings that cortical ACh levels track bouts of locomotion and pupil size and extended these findings across multiple cortical areas. After developing a method to account for on/off times of the sensor kinetics, we were able to precisely characterize the timing of ACh fluctuations surrounding locomotion onset/offset and dilation. By recording activity of CBF projections to the cortex simultaneously with the ACh sensor, we found that ACh is cleared quickly after it is released by these projections, and that the magnitude of ACh response diminishes as distance from the axon increases. Finally, we developed a model which allows accurate prediction of cortical ACh levels from CBF activity, locomotion speed, and pupil size.These findings suggest that the pupil and locomotion can be used as indirect readouts of ACh availability during waking periods, and shed light on how the neural features of cortical state could be induced on a rapid timescale via ACh.

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Poster

PSTR563. Circuits of Attention Across Species

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Program #/Poster #: PSTR563.10/RR7

Topic: H.01. Attention

Support: Stowers Institute for Medical Research
R01 DC008003
R01 DC014701
R01 DC016696

Title: Attention impinges on cholinergic-driven circuit modulation for rapid and accurate odor discrimination

Authors: *R. GARG[1], Q. QIU[1], R. YU[1,2];
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Abstract: Efficient allocation of limited cognitive resources is important for survival. Attention facilitates selective filtering of sensory information and optimizes decisions, but these circuit mechanisms remain unclear. Here, we show attention modulation of odor responses emerges from top-down cholinergic innervations of inhibitory dopaminergic short-axon cells (SACs) in the olfactory bulb. Cholinergic signal is valence dependent but not odor specific. Specificity arises from valence dependent differential learning. Attention impinges on the same circuit to bias sensory gating, leading to rapid and accurate decisions. Manipulation of cholinergic signal

or SAC activity recapitulates attention-like sensory response and behavioral improvement. Proficiency reduces cognitive demand and disengages attention, resulting in a Goldilocks zone where attention enhances performance. Thus, a disinhibitory circuit in the earliest sensory processing stage allows attention to increase sensory saliency. We suggest a floodlight model of attention as a broadcast signal where selective modulation of sensory responses arises from experience dependent synaptic modifications.

Disclosures: **R. Garg:** None. **Q. Qiu:** None. **R. Yu:** None.

Poster

PSTR563. Circuits of Attention Across Species

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.11/RR8

Topic: H.01. Attention

Support: CIHR FRN148365
NSERC CGSD

Title: Laminar microcircuitry underlying target selection in marmoset frontal cortex

Authors: *J. SELVANAYAGAM, S. EVERLING, K. D. JOHNSTON;
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Abstract: Attention allows for filtering irrelevant stimuli and selecting those relevant for behaviour. In foveate animals such as primates, visual attention and eye movements are closely linked and studies in the frontoparietal network of macaques have revealed neurons essential for these processes. However, our understanding of the intrinsic mechanisms shaping selection processes in these areas remains incomplete due to the challenges associated with conducting laminar investigations in key nodes of this network (e.g., FEF and LIP) as they are embedded within sulci in the macaque. To address this gap, we capitalized on the relatively lissencephalic cortex of the common marmoset (*Callithrix jacchus*). We conducted laminar recordings in two adult marmosets (1 female, 26-32 months), targeting regions with strong resting-state functional connectivity with the superior colliculus. Electrode tracts were observed in areas 8aV, 8C and 6DR, overlapping with marmoset FEF. Ultra-high-density laminar probes (neuropixels; 384 electrodes spanning 3.84mm) were used for the recordings as marmosets performed a visual target selection task, which required them to look to a target stimulus in the presence or absence of a distractor in the opposite hemifield. Marmosets displayed shorter saccadic reaction times and greater accuracy on single-target (median RT: 157.2 ms, mean accuracy: 90.9%) as compared to distractor trials (161.1 ms; 67.2%). We recorded the activity of 1491 single units, of which 276 exhibited significant visual or saccade-related activity, and 149 discriminated between target and distractor stimuli before a correct response was made. To examine the underlying mechanisms, we computed models evaluating the contribution of cortical layer and putative cell type to the population's visual and discrimination-related activity. We observed that visual

activity first emerged in granular narrow-spiking units, whereas discrimination-related activity first emerged in infragranular broad-spiking units. In our previous work in posterior parietal cortex, we found evidence supporting a canonical circuit, where visual activity emerged first in putative granular interneurons, followed by discrimination activity in putative supragranular pyramidal neurons. Surprisingly, our observations in frontal cortex challenge this model. While a similar pattern of visual activity was observed, discrimination activity was first observed in infragranular rather than supragranular pyramidal cells. In sum, our findings illuminate intrinsic mechanisms underlying visual attention in PFC and highlight the importance of studying attention across cortical regions.

Disclosures: **J. Selvanayagam:** None. **S. Everling:** None. **K.D. Johnston:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.12/RR9

Topic: H.01. Attention

Support: Brain and Behavior Research Foundation Young Investigator Award

Title: Sex-specific effects of Apoe over-expression on a touchscreen-based sustained attention task in mice

Authors: ***A. E. HARR**, L. RAMOS, S. R. ESSIG, G. J. KEMPSKIE, F. L. D. R. ZAKAS, N. A. FADIL, M. G. SCHMID, M. D. POMPY, M. C. CURLEY, L. A. GABEL, H. L. HALLOCK; Neurosci., Lafayette Col., Easton, PA

Abstract: Apolipoprotein E (ApoE) is a protein that is important for lipid storage, transport, and metabolism. APOE gene variants are associated with Alzheimer's disease (AD), as well as attentional function in healthy humans. Apoe transcription is increased following stimulation of the pathway between the locus coeruleus (LC) and frontal cortex (FC) in mice (see Ramos poster from our lab). This result suggests that Apoe may affect attentional function by virtue of its expression in circuits that control attention. Does Apoe causally regulate attention, or is its expression simply a byproduct of neuronal activity in the LC and FC? To answer this question, we synthetically induced Apoe transcription in the FC of male and female mice, and subsequently tested their ability to learn a touchscreen-based rodent version of the continuous performance test of sustained attention (the rCPT). To increase Apoe transcription, we injected a virus with a plasmid coding for an Apoe expression cassette (AAV8-CMV-Apoe-EGFP) into the FC (14 mice total - 7 male/7 female). Control mice received an injection of a virus with a plasmid coding for EGFP only (AAV8-CMV-EGFP | 14 mice total - 7 male/7 female). We found that increased Apoe transcription modestly attenuated rCPT learning in male mice, while increased Apoe transcription significantly accelerated rCPT learning in female mice. After mice reached performance criterion on the rCPT, we ran them on a probe session designed to increase

attentional demand by dramatically decreasing the probability that mice were presented with a target stimulus (20% probability), compared with a non-target stimulus (80% probability). Finally, all mice were given a 5-10 minute open-field session to measure locomotion and anxiety-like behavior. The results of this study provide insight into how Apoe causally regulates behavior, and point to Apoe supplementation as a potential therapeutic avenue for the treatment of attentional dysregulation in neuropsychiatric disorders.

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Poster

PSTR563. Circuits of Attention Across Species

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Program #/Poster #: PSTR563.13/RR10

Topic: H.01. Attention

Support: R56MH126233

Title: Activity patterns of mouse prelimbic cortical neurons during the continuous performance test.

Authors: *J. A. MIRANDA-BARRIENTOS[1], S. ADIRAJU[2], H. L. HALLOCK[3], G. CARR[4,5], K. MARTINOWICH[4,5,6];
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Abstract: Sustained attention, the ability to focus on a stimulus or task over extended periods, is crucial for higher level cognition, and is impaired in individuals diagnosed with neuropsychiatric disorders, including attention-deficit/hyperactivity disorder, schizophrenia, and depression. Accumulating evidence points to a role for the prelimbic cortex (PrL) in sustained attention. Electrophysiological studies using single-unit recordings demonstrated that neuronal activity in PrL correlates with sustained attention (Tonah et al., 2012; Wu et al., 2017), and we reported changes in local field potentials in the PrL in mice performing the rodent continuous performance test (rCPT) (Hallock and Adiraju et al., 2023), a translational sustained attention task. While the evidence correlating PrL electrical activity with sustained attention is compelling, the limitations on electrophysiological recording techniques, such as low sampling in single unit recordings, and the ambivalence of LFP source, preclude the determination of complex neural dynamics underlying the cellular mechanisms of sustained attention in the PrL. *In-vivo* calcium imaging using genetically encoded calcium sensors can facilitate these studies in behaving

animals since hundreds of neurons can be recorded simultaneously at single-cell resolution. In this study we expressed the genetically encoded calcium sensor GCaMP6f within the PrL (AP: +1.7; ML: ±0.3; DV: -1.7) and implanted Gradient Refractive Index (GRIN) lenses (AP: +1.7; ML: ±0.3; DV: -1.5) in C57BL/6 mice (n=8) for *in-vivo* calcium imaging recordings to characterize neural dynamics within the PrL in mice performing the rCPT. rCPT training consisted of three stages where mice learned to discriminate between a rewarded stimulus (S+) and a non-rewarded stimulus (S-), and where attention demands increased as mice advanced through the stages. rCPT performance was assessed using d', a ratio between correct and incorrect responses (screen touches) across trials, with higher d' reflecting higher sustained attention. We recorded PrL calcium activity at three time points of rCPT training that reflect different levels of cognitive demand and proficiency. PrL calcium activity was analyzed using Inscopix Data Processing Software with a custom MATLAB pipeline. We identified distinct groups of PrL neurons that increased activity prior to a correct or incorrect response. The composition of those groups changed across the recording time points, suggesting recruitment of distinct neuronal ensembles during rCPT training. Moreover, we identified a group of PrL neurons whose activity correlated with periods where mice were engaged with the task

Disclosures: J.A. Miranda-Barrientos: None. **S. Adiraju:** None. **H.L. Hallock:** None. **G. Carr:** None. **K. Martinowich:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

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Topic: H.01. Attention

Support: NIH R01MH128293
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Title: Artificial neural network models predict rodent performance on trial structure variations in an attentional set shifting task

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[1]Psychiatry, Columbia Univ., New York, NY; [2]Ctr. for Theoretical Neurosci., Columbia, New York, NY

Abstract: Understanding features of trial structure that impact learning can improve cognitive task design in animals and help elucidate how task parameters influence cognition. Adapting task difficulty is important for identifying the effect of experimental manipulations on cognitive performance. However, predicting task performance by intuitively changing the task structure is

difficult. We hypothesized that artificial neural network models (ANNs) would predict rodent performance on variations of task structure. We tested this hypothesis using the attentional set-shifting task (ASST), which is a well-established and ethologically relevant behavioral assay used to measure cognitive flexibility in rodent models. Multiple classes of ANNs including single-layer perceptrons, recurrent neural networks (RNNs), and RNNs with thalamocortical-like connectivity were trained on an adapted version of the ASST used in rodent models. Many ASST curricula were semi-randomly generated with varying trial orderings. Based on the performance in these models, we selected an easy and a hard trial curriculum. Wild-type mice were then tested on these curricula and elements of task performance, including number of trials to reach passing criteria and error features, were compared to that of the ANNs. We found evidence that task order influenced mouse performance as predicted by the models. Moreover, the vanilla RNN and thalmocortical-like RNNs best predicted mouse error features. Further investigation will attempt to identify features of curriculum trial ordering that influence task difficulty. These data show the validity of ANNs for optimizing task design in rodents. Employing computational modeling provides a novel strategy for trialing behavioral task sequences, reducing resource expenditure in early stages of experimentation. Additionally, this project can provide insights into the impact of task ordering on cognitive performance.

Disclosures: **I.M. Bravo:** None. **J.T. Stone:** None. **C. Kellendonk:** None.

Poster

PSTR563. Circuits of Attention Across Species

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Topic: H.01. Attention

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Duke Institute for Brain Sciences Germinator Award

Title: Using optogenetics in rhesus macaques to study the role of the claustrum-FEF projection in visuosaccadic behaviors

Authors: *H. G. EL-NAHAL[1], M. A. SOMMER[2];
[1]Biomed. Engin., [2]Biomed. Engineering, Neurobiology, Ctr. for Cognitive Neurosci., Duke Univ., Durham, NC

Abstract: The claustrum is a little-studied subcortical sheet of grey matter. It is the most densely connected brain structure by volume, with reciprocal projections to almost every cortical area, yet little is known about its functions. It has eluded characterization with classical techniques due to its narrow, irregular shape and deep location. Recent genetic technologies, like optogenetics, provide new hope for understanding the claustrum. We identified the retrograde virus rAAV2-retro as a good candidate for claustral study in rhesus macaques. Our injections of rAAV2-retro into the frontal eye field (FEF) of two macaques resulted in robust retrograde labeling in the

ipsilateral claustrum. Isolation of the claustrum-FEF projection would provide a defined circuit for understanding the claustrum's role in vision, eye movements, and related cognitive functions such as attention. Therefore, in this study, after training one macaque on a battery of visuosaccadic tasks including a memory-guided delayed saccade task, a visually guided delayed saccade task, and a step saccade task, we injected into its FEF a rAA2-retro viral vector encoding the inhibitory opsin Jaws. Next, we began behavioral experiments in which we applied laser illumination to the claustrum on randomly interleaved trials of each task to suppress the activity of claustro-FEF neurons. A laser pulse was applied to different task epochs, selected randomly by trial. The largest behavioral effects with laser illumination were in the memory-guided saccade task. On trials in which the target was in the hemifield contralateral to the injected FEF, laser illumination during target presentation (534 trials), during the delay period (1092 trials), and at the go cue (518 trials) resulted in a 14.2%, 26.9%, and 11.8% increase in saccade errors, respectively. Additionally, laser illumination during the delay period and go cue epochs resulted in a significant increase in the monkey's saccadic latency (15.6ms; 750 trials and 14.4ms; 382 trials, respectively). Laser illumination during the target presentation epoch resulted in a significant decrease in saccadic latency (11.6ms; 394 trials). On trials in which the target was in the hemifield ipsilateral to the injected FEF, laser illumination during the target presentation epoch resulted in a 7.9% decrease in saccade errors (482 trials) and a 28.7ms decrease in saccadic latency (365 trials). Saccadic latency also significantly decreased by 11ms when the laser was applied during the delay period (603 trials). These results suggest that the claustro-FEF projection may be necessary for aspects of visuosaccadic planning such as working memory or attention.

Disclosures: H.G. El-Nahal: None. **M.A. Sommer:** None.

Poster

PSTR563. Circuits of Attention Across Species

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Mt. Jade Young Scholarship Award from Ministry of Education, Taiwan

Title: Exploring attention function in individuals with schizophrenia based on functional connectivity networks of specific subcortical seed regions

Authors: *H.-Y. HSU[1], A.-C. YANG[1,2];
[1]Inst. of Brain Sci., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; [2]Dept. of Med. Res., Taipei Veterans Gen. Hosp., Taipei, Taiwan

Abstract: Attention dysfunction is a core cognitive symptom in individuals with schizophrenia, which can be interpreted by the network disconnection. Previous studies using resting-state functional magnetic resonance imaging (rs-fMRI) have indicated abnormal activation of the cortical brain network in schizophrenia, reflecting dysfunction in attention control. However, the relationship between subcortical brain networks and attention function in schizophrenia remains unclear. We aimed to investigate whether specific subcortical regions act as critical hubs that modulate attentional functional connectivity networks in individuals with schizophrenia. Participants included 167 individuals with schizophrenia (age: 43.41 ± 11.74; 55.1% female) and 167 healthy controls (age: 41.27 ± 10.95; 58.0% female) from the Taiwan Aging and Mental Illness cohort. First, four subcortical regions associated with attention were defined as seeds, including the insula, thalamus, anterior cingulate gyrus (ACG), and posterior cingulate gyrus (PCG). Second, the seed-based functional connectivity analysis was used to identify the rs-fMRI functional connectivity value between seed regions and each gray matter voxel. Finally, the general linear model was used to explore the relationship between various subcortical-seeded attention networks and clinical attention-related assessments (Mini-mental state examination; Digit span task; and Wisconsin card sorting test, WCST) in individuals with schizophrenia and healthy controls. We found that every subcortical-seeded functional connectivity network was reduced in schizophrenia compared to the healthy controls. Significant hypoconnectivity of attention networks were observed in the precuneus, supramarginal gyrus, cingulate gyrus, frontal, temporal lobe, and cerebellum ($P_{FWE} < 0.001$). Additionally, we found that the digit span task and WCST were positively associated with the functional connectivity between three seeds (insula, thalamus and ACG) and superior frontal gyrus, middle frontal gyrus, superior temporal gyrus, and supramarginal gyrus ($P_{FWE} < 0.05$). However, in comparison to healthy controls, no significant relationship between clinical attention scores and PCG-seeded network was observed in individuals with schizophrenia. Hypoconnectivity of specific subcortical-seeded functional connectivity networks may reflect the abnormal attention function of individuals with schizophrenia. Moreover, these findings could shed light on the clinical attention performance of schizophrenia.

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Poster

PSTR563. Circuits of Attention Across Species

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Program #/Poster #: PSTR563.17/RR14

Topic: H.01. Attention

Support: NSC 109-2923-H-006-002-MY3

Title: Advance preparation in cued crossmodal attention switching: evidence from BOLD fMRI

Authors: *P.-C. HUANG, S. HSIEH, W.-H. WU;
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Abstract: Our world is inherently multisensory, demanding seamless attentional shifts between different sensory modalities. A crucial aspect of successful attentional switching is advanced preparation. In this event-related fMRI study, we investigated the neural circuits associated with preparing and implementing attentional switches and repeats between visual and auditory modalities. Participants were engaged in a cued attention-switching paradigm, performing a spatial location task based on modality cues. Simultaneously presented lateralized visual and auditory stimuli demanded participants to determine the spatial location (left or right side) of the relevant modality target. By manipulating the cue-to-target interval (CTI, 200 ms vs. 2100 ms), we examined the impact of preparation on both behavioral task performance and fMRI-related measures. Our behavior results underscored the pivotal role of preparation in attentional switching. The preparation effect demonstrated its substantial influence in reducing the switching cost. Specifically, the response time (RT) significantly decreased from 42 ms to 16 ms, with a remarkable decrease in error rates from 3.4% to 1.6%. Notably, the preparation effect exhibited a greater magnitude in auditory trials than in visual trials (RTs: 130 ms vs. 91 ms; error rate: 5.7% vs. 0.5%). Analyzing the blood-oxygen-level-dependent (BOLD) response associated with the attention-switching task, we observed widespread activation in the frontal-parietal cortex, encompassing the superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, cingulate gyrus, insula, parahippocampal gyrus, cuneus/precuneus, inferior parietal lobe, and superior temporal gyrus. Further analysis of the interaction between task switch and preparation revealed no significant activation regions. This suggests that conceptual preparation involves a set of processes activated for both switch and repeat trials. In addition, the primary visual and auditory cortex showed stronger activation when contrasting well-prepared and less-prepared conditions (CTI2100 - CTI200). This suggests that these sensory-specific regions are crucial in facilitating successful attention shifts between modalities. Further analysis contrasting well-prepared and less-prepared switch conditions revealed stronger activation in the precuneus and superior parietal lobe during switch trials, emphasizing their involvement in the preparation process. Conversely, the bilateral superior frontal gyrus exhibited heightened activation without preparation, suggesting distinct networks supporting target-driven processes.

Disclosures: **P. Huang:** None. **S. Hsieh:** None. **W. Wu:** None.

Poster

PSTR563. Circuits of Attention Across Species

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Program #/Poster #: PSTR563.18/RR15

Topic: H.01. Attention

Support: Cole Fund UNH

Title: Dissociable effects of excitotoxic lesions along the rostro-caudal axis of the anterior, caudal anterior cingulate, and anterior mid cingulate cortex.

Authors: *J. A. MCGAUGHY[1], M. K. CLEMENT[1], L. A. NEWMAN[2];
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Abstract: In humans, dysfunction in the anterior cingulate cortex (ACC) has been linked to cognitive impairments in many neuropsychiatric disorders. Prior work to understand the basis of these impairments has rarely differentiated subdivisions within this region, which can be difficult to discern in humans. Research in non-human primates has shown functional and anatomical distinctions between the ACC and the region caudal to it, namely the anterior mid-cingulate cortex (MCC). These regions have yet to be explored to determine whether functional distinctions are present in rats. We used young, adult male Long-Evans rats to assess the effects on attention of circumscribed lesions along the rostrocaudal axis of the cingulate cortex in three subregions: rostral anterior cingulate (rACC:+2.7/+2.2), caudal anterior cingulate (cACC:+0.80), and anterior mid-cingulate (aMCC:-0.84). We used two previously validated translational tasks, the attentional set-shifting task (ASST) and the sustained attention task (SAT), to assess selective and sustained attention. Testing in the SAT also included variations to measure vulnerability to novel distractors in the same modality and cross-modal to the target. Performance in the ASST and SAT revealed unique cognitive impairments associated with each sub-region. Subjects with lesions of the rACC were selectively impaired in filtering distracting stimuli with a prior reinforcement history but had no impairments when presented with novel distractors that had not been previously paired with prior reinforcement. Lesions centered in the cACC located between the rACC and aMCC resulted in an inability to form an attentional set in the ASST but no impairments in filtering distractors regardless of prior reinforcement history. Lastly, lesions to the aMCC produced mild cognitive rigidity in the ASST, impaired target detection in the SAT, and increased susceptibility to novel but not previously reinforced distractors. These data show a clear functional delineation along the rostrocaudal axis of the rat cingulate cortex. These data are helpful to reconcile functional studies of rACC with anatomical studies of cACC. Future work is needed to understand how these regions contribute to other cognitive functions. The authors gratefully acknowledge the continued support of the Cole Fund.

Disclosures: J.A. McGaughy: None. **M.K. Clement:** None. **L.A. Newman:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.19/RR16

Topic: H.01. Attention

Support: JSAM "Jena School for Ageing medicine"

Title: The effect of STN-DBS on the reward system and cognitive function in Parkinson`s disease

Authors: *K. GERNER[1], A. BARTHEL[2], K. FINKE[3], P. BUBLAK[3], H. KOEHLER[4], A. SCHMIDT[4], C. KLINGNER[4], S. BRODOEHL[4], F. WAGNER[4];
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Abstract: Idiopathic Parkinson's disease is the second most common neurodegenerative disease worldwide. Besides the well-known motor symptoms, there are many non-motoric symptoms from which patients suffer. For Parkinson's patients in an advanced state of the disease, deep brain stimulation (DBS) is a viable option to improve motor function. However, DBS intervention is often associated with the risk of cognitive decline and an accumulation of obsessive-compulsive behavior. Therefore, a systematical exploration of the effects of DBS on cognition and the reward system via a test of visual attention (TVA) and functional imaging (MEG, EEG) will open up a better understanding of the neurological key mechanisms of DBS implantation. Since the localization and stimulation parameters seem to be crucial for the outcome and side effects of DBS, it is necessary to investigate the effects of the stimulation itself. For this project, we recruited three cohorts of patients diagnosed with idiopathic Parkinson's syndrome, each representing a different stage of DBS intervention. The first group consisted of 15 patients who were undergoing evaluation for DBS or were in the preoperative phase. Secondly, we included two groups of 15 patients each who had undergone DBS implantation, with one group having received the treatment within the past few months and the other group having had the DBS for over a year. All groups were age and gender-matched and had no diagnosis of any other neurological or psychiatric disorders. Firstly, questionnaires are used to evaluate the patients' non-motoric and motoric symptoms. Secondly, the patients undergo the test of visual attention (TVA) and a magnetencephalographic examination while completing a modified Monetary Incentive Delay Task. In the preliminary data, we have demonstrated the effect of DBS on both cognition and the reward system. Using paired t-test analysis and repeated measurement ANOVA, we were able to show a statistically significant decline in some cognitive parameters as measured by TVA. MEG data revealed a reduced reward sensitivity in post-DBS patients compared to the preoperative group. These findings support our hypothesis, however, further assessment of a larger patient cohort is necessary before drawing definitive conclusions. In conclusion, our aim is to optimize the treatment of Parkinson's patients post-DBS by systematically investigating relevant neurocognitive parameters. By taking these parameters into consideration during the adjustment of stimulation, it is possible to modify the simulation setting, thereby reducing cognitive decline and the incidence of obsessive-compulsive disorders in patients with IPS.

Disclosures: K. Gerner: None. **A. Barthel:** None. **K. Finke:** None. **P. Bublak:** None. **H. Koehler:** None. **A. Schmidt:** None. **C. Klingner:** None. **S. Brodoehl:** None. **F. Wagner:** None.

Poster

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Program #/Poster #: PSTR563.20/RR17

Topic: H.01. Attention

Support: NSF NRT Award 2152260
NIH EY014924
NIH EY029759
Brain and Behavior Research Foundation

Title: Inactivation of Parietal Cortex Neurons Increases the Intrinsic Timescales of the Frontal Eye Field

Authors: *O. SOYUHOS[1], T. MOORE[4], R. CHAUDHURI[2], X. CHEN[3];
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Abstract: Intrinsic neural timescales are critical for brain dynamics and information processing, coordinating firing patterns and facilitating precise information transmission. Traditionally, these timescales were thought to be governed mainly by connectivity within a local brain region. However, the specific role of interareal interactions in shaping intrinsic timescales remains unclear. In this study, we focused on the interactions between the Frontal Eye Field (FEF) and the Posterior Parietal Cortex (PPC) within the frontoparietal attention network. To understand the specific role of interareal interactions between PPC and FEF in shaping intrinsic timescales in FEF, we utilized the cryoloop cooling technique to reversibly inactivate the PPC in two macaques, while monitoring FEF neural activity using linear electrode arrays. Using cryoinactivation, we effectively diminished the PPC's input to the FEF. We assessed the changes to intrinsic timescales by comparing FEF neurons' autocorrelation profiles under PPC-control and PPC-inactivated conditions. Our results revealed a robust increase in autocorrelation duration, indicating slower intrinsic timescales and suggesting an elevated dependence on local recurrent processing after PPC inactivation. These results indicate that FEF neurons may extend their information retention period in response to diminished feedforward input from the PPC and highlight the causal influence of interareal connectivity on intrinsic neural dynamics. Overall, our findings expand our understanding of how the brain orchestrates information processing across neural circuits and offers potential insights into neurological disorders involving disruptions in frontoparietal attention networks.

Disclosures: O. Soyuhos: None. **T. Moore:** None. **R. Chaudhuri:** None. **X. Chen:** None.

Poster

PSTR563. Circuits of Attention Across Species

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR563.21/RR18

Topic: H.01. Attention

Title: Thalamic Reticular Nucleus and Dopamine in the modulation of a thalamo-cortical cue detection circuit

Authors: *K. RUNYON, S. MAZANEK, A. GREENWAY, A. HARTLE, K. MARSCHALKO, M. HOWE;
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Abstract: A deficit in the capacity to use instructive cues to guide ongoing behavior, "cue detection", is a feature of many neurodegenerative and neuropsychiatric disorders. Previous work in humans and model species highlights that circuitry including the mediodorsal thalamus (MD) and its projections to the prefrontal cortex (PFC) are critical for the control of this cognitive process. How activity in this circuit is sculpted to support these functions remains unclear. Here, we describe a set of multi-disciplinary studies designed to gain insight into this gap in our knowledge. First, using male and female wild-type mice and fiber photometry, we measured in vivo calcium dynamics (GCAMP7s) at both the local cell bodies (MD, n=3) and distal terminal fields (PFC, n=4) in mice undergoing Pavlovian cue-reward pairing. In both the cell bodies of the MD and terminals in the PFC, we observed significant increases in activity in response to a conditioned light stimulus. Interestingly, presentation of the primary reward evoked a transient increase in Ca+2 only in MD cell bodies, suggesting that information about the learned value of a predictive cue is selectively relayed to the PFC. We next sought to uncover mechanisms underlying this stimulus-specific encoding by thalamic input to the PFC. Output from PFC-projecting MD neurons is tightly controlled by the inhibitory thalamic reticular nucleus (TRN), a brain region that has been described as an "internal attentional searchlight" (Crick, 1984). Using transgenic mice and viral tracing techniques (n=4), we found that MD-projecting neurons are clustered in anterior dorsal TRN, a region that also receives a relatively dense input from dopamine neurons of the substantia nigra pars compacta. As midbrain dopamine systems are also known to mediate behavioral responses to reward-predictive cues, our on-going experiments measure and manipulate dopaminergic input to assess the role of dopamine in modulating thalamo-cortical circuits and cue detection.

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Poster

PSTR563. Circuits of Attention Across Species

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Program #/Poster #: PSTR563.22/RR19

Topic: H.01. Attention

Support: R01 DA031695

Title: Exploring the impact of HDAC5 underexpression in the anterior cingulate cortex on cognitive control

Authors: *D. VAZQUEZ, X. SCIARILLO, K. J. YEGANEH, C. XIA, X. LI, M. R. ROESCH; Univ. of Maryland, College Park, MD

Abstract: In previous studies, we have found that disruption of anterior cingulate cortex (ACC) signaling—via manipulations ranging from chemical lesions to optogenetic inhibition—results in attenuated attention during performance of a decision-making task. How these attentional disruptions may be repaired—using biologically relevant treatment—in the service of restoring decision-making impairments remains to be explored. One interesting molecular target for modulating cognition and neural activity is histone deacetylase 5 (HDAC5), an epigenetic enzyme involved in regulating gene expression. Studies have found that heightened HDAC expression contributes to memory impairments and cognitive decline (Dos Santos Sant'Anna, 2013; Taniguchi et al., 2018), and that expression of nucleus-localized HDAC5 in the nucleus accumbens reduces neuron excitability (Anderson et al., 2023). Taking these findings into account, we sought to explore whether chromatin remodeling resulting from HDAC5 underexpression might enhance attentional signals that are necessary for flexible decision-making. To this aim, we trained rats on a reward-guided decision-making task consisting of four sixty-trial blocks; reward value was manipulated by independently varying the delay to (Blocks 1 and 2) or size of reward (Blocks 3 and 4). Subsequently, we either knocked down (scAAV1-CMV-shHDAC5-GFP; n=8), or did not manipulate (control virus, scAAV1-CMV-shLuc-GFP; n=7) HDAC5 expression, and recorded ACC activity while rats performed the aforementioned task. Thus far, we have observed that HDAC5 underexpression in the ACC has enhanced signaling in a way that optimizes behavior—as operationalized by a bias towards high-value reward, and better performance on forced-choice trials. Continued recording from the ACC will provide insight into how HDAC5 underexpression may enhance attentional signals that are necessary for flexible and adaptive decision-making.

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Poster

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Topic: H.01. Attention

Support: Lundbeck Foundation R359-2020-2301
Lundbeck Foundation R266-2017-4331
Lundbeck Foundation R276-2018-792

Title: Determining the role of norepinephrine in attention - by chemogenetic manipulation and fiber photometry measurement of transmitter release dynamics

Authors: *J. COLL-MARQUES, L. P. POSSELT, K. KLIEM, S. H. JØRGENSEN, S. OVROM, F. HERBORG, A. T. SØRENSEN, U. GETHER;
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Abstract: Norepinephrine (NE) signaling in the frontal cortex is of high importance in facilitating attentive, adaptive, and goal-directed behavior. Dysregulation of NE signaling has been implicated in a broad spectrum of neurological and psychiatric disorders, including attention deficit hyperactivity disorder (ADHD). While the detrimental effects of NE deficiency on attention have been extensively studied, the impact of excessive NE levels on attentive behavior remains poorly understood. Gaining a deeper understanding of the neuronal mechanisms underlying NE signaling in attention could significantly improve the diagnosis and treatment of attentional deficits. To address this knowledge gap, we employed an excitatory Designer Receptor Exclusively Activated by Designer Drug (DREADD) approach in conjunction with a noradrenergic biosensor to examine NE release dynamics *in vivo*. By selectively activating NE neurons in the Locus Coeruleus via excitatory DREADD manipulation and expressing the biosensor in the medial prefrontal cortex, we observed a robust increase in basal NE fluorescence within the prefrontal cortex. Importantly, the increase in fluorescence exhibited a direct correlation with pronounced impairment in attentional performance. Notably, both the behavioral outcomes and fluorescence enhancement displayed a dose-dependent relationship, while gross motor functions remained unaffected. Furthermore, we sought to establish a direct link between NE dynamics at varying timescales and discrete behavioral outputs, aiming to gain deeper insights into the role of NE dynamics in attentive behavior. In conclusion, our findings provide evidence that excessive NE levels in the frontal cortex impair attentional performance. This study provides a foundation for understanding the intricate relationship between NE dynamics and attentive behavior, elucidating the temporal aspects of NE signaling in specific behavioral manifestations. Future studies will focus on elucidating the functional significance of NE dynamics in other brain regions involved in attentive processes.

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Poster

PSTR563. Circuits of Attention Across Species

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Program #/Poster #: PSTR563.24/SS1

Topic: H.01. Attention

Support: Lundbeck Foundation R359-2020-2301
Lundbeck Foundation R276-2018-792

Title: Identifying dopaminergic signatures of increased attentional demand across striatal subregions

Authors: *S. OVROM, S. H. JØRGENSEN, L. P. POSSELT, M. HERVIG, U. GETHER; Univ. of Copenhagen, Copenhagen, Denmark

Abstract: A key function of our brain is attention, which is the ability to select only the most important information for conscious perception. Dopamine has long been implicated in attention possibly by controlling motivation. However, we have little understanding of how different parts of the dopamine system might cooperate to support attentional functions. The densest dopaminergic projections include the mesolimbic and nigrostriatal pathways, which primarily project to the ventral striatum (VS) and dorsal striatum (DS), respectively. While the VS dopamine is suggested to affect behavior by encoding the salience value of objects and events, the dorsolateral striatum (DLS) is classically understood to have a closer role in the formation of habits, and the dorsomedial striatum (DMS) is understood to encode the association of actions, stimuli, and rewards. Here, we use multi-region fiber photometry together with the dopamine sensor dLight1.3b to investigate signatures of dopaminergic dynamics in striatal subregions of 22 male wildtype C57BL/6mice in the rodent Continuous Performance Test (rCPT), a reward-based learned attentional touchscreen task. We record dopamine dynamics in the DLS, DMS, and VS, which have differential characteristics from one another and have distinct signatures corresponding to separate events in the task. We then manipulate the task to increase the attentional requirement, which results in increased dopaminergic release. Further task manipulation, which alters reward prediction, results in unique changes in each subregion during each event in the task. Our results show evidence that attentional performance is supported by highly cooperative and differentially timed dopamine release dynamics across the DLS, DMS and VS.

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Poster

PSTR563. Circuits of Attention Across Species

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Program #/Poster #: PSTR563.25/SS2

Topic: C.01. Brain Wellness and Aging

Support: R01 AG062667
P01 AG036694

Title: Dorsal Attention Network Functional Connectivity is Associated with Time Spent in N2 Sleep in Older Adults

Authors: C. E. SCANLON[1], R. T. BOYLE[1], L. STANKEVICIUTE[1], S. HSIEH[1], Z. SHIRZADI[1,2], S. A. SCHULTZ[1], W.-Y. W. YAU[3], M. J. PROPERZI[1], R. F. BUCKLEY[1], K. A.

JOHNSON[1], R. A. SPERLING[1], S. REDLINE[4], S. M. PURCELL[4], I. E. DJONLAGIC[4], A. P. SCHULTZ[1], *J. CHHATWAL[1];
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Abstract: Individual variations in time spent in each stage of sleep are correlated with differences in behavior, cognition, and brain structure. However, the link between sleep stages and functional connectivity of cortical networks has not been well studied, and prior work has been complicated by varying and relatively long intervals between polysomnogram (PSG) recordings and resting-state functional magnetic resonance imaging (rs-fMRI) scans. We sought to investigate this association in older adults with rs-fMRI scans collected the morning after overnight PSG completion. 17 cognitively unimpaired older adults (9 women, Mean age = 75.5 ± 8.42 years) from the Harvard Aging Brain Study underwent an overnight, in-home PSG and an rs-fMRI scan the next morning. We calculated the proportion of time spent in each sleep stage (N1, N2, N3, and Rapid Eye Movement [REM]) and metrics of slow and fast sleep spindles during the N2 stage (density, frequency, mean integrated spindle activity, and frequency modulation of global spindles) from the PSG data. We obtained measurements of whole network functional connectivity in 6 cortical networks (Default Mode [DMN], Dorsal Attention [DAN], left and right Frontoparietal Control [FPCN], Primary Visual [PriVis], Extrastriate Visual [ExVis], and Motor networks) using Template-Based Rotation. We calculated Pearson's correlations between percent time in each sleep stage and functional connectivity of each network. For sleep stage and networks with significant associations, we then used hierarchical regressions to assess their effect size, adjusting for age and head motion. We then examined whether networks with independent sleep stage associations were correlated with sleep spindle characteristics. Percentage of sleep in the N2 stage (N2%) was positively associated with functional connectivity of the DAN ($r = 0.637$, $p = 0.006$) and PriVis network ($r = 0.54$, $p = 0.025$). Percentage of sleep in the REM stage was negatively associated with DAN connectivity ($r = -0.506$, $p = 0.038$). N2% explained 41.5% of the variance in DAN connectivity ($p = .007$). No other associations remained significant after adjusting for covariates. There were no statistically significant associations between DAN connectivity and sleep spindle characteristics from the N2 stage. Our findings indicate that N2% is strongly correlated with DAN connectivity, suggesting that brainstem nuclei involved in promoting N2 may also promote DAN integrity during wakefulness. Future work with a larger sample will enable investigation of how sleep and DAN functional connectivity may be related to cognitive performance and Alzheimer's disease pathology.

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Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.01/SS3

Topic: H.01. Attention

Support: Partial support by Brainwave Science, Inc.

Title: Measuring the effectiveness of mantra-based meditation using EEG data analysis

Authors: *A. LI[1], P. PRADHAN[1], A. WOZNIAK[1], B. H. COHEN[3], S. RAVISHANKAR[1,2]; [1]Dept. Computat. Mathematics, Sci. and Engin., [2]Dept. Biomed. Engin., Michigan State Univ., East Lansing, MI; [3]Dept. Applied Psychology, New York Univ., New York, NY

Abstract: Our study explores the effects of mantra meditation on cognitive abilities, shedding light on its potential therapeutic value for mental health. By examining a less-explored form of meditation, we aim to provide an alternative non-pharmacological approach that improves attentional focus.Specifically, P300 (a widely used measurement to reflect the cognitive process) results were collected by iCognative technology (made by Brainwave Science, Inc.) on a total of twenty-three pre-screened participants. The mantra-based meditation (MBM) group (n_{male} = 10, n_{female} = 2, median age or M_{age} = 31.5 years, SD = 3.9 years) comprised individuals who had undergone over 2 years of practice, mainly chanting the “Hare Krishna" mantra. For purposes of comparison, a novice group (n_{male} = 8, n_{female} = 3, M_{age} = 23 years, SD = 5.7 years), who had no experience with meditation, was included.The P300 speller test is conducted by presenting a grid of characters on a computer screen to the participant. The characters are flashed in a random order and the participant is instructed to count instances of a specific target character. In the test, EEG data are divided into epochs, which are time segments corresponding to the presentation of each character. The epochs are then averaged across multiple trials to enhance the signal-to-noise ratio.The empirical findings revealed a substantial reduction in latency for the MBM group (**$M_{latency}$ = 366.4 ms**, SD = 17.48 ms) compared to the novice group (**$M_{latency}$ = 414.15 ms**, SD = 23.84 ms). An unpaired t-test demonstrated a significant between-group difference, affirming the distinct impact of mantra-based meditation on latency during the P300 test (p-value = 0.0022). Based on our observations, the MBM group showed superior sustained attention and task engagement compared to novices, as evidenced by their enhanced accuracy in counting letter flashes during the task. The significant difference in latency between MBM subjects and novices suggests a positive impact on cognitive processing. MBM may enhance neural processing and response generation, resulting in reduced P300 latency. These findings support MBM as a potential intervention for improving cognitive abilities. Limitations of this study include that it was cross-sectional (meditators and novices were selected from pre-existing groups) and that the meditators were considerably older than the novices. Future research should explore other meditation techniques, employ larger, age-matched, samples, and investigate long-term effects, including measures of mental well-being. In conclusion, MBM shows promise for enhancing cognitive abilities, especially attentional focus.

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Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.02/SS4

Topic: H.01. Attention

Support: UKRI MRC funding
Gates Cambridge Scholarship

Title: The causal roles of sustained potentials and alpha oscillations in coding task-relevant information during selective attention

Authors: *R. LU[1], E. MICHAEL[1], C. L. SCRIVENER[2], J. B. JACKSON[1], J. DUNCAN[1,3], A. WOOLGAR[1];
[1]MRC Cognition and Brain Sci. Unit, Univ. of Cambridge, Cambridge, United Kingdom; [2]Univ. of Edinburgh Sch. of Psychology, Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Dept. of Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom

Abstract: Selective attention is a fundamental cognitive mechanism that allows people to prioritize task-relevant information while ignoring irrelevant information. While previous research has suggested key roles of sustained potentials like N2pc and alpha power in coding spatial information (i.e., where to attend) during selective attention, their roles in coding additional attentional information (e.g., what to attend to, visual feature information) remains less clear. Also, the question of whether sustained potentials and/or alpha power causally influence the coding of different task-relevant information during selective attention is yet unresolved. In our two-part study, we used concurrent TMS-EEG to causally manipulate both alpha power and N2pc amplitudes and investigated their roles in coding task-relevant information in a selective attention task. In the first part, using EEG-only data, we investigated the decodability and temporal dynamics of sustained ERPs and alpha power in coding different facets of selective attention (i.e., where to attend, what to attend to, and visual feature information). We found sustained potentials coded all three types of task-relevant information with distinct temporal dynamics, whereas alpha power only carried information regarding where to attend and what to attend to. In the second part, we applied rhythmic-TMS (rTMS) at individual alpha frequency targeting the right intraparietal sulcus (IPS), examining the causal roles of sustained potentials and alpha oscillations in selective attention. Compared with the control arrhythmic-TMS, alpha rTMS increased both power and inter-trial phase coherence in alpha band and induced more negative N2pc amplitudes. Moreover, alpha rTMS specifically caused higher multivariate decoding accuracy of the information about where to attend (but not what to attend to or feature information), and TMS-induced changes in decoding information about where to attend predicted TMS-induced changes in behavioural errors and reaction time. Together, these findings illuminate the different temporal profiles of sustained potentials and alpha power in coding various task-relevant information during selective attention. Moreover, they revealed specific and causal roles of IPS-controlled N2pc and alpha power in coding spatial information about where to focus attention.

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Poster

PSTR564. Attention II

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Program #/Poster #: PSTR564.03

Topic: H.01. Attention

Title: Is the attentional template held in visual working memory, or can it be indexed by frontal-to-posterior theta-gamma coupling?

Authors: *J. SCHMIDT[1], M. X. COHEN[2], M. T. MIUCCIO[1];
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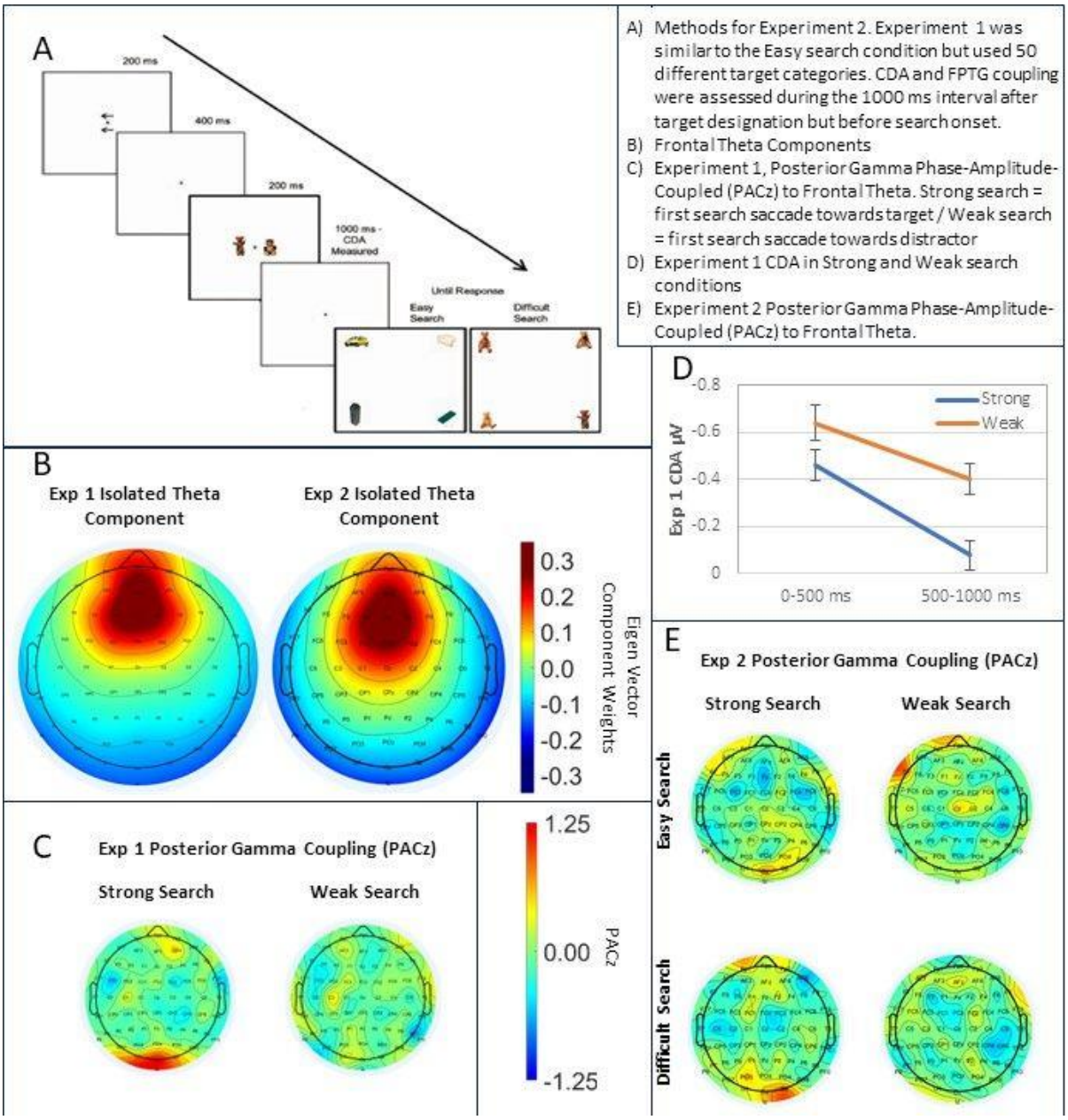
Abstract: To guide spatial attention to potential target regions in our environment, we must use a mental representation of a target to match to incoming perceptual information (e.g., Zelinsky, et al. 2013). Decades of work argue that this representation, or attentional template, is stored in visual working memory (VWM). Models of search suggest that more target visual details should more efficiently direct attention (e.g., Zelinsky, 2008; Wolfe, 2021). However, the evidence is mixed, with most work suggesting higher target-related VWM load results in poorer search performance (Carlisle et al., 2011; Schmidt et al., 2014; Woodman et al., 2013; but see, Schmidt & Zelinsky, 2017). This presents a conundrum; how can the attentional template be stored in VWM, but search performance improve when target details leave VWM? We argue that the attentional template is not stored in VWM, but is represented by neural communication from frontal to posterior brain regions. The classic biased competition model argues that the attentional template is stored in VWM, but the information is communicated to frontal regions, which in turn bias early visual areas to preferentially process target-related visual features (Desimone & Duncan, 1995). However, this neural communication was never formally demonstrated; it was inferred from local firing rates. Here, we show that this neural communication is related to later deployments of spatial attention, demonstrating that it is an accurate index of the attentional template. Using eye movement and Phase-Amplitude Coupling (PAC) EEG analyses across two experiments, we show that pre-search, target-related frontal-to-posterior theta-gamma coupling (FPTG; Canolty et al., 2006; Cohen, 2017) is consistently stronger when attention (as indexed by the direction of the first search saccade) is directed to the search target; whereas VWM load, as indexed by contralateral delay activity, is inconsistently related to search performance. Given this, we argue that neural communication, as indexed by FPTG coupling, is the attentional template rather than what is stored in VWM.



Disclosures: **J. Schmidt:** None. **M.X. Cohen:** None. **M.T. Miuccio:** None.

Poster

PSTR564. Attention II

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Program #/Poster #: PSTR564.04/SS5

Topic: H.01. Attention

Title: Evidence for Separable Mechanisms Underlying Enhancement Versus Suppression Using Individual Differences

Authors: *N. KHODAYARI[1], H. EGETH[1,2,3], S. M. COURTNEY[1,2];
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Abstract: In a previous study, we used a visual search task with targets defined by shape and with color singleton distractors present on some trials. In this statistical learning task, using an individual differences approach, we found evidence that target enhancement and distractor suppression utilize separable mechanisms. Reaction time (RT) differences for trials in which targets appeared in frequent versus infrequent locations ("target enhancement") did not correlate across individuals with RT differences for trials in which distractors appeared in frequent versus infrequent locations ("distractor suppression"). This lack of correlation was specific to the comparison of enhancement versus suppression. To make strong claims from such results, however, requires internally-reliable individual differences measures. In that previous experiment, individual differences in target enhancement were reliable and stable across task contexts. In contrast, reliability of individual differences in distractor suppression was sensitive to context and reliable only when search strategy was constrained. The goal of the present study was to 1) attempt to replicate these prior findings, further supporting the hypothesis that enhancement and suppression utilize separable mechanisms, and 2) improve reliability of our individual differences measures by relaxing performance exclusion criteria, to enable a greater range of individual differences in performance. Using the same task, we replicated the findings that individual differences in target enhancement were reliable and that there was no correlation between target enhancement and distractor suppression. Bayesian results also replicated, with anecdotal evidence in favor of the null for the correlation between target enhancement and distractor suppression. Unlike for this task in the previous study, however, individual differences in distractor suppression were not reliable, despite a greater range of performance in this participant cohort. This lack of internal reliability for suppression limits our ability to draw conclusions from our subsequent lack of correlation between enhancement and suppression. The difference in internal reliability for enhancement versus suppression, however, is consistent with the hypothesis of separable mechanisms.
In conclusion, we found additional evidence for the hypothesis that enhancement and suppression utilize separate mechanisms. Additional research will be needed, however, to identify factors in experimental design and participant characteristics that affect suppression reliability.

Disclosures: **N. Khodayari:** None. **H. Egeth:** None. **S.M. Courtney:** None.

Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.05/SS6

Topic: H.01. Attention

Title: Influence of smartphone presence on event-related potential and spectral power indices of attention

Authors: C. A. GLAND, C. E. KIDWELL, E. L. KERR, B. E. STEWART, C. G. SHIPMAN, E. M. WALKER, M. N. BLIEK, ***D. S. LELAND**;
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Abstract: Previous research suggests that the mere presence of one's smartphone can negatively impact behavioral performance on an attention-related task, particularly among those more reliant on their phones. Our research, in progress, investigates whether simply having one's smartphone present and visible (although shut off) influences attention-related EEG activity. We administered a survey of smartphone use patterns to undergraduate college students and recruited EEG participants from among those scoring above the median on at least 2 of 3 phone-related survey measures (hours of usage, phone reliance, fear of being unable to use one's phone). During EEG recording, subjects first perform an oddball task, which requires attention and responses to rare target stimuli ("oddballs") among many frequent but task-irrelevant stimuli ("standards"). The P3, a late component of the event-related potential, is typically larger to oddballs (which receive more attention) than standards; we predict an attenuation of this oddball effect when one's smartphone is in peripheral view, as compared to a non-phone control object (tile). After the oddball task, subjects passively view, one at a time, their centrally-presented smartphone and the control object, and we quantify average EEG power in the beta range (13-32 Hz) at left and right anterior and posterior scalp sites. Prior research shows that current cigarette smokers show increased beta activity while passively viewing a cigarette as compared to a control stimulus (pen); similarly, we predict greater beta power in the phone condition. Preliminary findings (n=9) suggest that beta power is indeed greater while viewing one's smartphone versus the control stimulus, but not that the smartphone influences the P3 oddball effect.

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Poster

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Topic: H.01. Attention

Support: NSF Grant 2120539
Searle Scholars Program

Title: External sampling of the environment during selective attention and internal sampling of working memory representations compete for neural resources

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Abstract: While selective attention (SA) is often characterized as the neural mechanisms through which behaviorally relevant information is prioritized and preferentially sampled from the external environment, recent work has tested how these same neural mechanisms contribute to maintaining and sampling internal representations during working memory (WM). Many studies have used dual-task paradigms to investigate the interaction between SA and WM, but the extent to which these processes engage the same neural resources remains an open question. Here, we are using human EEG to test how the brain coordinates external and internal sampling of behaviorally relevant information under conditions that require subjects to deploy them either separately or simultaneously. We focus on how functional specialization (i.e., unique neural resources) and temporal coordination of overlapping neural resources might facilitate such multitasking. Our preliminary results support previous findings by showing topographical specificity of alpha-band amplitude (8-12 Hz) to the spatial location of to-be-remembered items or the spatial location of upcoming targets. Changes in the distribution of alpha-band amplitude across the scalp have been repeatedly shown to reflect the selection of behaviorally relevant information and the suppression of potentially distracting information. Here, we observed that changes in the distribution of alpha amplitude during a memory delay (i.e. during an internal sampling task) differs from that during a cue-target delay (i.e., during an external sampling task). During a memory delay there is primarily an increase in alpha amplitude relative to baseline, ipsilateral to the locus of internal sampling, whereas during a cue-target delay there is primarily a decrease in alpha amplitude, contralateral to the locus of external sampling. During trials that required simultaneous internal and external sampling, we observed that the contralateral decrease in alpha amplitude was lower than that observed during external-only trials, yet the ipsilateral increase in alpha amplitude was the same as that observed during internal-only trials. This fits with behavioral performance, where we observed reduced performance in the external sampling task on trials that required both internal and external sampling, but no difference in performance for the internal sampling task. These preliminary results support the notion that internal and external sampling compete for control of neural resources. We will next test whether this competition for neural resources results in the temporal coordination of internal and external sampling.

Disclosures: P.J. Cavanah: None. **I.C. Fiebelkorn:** None.

Poster

PSTR564. Attention II

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Program #/Poster #: PSTR564.07/SS8

Topic: H.01. Attention

Support: NIH grant R01EY033329
NINDS grant R25NS112130

Title: Decoding Attended and Unattended Letters from Neural Signals

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Abstract: This study applied multivariate pattern analysis (decoding) to ERP data to investigate the impact of attention on perception and working memory. Previous research has demonstrated that the identity of an object being perceived or held in working memory can be decoded from the distribution of voltage over the scalp. Building on this, our study aimed to examine how attention influences the processing of simple letters when the visual system is overloaded by a fast stimulation rate. We predicted that the brain would extract letter identity more effectively for attended letters, leading to greater decoding accuracy for attended letters than for unattended letters. We recorded scalp EEG signals from 24 young adults who were presented with long sequences of foveal letters at a rapid rate (10 letters per second). Each letter was presented in one of 4 colors, and we used 8 letter identities (G, J, T, S, M, K, P, or Z). One color was designated the target for a given block of trials, and participants were instructed to focus on target letters and press a button when the current target letter matched the identity of the previous target letter. We used support vector machines with error-correcting-output codes to decode the identity of each letter at each moment in time following letter onset on the basis of the distribution of voltage over the scalp at that time point. Despite the very fast stimulation rate, we found that we could decode which of the 8 letters was presented. Decoding accuracy was greater when the letter appeared in the attended color than when it appeared in an unattended color, indicating enhanced information extraction. Moreover, decoding performance was more sustained for attended-color letters, indicating enhanced storage in working memory. Whereas previous ERP studies showed that the neural response is larger for attended than for unattended stimuli, the present results indicate that attention also enhances the amount of information extracted about shape identity.

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Poster

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Program #/Poster #: PSTR564.08/SS9

Topic: H.01. Attention

Support: ONR Grant N0014-22-1-2123

Title: Handoff between cerebral hemispheres during attentional tracking with different loads

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Abstract: Human attentional tracking performance is better when targets are distributed across both visual hemifields, compared to when confined to one hemifield. This suggests that each

cerebral hemisphere has separate limited attentional resources. These attentional resources should account for the transfer of information between brain hemispheres, such as when a tracked object moves between visual hemifields. With electroencephalography (EEG), we tested if the number of tracked objects (i.e., tracking load) modulates this handoff. While holding central fixation, participants tracked one or two moving targets among several moving distractors. Targets either moved within or between visual hemifields. We measured the contralateral delay activity (CDA) - the difference in EEG activity recorded from contralateral and ipsilateral electrodes in occipitoparietal regions. The CDA was sensitive to tracking load (larger amplitude for two targets than for one target) and inverted in polarity when objects shifted between visual hemifields. This inversion reflected the transfer of attention between brain hemispheres. Our results suggest that the handoff between hemispheres differed for one versus two targets crossing vertical midline. The rate of CDA inversion was slower for one target compared to two targets, suggesting both hemispheres represented the moving object for longer during handoff of one target compared to the handoff of two moving targets. This supports the idea that attentional tracking resources are hemisphere specific - hemisphere-specific resources are limited when handing off a higher load, resulting in rapid transfer between the hemispheres.

Disclosures: **P. Styrkowiec:** None. **E.K. Vogel:** None.

Poster

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Topic: H.01. Attention

Support: DFG Grant AL 2408/1-1

Title: Hemodynamic and electrophysiological connectivity of attentional-control brain networks underlie individual differences in selective listening behavior

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[1]Univ. of Lübeck, Lübeck, Germany; [2]Univ. of Luebeck, Univ. of Luebeck, Luebeck, Germany

Abstract: Complex auditory scenes come with perceptual challenges that render a listener slower and more uncertain in their perceptual decisions. Here, using a selective pitch discrimination task, we aim to explain such behaviors by the dynamics of cortical networks derived from functional magnetic resonance imaging (fMRI) and source-localized electroencephalography (EEG). During the task, a spatial cue prompted the listeners to attend to one of two concurrent tone streams and to judge the pitch difference (increase/decrease) in the target stream. Listeners additionally reported their confidence in own decisions. Individual titration of the pitch difference throughout the task retained the listeners' accuracy at ~70% but yielded considerable inter-individual variability in response speed and confidence. The fMRI study (N=40, young listeners) allowed us to characterize a significant modulation of

interconnectivity between cinguloopercular network and each auditory and dorsal attention network, the degree of which were predictive of individual differences in response speed and confidence. The EEG study (N=33/35, young/older listeners) revealed a significant increase in frontoparietal connectivity within low-beta frequency range (16-24 Hz) during processing of the auditory spatial cue, the degree of which was significantly stronger in younger than older listeners. Our findings support the functional significance of large-scale brain networks beyond auditory cortex in attentional control during selective listening. The connectivity dynamics of these networks can account for interindividual differences in objective and subjective measures of listening behavior.

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Poster

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Program #/Poster #: PSTR564.10/SS11

Topic: H.01. Attention

Support: NIH Grant MH112558

Title: Effects of Emotional Distractors on the Processing of Motion Stimuli

Authors: ***C. XIONG**[1], K. BO[2], N. M. PETRO[1], A. KEIL[3], M. DING[3];
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[3]Univ. Florida, Univ. Florida, Gainesville, FL

Abstract: The human visual system has limited processing capacity. Both behaviorally relevant information and behaviorally irrelevant information compete for representations in this capacity-limited system. It has been further suggested that distractors that are motivationally significant have stronger distracting influence. We examined the neural underpinnings of these ideas by recording fMRI from participants who detected instances of coherent motion in a random dot kinematogram (RDK) overlayed on three categories of affective images from the International Affective Picture System (IAPS): pleasant (erotic couples), neutral (workplace people), and unpleasant (bodily mutilations). Behaviorally, we found that there was no significant difference in the subject's ability to detect coherent motion for the three classes of distractors. Applying machine learning (support vector machine) to BOLD responses in two important areas of visual motion processing: ventral visual cortex (VVC) and MT cortex, we found that the decoding accuracy of both pleasant-vs-neutral distractors and unpleasant-vs-neutral distractors was above chance level in VVC and MT and importantly, the distractor decoding accuracy in VVC and MT was negatively associated with the subject's ability to detect coherent motion. These results suggest that emotional and neutral distractors have similar influences on the processing of the attended motion stimuli and stronger neural representations of distractors in the motion-processing areas of the brain reduce the ability to detect coherent motion in the RDK task.

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Poster

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Program #/Poster #: PSTR564.11/SS12

Topic: H.01. Attention

Title: Does a brief and simple 100% oxygen treatment improve sustained attention levels?

Authors: J. RODRIGUE[1], Z. WANG[2], G. SPIELMANN[1], B. A. IRVING[1], F. GREENWAY[3], *M. DALECKI[4];
[1]Louisiana State Univ., Baton Rouge, LA; [2]Mayo Clin., Rochester, MN; [3]Pennington Biomed. Res. Ctr., Baton Rouge, LA; [4]German Univ. of Hlth. and Sports, Berlin, Germany

Abstract: Previous work in our lab showed that a 100% oxygen treatment improves motor learning processes. A possible mechanism lies in enhanced cognitive functions, e.g., improved memory processes or increased processing speed. Sustained attention is dependent on such processes and critical for learning and memory formation, however, the effects of an oxygen treatment on sustained attention levels is unknown. Therefore, the current study aims to identify whether a 100% oxygen treatment improves sustained attention levels. 28 healthy young adults performed a computerized D2 sustained attention task, randomly assigned to an oxygen group (N=14, M=20.43 yrs.), or an air group (N=14, M=22.07 yrs.). The oxygen group received a 100% oxygen treatment and the air group regular air via a nasal cannula (3l/min). The D2 task comprised random sequences of nine letters (d and p), with a dash or dashes contained over or under the letters, for an overall of eight 30 second blocks of sequences. Participants were instructed to press the left arrow key when seeing a “d2 target” (letter d surrounded by two total dashes), and the right arrow key for a non-target character (‘not-d2’). Participants first completed a brief training for task familiarization and then performed the D2 task three times: A baseline test without gas flow, a treatment test with gas flow, and a post-treatment test without gas flow. Gas flow was given for 3-minutes after the baseline test and continued throughout the treatment test. Then, the gas treatment was shut off, and a post-treatment test was run after a 5min break, with no gas flow. Performance variables were concentration score (CS) (Correct D2 - Incorrect D2 responses), response time (RT), error percentage, and marked characters. Normalized data was calculated by subtracting treatment and post-test scores from baseline data. T-test´s revealed a small trend of a larger CS performance improvement in the oxygen group (+24 correct D2 responses compared to baseline) than in the air group (+18 correct D2 responses compared to baseline) during the treatment test ($p=0.10$), and a strong trend for more correctly marked D2 letters in the oxygen group ($p=0.06$). However, the trends did not sustain into the post-treatment test, for both variables ($p>0.10$). Our results suggest that a brief treatment with 100% oxygen tends to improve sustained attention levels during the gas flow period, potentially by improving decision-making processes. However, the effect did not translate into the post treatment phase,

potentially due to treatment duration or task nature. Future research could scrutinize whether a longer treatment would lead to stronger effects and better consolidation for this task.

Disclosures: J. Rodrigue: None. **Z. Wang:** None. **G. Spielmann:** None. **B.A. Irving:** None. **F. Greenway:** None. **M. Dalecki:** None.

Poster

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Program #/Poster #: PSTR564.12/SS13

Topic: H.01. Attention

Support: NIH/NIA Grant P01 AG055365

Title: Attention mobilization as a modulator of listening effort: Evidence from pupillometry

Authors: *M. A. JOHNS[1], R. C. CALLOWAY[1], L. P. DECRUY[1], I. KARUNATHILAKE[2], S. B. ANDERSON[3], J. Z. SIMON[2,4,1], S. E. KUCHINSKY[5];
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Abstract: Listening to speech in noise requires substantial mental effort, even among young normal-hearing adults. While numerous studies have examined listening effort in response to single words or sentences in noise, few have examined the trajectory of listening effort across longer stretches of speech or how sustained listening may interact with listeners' expectations about upcoming difficulties—both of which may be more representative of real-world listening. In the present study, 17 younger normal-hearing adults (aged 18.5 to 26.1 years) listened to 60-s long audiobook passages while pupil size was recorded. Changes in pupil size have been found to track changes in both attention mobilization—how listeners prepare their attention, reflected by baseline pupil size (BPS)—and effort allocation—how listeners deploy their cognitive resources, reflected by the task-evoked pupil response (TEPR). Participants were instructed to attend to one of two competing speakers, with the target speaker presented at either the same level (0 dB SNR) or a quieter level (-6 dB SNR) than the non-target speaker. Passages were blocked by SNR, and each passage was repeated three times in a row. Generalized additive mixed models were used to analyze the time course of the TEPR for each trial as a nonlinear function of BPS (the median pupil size during a 2-s neutral period before each passage), SNR, and presentation (first, second, or third). The model revealed a significant modulation of the TEPR by BPS that differed by both SNR and presentation. At lower BPS values, the TEPR was significantly larger in the harder -6 dB compared to the 0 dB SNR condition for all three presentations, suggesting that under-mobilization of attention led to increased listening effort despite individuals being able to anticipate the difficulty of the subsequent presentations. At intermediate BPS values, reflective of more optimal attention mobilization, differences between

the two SNR conditions were largely absent. Lastly, at higher BPS values, the TEPR was significantly reduced in the -6 dB compared to the 0 dB SNR condition for the second and third presentations, with a larger and more sustained difference in the second than in the third presentation. This suggests that, in an over-mobilized and more distractible state, listeners initially 'gave up' or under-allocated listening effort to the harder SNR condition before ultimately recovering and allocating listening effort more appropriately. Together, these findings suggest that how listening effort unfolds over time depends on the extent to which individuals successfully mobilize their attention in anticipation of difficult listening conditions.

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Poster

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Program #/Poster #: PSTR564.13/SS14

Topic: H.01. Attention

Title: Neurostructural Correlates of Attention Bias

Authors: *J. YANG[1], D. KASSEL[2], L. FANG[2], J. CARLSON[2];
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Abstract: The amount of information the brain can process is limited, and because of this limitation, the brain has developed mechanisms to prioritize certain stimuli over others, which is referred to as attention bias. An attention bias toward negative stimuli is a phenomenon observed in individuals with anxiety and is thought to contribute to the development and maintenance of anxiety-related disorders. Previous research has linked attention biases to gray matter volume (GMV) clusters in the anterior temporal lobe (ATL), dorsolateral prefrontal cortex (dlPFC), and anterior cingulate cortex (ACC). However, the existing MRI research mostly consists of reaction time measures of attention bias, which have shown low reliability. Eye-tracking measures show superior reliability but have not been used previously to assess the structural correlates of attention bias. Therefore, this study aimed to identify the brain structures associated with attention bias, measured by using the eye-tracking methodology. A sample of thirty-nine healthy adult participants (N_{Female} = 27; M_{Age} = 21.4; SD = 5.65) completed the Scrambled Sentence Task (SST) to measure attention biases to emotional information and had whole-brain structural T1-weighted MRIs within two weeks of completing the task. During the SST, participants were presented with a set of words and were instructed to create a grammatically correct sentence using those words. The eye-tracker was used to obtain the dwell time on each word, and attention bias was indexed by the difference in dwell time between negative and positive words. MRI images were then processed using CAT12. To assess the GMV correlates of attention bias behavior, a Voxel-Based Morphometry (VBM) approach was used. Our VBM multiple

regression model included attention bias scores, age, and total intracranial volume as covariates in SPM12. At a threshold of $p = 0.001$ and $k = 10$, we found that attention bias was positively correlated with GMV clusters in the ATL ($t = 5.04$, $p < 0.001$, $k = 235$), somatosensory cortex/posterior insula ($t = 4.14$, $p < 0.001$, $k = 284$), posterior cingulate cortex ($t = 3.99$, $p < 0.001$, $k = 139$), parahippocampal gyrus/ medial temporal lobe ($t = 3.85$, $p < 0.001$, $k = 62$), and the dlPFC ($t = 3.51$, $p < 0.001$, $k = 12$). These results replicate previous findings, which showed a correlation between attention bias and GMV in the dlPFC and ATL. However, our results did not show a correlation between GMV in the ACC and attention bias. We also provide novel evidence for additional structures being involved in attention bias. In sum, GMV in brain regions involved in sensory processing, affective regulation, and cognition is associated with attention bias behavior.

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Poster

PSTR564. Attention II

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Program #/Poster #: PSTR564.14/SS15

Topic: H.10. Human Learning and Cognition

Support: BBRF Young Investigator Grant

Title: Leveraging the successor representation for causal inference and efficient planning

Authors: *A. GUNGI[1], P. SEPULVEDA[2], K. IIGAYA[3];
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Abstract: The ability to discover causal structures in the world is a hallmark of human intelligence. However, the computation mechanisms enabling such causal structure learning remain elusive. Most existing computational models, such as standard model-based reinforcement learning, assume pre-determined world structures. While Bayesian inference models aim to learn complete, probabilistic world structures, they often lack biological plausibility due to their high computational demands. Moreover, these models fail to capture the human inclination to infer deterministic structures even in their absence[Steiner+Stewart 2016, Khaw et al., 2021].

Here, we propose a novel computational model that captures such deterministic structure learning. We leverage a variant of successor representations, a concept developed in reinforcement learning paradigms[Dayan,1993] that has been demonstrated to capture key aspects of navigation and planning[Momennejad et al., 2017, Geerts et al., 2022]. Our model dynamically constructs deterministic causal graphs based on successor representations, requiring minimum prior knowledge of the world's structure. We demonstrate how our model can provide a unified account for diverse behavioral and neural data, including phenomena like blocking in classical

conditioning, apparent discrepancies between neural signals and behavioral outputs, and the preference for determinism. Our study provides insights into biologically plausible computation for causal inference, which enables efficient planning and decision-making in complex environments with limited computational resources.

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Poster

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Topic: H.01. Attention

Support: Toyota Motor Europe Grant CI6585379

Title: The role of perceptual load in sustained attention

Authors: *L. BARNE, N. LAVIE;
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Abstract: Perceptual load is a well-established determinant of focused attention: increased perceptual load in a task (e.g. through manipulations of search set size or the demands on perceptual discrimination) has been shown to result in attentional modulations of neural signals related to attended versus unattended processing (e.g. Gutteling et al. (2022) for a recent demonstration). A separate body of research has examined the neural correlates of sustained attention (e.g. increased alpha with time-on-task (Pershin et al., 2023) across all cortical regions). However sustained attention paradigms have typically involved only low levels of perceptual load so far, such as monitoring for feature-based target (e.g. Esterman et al., 2013). Here we used EEG to investigate the neural markers of the effects of perceptual load on sustained attention. Participants (n=34) performed a gradual continuous performance (GradCPT) task, monitoring eight-minutes long streams of natural scenes of gradual onsets (through morphing) for infrequent (10%) target scenes (mountains among cities). Eight GradCPT streams of either high or low perceptual load (with or without overlaid salt and pepper noise masks throughout a stream, respectively) were run. Response variability increased, and discrimination sensitivity decreased with time on task in each stream, accompanied by increase in alpha power, replicating previous results. Importantly, by fitting 1/f aperiodic component over the power spectrum density function, we found an interaction of perceptual load and time on task on the exponent of the aperiodic signal in right-lateralised channels, which reflected a steeper 1/f slope with time on task in high (vs. low) perceptual load. Together with the behavioural findings of a greater decrement of discrimination sensitivity over time in high (vs. low) perceptual load, these findings support an account of perceptual load effect on sustained attention in terms of a change in the Excitation/Inhibition neural balance. Specifically, on this account the reduced ratio of excitation over inhibition in a right lateralised attention network, is mediating the greater time-

related deterioration of sustained attention, and hence detection ability, in tasks of high perceptual load. Our account is consistent with findings that reduced conscious awareness is also associated with reduced E/I ratio (Gao et al., 2017).

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Poster

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Topic: H.01. Attention

Support: STI2030-Major Projects (2021ZD0203803)
National Natural Science Foundation of China (32200840)

Title: Shared and distinct neural signatures of feature and spatial attention

Authors: *A. YANG[1,2], L. ZHOU[2], K. ZHOU[2];
[1]Univ. of Chicago, Chicago, IL; [2]Fac. of Psychology, Beijing Normal Univ., Beijing, China

Abstract: The debate regarding whether feature attention (FA) and spatial attention (SA) share a common neural mechanism continues to persist. Previous studies have identified frontoparietal regions consistently activated during different visual attention tasks. However, these studies had limited sample sizes and methodological constraints inherent in univariate analysis. To address these limitations, the current study employed a machine-learning-based multivariate analysis approach, leveraging a large sample size of 235 participants. This enabled a comprehensive investigation into the shared and distinct neural signatures associated with FA and SA. Our findings revealed that both neural signatures exhibited the ability to predict each other, although inter-task prediction performance was notably weaker compared to intra-task prediction. The findings lend support to the presence of both shared and distinct neural mechanisms underlying FA and SA. Notably, the frontoparietal network emerged as the most predictive network for FA, while the visual network played a primary role in SA. Moreover, we observed overlapping areas between the two attention tasks. Specifically, through cluster-level analysis with single-cluster prediction, we identified four regions located at the temporal/occipital lobes (i.e., the left Lingual Gyrus (LG), right Occipital Pole (OcP), right Occipital Fusiform Gyrus (OFG), and left Temporal Occipital Fusiform Cortex (TOF)) that exhibited similar patterns on both FA and SA. Further analysis with 'virtual lesion' techniques revealed that no single cluster was indispensable for predicting either FA or SA, suggesting a distributed neural network responsible for supporting both attention tasks. Additional voxel-level analysis comparing the neural signature patterns further corroborated the distributed nature of FA and SA. In sum, by utilizing a machine-learning-based multivariate analysis approach with a large sample size, our study provides comprehensive evidence regarding the shared and distinct neural mechanisms

underlying FA and SA. This approach overcomes previous limitations and sheds new light on the intricate nature of attentional processes.

Disclosures: **A. Yang:** None. **L. Zhou:** None. **K. Zhou:** None.

Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.17/SS18

Topic: H.01. Attention

Title: Immersion in nature enhances aspects of human attention

Authors: *A. S. MCDONNELL, D. L. STRAYER;
Psychology, Univ. of Utah, Salt Lake City, UT

Abstract: The United Nations projects that by 2050, 70% of the world's population will reside in urban centers. While urbanization provides access to education and health care, some characteristics of these environments (e.g., pollution, artificial light, stress, and heightened attentional demands) negatively impact our health and cognition. Exposure to nature is thought to buffer against some of the detriments associated with urban environments. Attention Restoration Theory (Kaplan, 1995) posits that our modern, urban environments place high demand on executive attention, which is limited in capacity and can be depleted over time, leading to impairments in performance. Natural environments, on the other hand, provide relief from this demand by allowing our attention networks to rest and be replenished. While these claims have been evaluated with self-report and behavioral measures, there is limited understanding of the neural mechanisms underlying the attentional benefits of exposure to nature. The present experiment used scalp electroencephalography (EEG) to fill this gap in the literature. Because attention is multifaceted, we explored three attention networks in the brain—alerting, orienting, and executive control—using the Attention Network Task (Fan et al., 2002). In this randomized control trial, human participants (N=92) were assigned to walk for 40-minutes in either nature or an urban environment. We recorded behavioral and EEG measures of performance on the Attention Network Task, as well as resting brain activity, before and after the walk. Linear mixed-effects models and likelihood ratio tests revealed a significant interaction between Time (pre-walk vs. post-walk) and Condition (nature vs. urban) on amplitude of the error-related negativity (ERN)—an event-related potential that indexes executive control—such that ERN amplitude increased after the nature walk ($\chi^2(1)=4.47$, p=.034; Cohen's d=0.41) but not after the urban walk. Immersion in nature also dampened frontal theta power when at rest, a frontally-distributed theta (4-8 Hz) oscillation in the brain that increases with attentional demand and fatigue ($\chi^2(1)=4.06$, p=.044; Cohen's d=-0.25). Consistent with Attention Restoration Theory, we conclude that immersion in nature enhances executive control and buffers against the attentional demands associated with urban environments. This work demonstrates the power of utilizing EEG to uncover the benefits of nature on human attention and provides a framework for

future work to explore which *types* of nature enhance attention (e.g., desert, mountains, forest) and how *much* time in nature is needed to do so (e.g., minutes, hours, days).

Disclosures: A.S. McDonnell: None. **D.L. Strayer:** None.

Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.18/SS19

Topic: H.10. Human Learning and Cognition

Support: National Science and Technology Innovation STI2030-Major Project (2021ZD0204103 to H.L.)
National Natural Science Foundation of China (31930052 to H.L.)

Title: Compressive learning: brief pre-learning of essential paths facilitates scale-free knowledge network learning in humans

Authors: X. REN[1,2,6,7], ***M. WANG**[1,2,3], T. QIN[4], A. LI[4,5], H. LUO[1,2,3];
[1]Sch. of Psychological and Cognitive Sci., [2]PKU-IDG/McGovern Inst. for Brain Res., [3]Beijing Key Lab. of Behavior and Mental Hlth., [4]Ctr. for Systems and Control, Col. of Engin., [5]Ctr. for Multi-Agent Research, Inst. for Artificial Intelligence, Peking Univ., Beijing, China; [6]Max Planck Res. Group NeuroCode, Max Planck Inst. for Human Develop., Berlin, Germany; [7]Inst. of Psychology, Univ. Hamburg, Hamburg, Germany

Abstract: The essence of knowledge learning is inferring hidden relationships (edges) from fragmented data (nodes). Meanwhile, this process typically relies on random exploration within knowledge networks and is cognitively demanding and inefficient. Here, motivated by network theory, we examined whether pre-learning of certain paths that prioritize the core network structure could aid subsequent network learning. Human subjects performed a probabilistic sequential prediction task on 16-image sequences. Unbeknownst to them, the transitional link between images comprises a scale-free network, a representative type of knowledge system. Scale-free networks have inhomogeneous connections between nodes, wherein hub and leaf nodes possess more and fewer connections (degrees of freedom), respectively. We designed three pre-learning paths (HubToLeaf, LeafToHub, RandomWalk) and applied them to three subject groups (N = 80, 80, 78), respectively. Specifically, the HubToLeaf path visits Hub images first, while the LeafToHub path visits Leaf images first, and the RandomWalk path follows a random walk profile. After 200-trial pre-learning, the three groups performed network learning on the same 800 random-walk trials, and their learning performances were compared. Interestingly, although the three groups had comparable learning performance at the beginning, the HubToLeaf group gradually outperformed the other two groups. This supports the modulation effect of pre-learning paths on network learning. Furthermore, we recruited two new groups (N =20 for each) for HubToLeaf and LeafToHub paths respectively and recorded their

magnetoencephalography (MEG) activities during network learning. Representational dissimilarity analysis revealed that the HubToLeaf group displayed enhanced neural representation of network structure compared to the LeafToHub group at a later time range. Together, we provide converging behavioral and neural evidence supporting that pre-learning of paths that capture the core network structure could facilitate network learning and enhance representations of knowledge structures in the brain.

Disclosures: **X. Ren:** None. **M. Wang:** None. **T. Qin:** None. **A. Li:** None. **H. Luo:** None.

Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.19/SS20

Topic: H.01. Attention

Support: NIH grant MH117991

Title: Visual Spatial Attention Both Enhances and Suppresses Neuronal Responses in Visual Cortex

Authors: *Q. YANG[1], S. MEYYAPPAN[2], G. R. MANGUN[2], M. DING[1];
[1]Univ. of Florida, Gainesville, FL; [2]Univ. of California Davis, Yolo County, FL

Abstract: Past fMRI studies have shown that lateralized visual stimuli evoke a neuronal pattern across the visual cortex that resembles a Mexican hat, where positive activation in the center is flanked by negative activation (deactivation) in the surround. We examined (1) how visual spatial attention modulated the Mexican hat activation profile, and (2) whether attended information is better encoded in the enhanced center or the suppressed surround. The participant was cued in the beginning of each trial to deploy covert attention to the left or the right visual field. Following a random time delay, a vertical grating was presented in one of the two visual fields, and the participant was asked to discriminate the frequency of the grating (low vs high) in the attended visual field and ignore the grating in the unattended visual field. Analyzing the fMRI data recorded from the experiment, we found that relative to the unattended stimulus, the attended stimulus evoked both stronger center activation and stronger surround deactivation. Applying MVPA analysis to decode high versus low spatial frequencies of the attended stimulus, we found that while the decoding accuracy is above chance in both the activated center and the deactivated surround, it is higher in the activated center than the deactivated surround. These results suggest that visual spatial attention both enhances and suppresses neural activity in the visual cortex by accentuating the Mexican hat response profile such that attended information is more efficiently represented in the activated center than the deactivated surround.

Disclosures: **Q. Yang:** None. **S. Meyyappan:** None. **G.R. Mangun:** None. **M. Ding:** None.

Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.20/SS21

Topic: H.01. Attention

Title: Single neuron signatures of spatial attention in the human lateral occipital complex

Authors: *J. GARCIA RAMIREZ, M. VANHOYLAND, P. JANSSEN, T. THEYS;
KU Leuven, Leuven, Belgium

Abstract: Selective visual attention plays a crucial role in helping us making sense of busy scenes by filtering out distractors. Studies in animal models have demonstrated that when two stimuli are present inside the receptive field (RF) of one higher-level visual neuron they engage in a competition. When attention is focused on one of those stimuli, the neuron responds as if only the attended stimulus was presented in isolation. This phenomenon has been modeled as an amplification of the synapses that carry the responses of the attended stimulus, which might be implemented by selective neuronal gamma-band synchronization. However, this model of attention remains untested in the human higher-order visual cortex. We had the unique opportunity to record intracortical neuronal activity (96-electrodes Utah arrays; 244 visually responsive multi-unit sites, $p<0.01$) from the lateral occipital complex (LOC) in three epilepsy surgery patients during a spatial attention task. In this task, we presented two images of objects simultaneously, and cued subjects to covertly attend to a particular location in the visual field to signal the presence of a specific object with a button press. The RFs of the recorded populations covered either the two stimuli (In/In configuration, array 1 and 2) or only one (In/Out configuration, array 3), which allowed us to compare the neural correlates of attention in the presence or absence of competing stimuli inside the RF. We employed linear decoding techniques to decode (1) the identity of the object at the attended location and (2) the locus of attention. We found significant (permutation tests, $p<0.005$) information about the object at the attended location only for the In/In configuration. In contrast, the In/Out configuration only yielded significant (permutation tests, $p<0.005$) decoding of the object in the RFs of the recorded population, irrespective of the focus of attention. Moreover, the locus of attention could only be reliable decoded (permutation tests, $p<0.005$) for the arrays in the In/In configuration. Overall, our results are in line with previous single-cell studies on attention that posit that the main role of attention is to disambiguate competition between stimuli inside the RF of visual neurons. Secondly, we computed spike-field coherence (SFC), which measures phase synchronization between spikes and LFP as a function of frequency. In the In/Out configuration, we found an increased SFC in the gamma band when attention was directed towards the RF compared to when it was directed outside the RF. This result aligns with the idea of gamma-synchronization as facilitating the processing of the attended stimulus for downstream areas.

Disclosures: J. garcia ramirez: None. **M. Vanhoyland:** None. **P. Janssen:** None. **T. Theys:** None.

Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.21/SS22

Topic: H.10. Human Learning and Cognition

Support: Gallaudet University Seed Fund

Title: Impact of American Sign Language Fluency on Response Time and Accuracy in Visual-Spatial Tasks

Authors: *M. SCHWENK[1], L. C. QUANDT[2];
[1]Educational Neurosci., [2]Gallaudet Univ., Gallaudet Univ., Washington, DC

Abstract: Visual spatial perception plays a vital role in cognitive processes like problem-solving, navigation, and spatial understanding. Deaf and Hard of Hearing (DHH) people, who rely heavily on visual cues, may exhibit enhanced visual spatial abilities. While studies have examined visual spatial abilities in DHH individuals, further investigation is required to differentiate effects of hearing status from sign language experience. This study aimed to compare the performance of DHH and Hearing American Sign Language (ASL) users on visual spatial tasks, specifically the Block Design (BD) Subtest. Two hypotheses were tested: (H1) DHH will outperform hearing ASL signers in 4-block and 9-block tasks in response time and accuracy, and (H2) DHH individuals with early ASL acquisition will exhibit faster, more accurate responses across BD trials.The ASL Comprehension Test (ASLCT) and the BD were administered to DHH ASL signers (n=28) and Hearing ASL signers (n=12). To examine how hearing status, ASL fluency, and age at acquisition (AoA) influence response time and accuracy, and how ASL fluency affects BD performance, ANOVAs and correlations were conducted.Supporting H1, ANOVA results revealed being DHH ($p<.001$), having early AoA ($p<.001$), and higher ASLCT score ($p<.001$) resulted in higher scores on the 4-block task, as well as significant main effects of AoA ($p<.001$) and a hearing status by Early AoA interaction on the 9-block task ($p=.011$) for DHH participants. Correlation analysis supported H2, with a positive correlation between BD performance and ASL fluency ($p.<001$). Participants who are DHH, early AoA, and fluent in ASL, showed faster, more accurate responses than their late AoA peers showing a trend towards significance in 4 blocks ($p = .066$) and significance in the 9 block questions ($p < .001$), indicating higher BD scores on average. This finding highlights the significance of early exposure to ASL and its long-term effects on fluid reasoning skills during complex visual-spatial tasks.DHH individuals with higher ASL fluency exhibit superior performance on the BD task, characterized by faster response times and higher accuracy. To support cognitive skill development, it is crucial to provide early exposure to ASL and encourage DHH children to become fluent signers.

Disclosures: M. Schwenk: None. **L.C. Quandt:** None.

Poster

PSTR564. Attention II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR564.22/SS23

Topic: H.01. Attention

Title: Investigating the Impact of Meditation on Neural Processes: Insights from EEG and Sleep Analysis

Authors: D. CHAO, S. GUDISAY, U. AVADHANI, S. PASALA, S. CHAKRABORTY, A. CHOWDHURY, ***S. JAHANIKIA**;
The Aspiring Scholars Directed Res. Program, Fremont, CA

Abstract: The research on the effects of meditation on the brain using electroencephalography (EEG) has garnered considerable attention in recent years, building upon decades of prior investigation. Despite this extensive body of work, the precise influence of meditation on neural processes within the body remains elusive. In this study, we sought to address this knowledge gap by leveraging OpenNeuro, an open web resource providing access to EEG meditation datasets. Our objective was to gain a comprehensive understanding of how individuals respond to different meditation practices, specifically focusing on mantra repetition meditation and open awareness thinking. To analyze the collected data, we employed well-established software tools such as MatLab and EEGLab. Sleep data, an integral component of our study, was categorized into distinct stages. We utilized waking state brain frequencies as a reference point, which are characterized by high-frequency beta activity 15-60 Hz. Stage 1 non-REM sleep was identified by a decrease in EEG frequency, manifested as theta waves 4-8 Hz. As sleep progressed to Stage IV, we observed the emergence of slow delta waves 0.5-2 Hz. Subsequently, individuals transitioned into the rapid eye movement (REM) sleep phase, during which brain activity exhibited high-frequency patterns akin to those observed during wakefulness. Our analysis specifically focused on deciphering the sleep non-REM pattern associated with open awareness thinking. To discern and delineate the sleep stages present within the meditation data, we employed the SSAVE (Sleep Cycle and Spectrogram Analysis and Visualization for Electroencephalography) python algorithm. Throughout our data processing and preliminary analysis, we made intriguing observations. Notably, our findings indicated a significant influence of NREM sleep patterns, characterized by delta waves 0.5-2 Hz, on individuals practicing open awareness thinking, a trait associated with Tibetan Buddhist meditation.As we advance our investigation, we aim to unravel the intricate relationship between meditation and neural processes, particularly within the context of sleep. By shedding light on the interplay between meditation, brain activity, and sleep patterns, we aspire to deepen our understanding of the direct effects of meditation, while exploring its potential benefits for cognitive functioning, stress reduction, and overall physical wellbeing.

Disclosures: D. Chao: None. **S. Gudisay:** None. **U. Avadhani:** None. **S. Pasala:** None. **S. Chakraborty:** None. **A. Chowdhury:** None. **S. Jahanikia:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR565.01/SS24

Topic: H.05. Working Memory

Support: NIH Grant R01 MH123679
NIH Grant R01 MH124998-01A1

Title: Bridging gaps between primate and rodent studies: developing a mouse paradigm that matches primate working-memory tasks

Authors: *I. IBARRA-LECUE[1,2], A. Z. HARRIS[1,2], S. HAEGENS[3,1,2];
[1]Columbia Univ., New York, NY; [2]New York State Psychiatric Inst., New York, NY; [3]Ctr. for Cognitive Neuroimaging, Donders Inst. For Brain, Cognition & Behaviour, Nijmegen, Netherlands

Abstract: In daily life, we often make decisions based on sensory input from the environment. However, how the brain sets up the functional neural architecture to support these decisions is currently unknown. Working memory (WM), the capacity of transiently holding and/or manipulating information that is no longer present in the environment, has received substantial interest. However, there are big discrepancies in the design of WM paradigms, especially in the time-scales, between rodent and primate studies, which make comparisons between studies in different species challenging. Here, we designed a task that relies on the ability of mice to maintain sensory information over a few seconds. The approach consists of multiple training stages, where mice first consecutively learned to associate two distinct tones (1 kHz and 11 kHz, 1.5 s) to the retrieval of rewards (saccharin water, 0.05%) in the two opposite arms of a T-maze. After mice met learning criterion (discrimination index > 0.7 / d' > 1.5 for 2 consecutive sessions; 8±0.8 sessions per tone training; approx. 65 trials/session; n=6, 3 females; C57BL/6 strain), they were trained on the two tones in an interleaved, randomized manner (i.e., tones were not predictable). After meeting criterion (discrimination index > 0.6 / d' > 1 for 2 consecutive sessions; 7.8±0.7 sessions), animals learned to self-initiate each trial by poking at a non-rewarded third location (discrimination index > 0.6 / d' > 1 for 2 consecutive sessions; 5.2±0.8 sessions) before reaching the final test period, where an increasing delay (0.5 to 3 s) was added between the tone offset and the decision period. As a group, animals were able to reach stable, above-chance performance after 11 sessions (discrimination index = 0.25±0.1 / d' = 0.5±0.1). These behavioral results open new possibilities of neuronal recordings in freely moving mice to assess key brain circuitry involved in WM in a more inter-species comparable way.

Disclosures: I. Ibarra-Lecue: None. **A.Z. Harris:** None. **S. Haegens:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR565.02/SS25

Topic: H.05. Working Memory

Title: Disentangling visuospatial and motor working memory

Authors: *H. K. HILLMAN, A. D. FORRENCE, T. N. MCCLURE, S. D. MCDOUGLE; Psychology, Yale Univ., New Haven, CT

Abstract: Working memory (WM) research has largely focused on systems like language and vision, but little is known about how motor information is maintained and manipulated in WM. Here, we present research on how motor information (e.g., kinematics of recent movements) is encoded and maintained in working memory. To that end, we employ a nonvisual reaching paradigm, in which participants passively encoded movement trajectories and then actively recalled those movements, with either the same or different arm, allowing us to dissociate between information tied to the encoding arm (effector-specific) and abstracted knowledge which could inform recall by the non-encoding arm (effector-independent). We build on our previous work using this design to test motor working memory under various load, delay, and interference conditions, with a focus on the intersection of visuospatial WM and motor WM. We found evidence that effector-specific representations are corruptible by additional movements, even when they are irrelevant to the task, while the effector-independent representation appears relatively robust to sensorimotor interference. To examine the relationship between motor and visual WM, we interleaved our non-visual motor WM task with a visuospatial WM task. Our results indicate that an increased visuospatial information load held in working memory does not have a significant interaction with hand-switch condition, suggesting that motor WM codes are distinct from visuospatial WM. This may have important implications concerning domain specificity and the neural correlates of motor working memory.

Disclosures: H.K. Hillman: None. **A.D. Forrence:** None. **T.N. McClure:** None. **S.D. McDougle:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR565.03/TT1

Topic: H.05. Working Memory

Support: NHMRC Ideas Grant

Title: The effect of betacellulin knockdown on the trial unique non-matching to location (TUNL) and pairwise discrimination (PD) cognitive tasks

Authors: *S. RAJU, S. SUNDRAM, R. A. HILL;
Psychiatry, Monash Univ., Clayton, Australia

Abstract: Schizophrenia is a severely debilitating psychiatric disorder characterised by psychotic symptoms, mood-related symptoms, and cognitive impairment. Unfortunately, current antipsychotic treatments provide symptomatic relief for psychosis-related symptoms and limited efficacy in treating mood-related symptoms and cognitive deficits. Our laboratory found that a protein called betacellulin (BTC) is reduced in the serum and dorsal-lateral-prefrontal-cortex of people with schizophrenia and that low expression was associated with poorer cognitive outcomes. However, it is unclear if reduced BTC is causative to altered cognitive function or a result of the illness. Our aim was to understand the role of BTC within the specific cognitive domains using a BTC knockout(KO) mouse model. We hypothesised that BTC KO mice display disruptions in working memory and cognitive flexibility compared to wildtype (WT) controls. To assess spatial working memory and cognitive flexibility though reversal learning, mice were tested in the Trial-Unique-Non-matching-to-location (TUNL) and the Paired-discrimination (PD) touchscreen tasks. During TUNL, mice were trained to identify a non-matching square from a familiar square after a short delay, with % accuracy measured. During PD, mice were trained to differentiate a correct image from an incorrect image and days taken to acquire the task was recorded. The rule was then switched, and mice relearned the new correct image. Days taken to successfully reverse the rule was recorded. Male and female BTC KO and WT mice (n=9-12/group) were assessed. Results showed that BTC KOs take longer to complete the TUNL task in the early training phase ($p = 0.03$). During the test phase male BTC KOs showed significantly reduced % accuracy compared to WTs at the easiest delay of 0 seconds ($p=0.01$). Similarly, female BTC KOs showed reduced accuracy compared to WT but only at the most difficult delay of 3 seconds. During the acquisition phase of PD, both sexes of BTC KOs took a similar amount of time to acquire the task as WTs. However, male BTC KOs showed significantly increased latency when selecting correct responses compared to WTs ($p = 0.03$). During reversal, only female BTC KOs were quicker to reverse the rule than WTs ($p = 0.03$). BTC KO males were significantly faster than WTs in selecting the incorrect images ($p = 0.03$), suggestive of impulsive-like behaviour. Overall, the data provides impactful new insight into the contribution of BTC to working memory, reversal learning and impulsivity- which are key domains known to be disrupted in schizophrenia and highlights several sex-specific phenotypes that are important when translating to clinical findings.

Disclosures: S. Raju: None. **S. Sundram:** None. **R.A. Hill:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR565.04/TT2

Topic: H.05. Working Memory

Support: John Carroll University

Title: The effect of the 5:2 fasting diet on body weight, food intake, and spatial working memory in rats

Authors: *H. MURPHY, C. WIDEMAN;
Interdisciplinary Neurosci. Program, John Carroll Univ., University Heights, OH

Abstract: The American lifestyle consists of sedentary tendencies, foods with preservatives, and sleep deprivation which may contribute to the obesity epidemic. Specific diets have been employed in recent years to combat this epidemic and allow individuals to pursue a healthier lifestyle. One diet, the 5:2 method, instructs individuals to limit their caloric intake to a quarter of their daily intake on two non-consecutive days. In the remaining five days, individuals can consume their average diet with no restrictions. Additionally, research has found a direct correlation between dieting and other benefits such as memory improvements and managing chronic illnesses. The present experiment sought to investigate the effect of this diet on cognitive function in rats. Experimental and control rats were housed individually. The variables studied were body weight, food intake, and performance in the Morris water maze (MWM), utilized to test spatial working memory. The study was divided into three periods: 1) habituation, 2) experimental - during which experimental animals were provided with a reduced diet (5:2 method), and 3) withdrawal. In the MWM, the animals were provided with two trials in each session: 1) a sample trial to find the location of the platform for the first time and 2) a test trial in which they remembered the location of the platform. At the weekly sessions, the location of the platform changed. As time progressed throughout the experiment, the weight of the control group increased in a steady manner. The experimental group experienced fluctuations in their weight with a steady decrease around week 3 and a steady increase throughout the withdrawal week. The control group increased their food consumption at a steady rate. The experimental group did not consume the food in a uniformed manner due to the nature of their restricted diet in weeks 2-4. In the withdrawal week, the experimental rats increased their consumption at a steady rate. With the MWM, there was a significant difference in mean test trials for week 1 with the experimental group reaching the platform in the test trial at a faster rate than the control group. During the withdrawal period, there was no significant difference between the experimental and control groups. This study supported the hypothesis that, when compared to the control group, rats eating on the 5:2 diet had higher positive effects of cognitive function, specifically spatial working memory, when performing the MWM. The positive effects of the 5:2 diet on body weight, food intake and cognitive performance were noted, however, only when the animals remained on the diet and disappeared when returned to a normal diet during the withdrawal period.

Disclosures: H. Murphy: None. **C. Wideman:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR565.05/TT3

Topic: H.05. Working Memory

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Title: Overflowing items from working memory are retrieved rhythmically

Authors: *T. IDERIHA[1], J. USHIYAMA[2];
[1]Keio Univ., Tokyo, Japan; [2]Fac. of Envrn. and Information Studies, Keio Univ., Fujisawa, Japan

Abstract: Working memory serves as a storage system for actively maintaining information related to the current task, and its capacity is strictly limited. In this research, we report the phenomenon wherein memories that overflow from working memory are recalled in a rhythmic manner, while those remaining within working memory are recalled smoothly. In our experiment, we analyzed the reaction time of participants when recalling an alphabet that is paired with a colored circle. We varied the memory difficulty by changing the number of pairs from two to five (n = 15 each). In the most difficult condition of five pairs, the distribution of reaction times to recall non-recent memories showed a rhythmic pattern in the theta (4-7 Hz) to alpha (8-13 Hz) band. The power of the rhythmicity had individual differences, and it was demonstrated that participants with lower recall performance had stronger rhythmicity. These results demonstrate that memories overflowing from working memory are recalled rhythmically. Taken together, it is suggested that the brain, when struggling to retrieve memories, may utilize rhythmic neural oscillations to realize memory recall, as if sifting through items rhythmically.

Disclosures: T. Ideriha: None. **J. Ushiyama:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

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Topic: H.05. Working Memory

Support: Seoul National University Grant 339-20220013
the National Research Foundation of Korea NRF-2021R1F1A1052020

Title: The more alternatives we have, the more strongly our current choice assimilates to the previous choice

Authors: *H. LEE, J. LIM, S.-H. LEE;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: When making perceptual decisions, our previous experiences can also influence our choices along with the current stimulus. One form of such influence is the ‘choice assimilation

bias, a tendency to make choices similar to previous choices. Researchers have extensively studied the choice assimilation bias, seeking computational and mechanistic accounts. However, although the granularity of choice space (i.e., the number of alternatives) varies over a spectrum in everyday decision-making, previous studies on the choice assimilation bias have focused on the two extreme ends of the spectrum of choice granularity: binary or continuous choices, referring to the choice assimilation bias in the former and latter cases as the 'choice repetition bias' and 'serial dependence,' respectively. As a result, the understanding of the choice assimilation bias is currently lacking for the intermediate range of choice granularity, which makes it difficult to offer a unified account of the bias.
To fill this gap, we conducted a series of experiments where observers had to choose between multiple alternatives. Importantly, we varied the choice granularity to assess how it affects the choice assimilation bias. Over multiple daily sessions, fifty-eight human participants classified ring sizes and sound pitches into two, four, or eight classes (i.e., the choice granularity = [2, 4, 8]). Regardless of the choice granularity conditions, stimuli were always randomly sampled from the same continuous distribution.
We found that the choice assimilation bias increases as the choice granularity increases. This choice granularity effect was observed in both visual and auditory domains. Further, there was a strong inter-participant correlation between the visual and auditory domains in the choice granularity effect. This suggests that the relationship between granularity and assimilation is not confined to a particular sensory modality but reflects a higher-level cognitive process. Additional analyses and experiments revealed that the choice granularity in the "previous" trial is the main factor contributing to the relationship between assimilation and granularity. Other potential explanations such as decision difficulty, motor-dependent heuristics, and the granularity of the current choice were ruled out.
Our findings bridge the gap in the current understanding of the choice assimilation bias by revealing the systematic influence of choice granularity on the bias and by implicating the mnemonic source of that influence.

Disclosures: **H. Lee:** None. **J. Lim:** None. **S. Lee:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR565.07/TT5

Topic: H.05. Working Memory

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Gatsby Charitable Foundation (GAT3755)

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Title: Characteristics of visual short- and long-term memory: evidence from the any-back task

Authors: *V. KLAR[1], S. MANOHAR[2], A. SAXE[3], M. HUSAIN[2];
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Abstract: Visual long-term memory (LTM) shows a surprisingly high capacity for objects, with participants being able to distinguish familiar from novel items in thousands of objects even after long delays. Visual short-term memory (STM), in contrast, has been considered to be severely limited, storing high-precision information of only a few items at a time. An open question is how feature memory is sustained from short to long delays when bound to an object.
To bridge this gap, we developed a novel any-back task. Participants (N = 57) were presented with a continuous stream of four different trial types. On encoding trials, they saw a new object to remember, colored with shades randomly drawn from a color wheel. On retrieval trials, a memory item was probed, requiring participants to make an old/new recognition judgment, and then estimate the object's color. Unbeknownst to participants, retention intervals ranged uniformly from 1.5s (immediate retrieval) to an average of 83.48s (retrieval after 30 trials). Trials were interspersed with lure items and filler trials (a simple perceptual judgment).
The results show high performance on object recognition (M = 91.77%, SD = 0.27%) and color estimation error (M = 48.00°, SD = 47.50°). Mixed-effects regressions indicate that performance on both measures decreased with increasing retention intervals (recognition: $p < 0.001$; color: $p < 0.001$), but remained well above chance even for the longest retention interval of 83.48s (recognition accuracy M = 94.82%, chance = 33.3%; color error M = 52.61°, chance = 90°). A Gaussian mixture model revealed clusters in color responses, suggesting that participants retained categorical color information. However, clusters were too narrow (mean SD = 17.78°) to exhaustively explain sustained above-chance color performance. Performance measures were differentially affected at shorter retention intervals, with an immediate decrement in color estimation accuracy after one intervening trial ($p < 0.001$), while recognition accuracy was unaffected ($p = 0.42$). Further, a higher number of items in memory (items encoded and not retrieved) led to reduced object recognition accuracy above and beyond the effect of retention interval ($p < 0.05$) and generally longer reaction times ($p < 0.05$); but did not affect color error (color error $p = 0.96$, reaction time $p = 0.5$).
Taken together, our results provide compelling evidence for sustained visual STM for color when bound to an object, across varying retention intervals and numbers of interfering items. This is unexpected considering long-standing research on the capacity limits of STM and can only partially be attributed to retention of broad color categories.

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Poster

PSTR565. Working Memory: Behavior and Physiology

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Program #/Poster #: PSTR565.08/TT6

Topic: H.05. Working Memory

Support: NIH Grant R01MH126954

Title: Discovery of a presynaptic actin-signaling mechanism crucial for working memory

Authors: *J. KIM[1], W. C. WETSEL[2], S. H. SODERLING[1];
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Abstract: Working memory, a cognitive function that governs the ability to retain and manipulate information over short periods, plays a pivotal role in various complex cognitive tasks, such as learning and reasoning. Impairments in working memory are evident in several neurological and psychiatric disorders, including Alzheimer's disease, schizophrenia, and ADHD. Therefore, understanding the mechanisms underlying working memory could have substantial therapeutic implications. Previous studies have proposed a relationship between short-term plasticity (STP) and memory, but validating this correlation has been challenging. Our earlier analysis revealed a novel mechanism wherein presynaptic Rac1, a Rho GTPase, influences STP by negatively regulating synaptic vesicle replenishment rate, while leaving other synaptic properties largely unaffected. This provided the impetus to use presynaptic Rac1 modulation as a unique tool for exploring STP's role in behaviors, with specific emphasis on various forms of learning and memory. To suppress presynaptic Rac1 activity *in vivo*, we engineered adeno-associated viruses (AAVs) carrying a fusion protein of a confirmed Rac1 inhibitory peptide (W56) and a presynaptic protein (Synapsin1a). A control AAV, with a scrambled sequence (Scr) in place of W56, served as a comparator. These AAVs were introduced into the hippocampi of C57BL/6J mice, and following a four-week period, we conducted behavioral experiments emphasizing spatial memory performance. The working memory tasks consistently showed impaired spatial working memory following inhibition of presynaptic Rac1. In the eight-arm radial arm maze, the W56 group entered fewer arms and took less time when they first revisited a previously visited arm, compared to the control group. In the spontaneous alternation Y-maze test, the relative number of spontaneous alternations, or the consecutive entries into the three arms, was also lower for the W56 group. The delayed nonmatch-to-place T-maze test showed that the number of successful choice trials increased over time up to 80% for the Scr group but remained around 50% for the W56 group. Notably, other cognitive experiments, such as Morris Water Maze and Fear Conditioning, demonstrated that other forms of memory remained unaffected. Our findings suggest that presynaptic Rac1 in the hippocampus specifically affects working memory while leaving short- and long-term forms of memory intact. These data reveal that distinct synaptic plasticity mechanisms govern different types of memory and that presynaptic Rac1-mediated modulation of presynaptic physiology in the hippocampus is essential for working memory performance.

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Poster

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Topic: H.05. Working Memory

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Title: Awareness of the uncertainty of multiple spatial working memory representations

Authors: *A. Y. LI, S. FERRER, P. BALAGURU, T. SPRAGUE;
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Abstract: Working memory (WM) refers to the capacity to maintain and manipulate information that is no longer present in the surrounding environment. During delay periods, the brain retains WM representations through noisy activation patterns across various regions of the cortex (Curtis & Sprague, 2021), and participants can make accurate behavioral reports about the quality of a single item's neural WM representation (Li et al., 2021; Geurts et al., 2022). Furthermore, individuals can introspectively assess the relative precision of multiple WM representations (Suchow et al., 2017; Adam & Vogel, 2017; Li & Sprague, 2023). However, it remains unknown whether people have metacognitive knowledge of trial-by-trial fluctuations of WM quality for multiple spatial locations simultaneously. Here, we tested participants' ability to introspectively compare and report the quality of working memory representations for spatial locations. Participants (n = 10) completed a memory-guided saccade task, remembering 1 or 2 locations over a 3.5 s delay period and reporting the location of one item with a saccade. Extending our previous study, they were either instructed to report a cued item or choose the item they believed they remembered best with a saccade (Li & Sprague, 2023). After the memory report, participants reported their uncertainty about the reported location by adjusting the extent of an arc (as in Li et al, 2021). If participants can accurately introspect the relative quality of the spatial WM representations, then recall error will be lower when participants can choose which representation to report, reported uncertainty (based on arc size) should be decreased, and uncertainty reports should track trial-by-trial recall accuracy. Results showed that when participants selected their best-remembered item, recall error was significantly lower compared to cued trials ($p < .05$). Uncertainty reports were also lower in self-selected trials compared to randomly cued trials ($p < .05$). Furthermore, participants' binned uncertainty reports only significantly correlated with their average recall error when participants were asked to remember only 1 item ($r = .55$, $p < .05$) and the best remembered item within the 2 item conditions ($r = .60$, $p < .05$), indicating accurate introspection of WM representation quality. These findings suggest that participants can simultaneously compare and report the quality of multiple remembered locations, and reveal a disconnect between trial-by-trial readout of memory quality when a participant chooses which WM representation to report compared to when cued by the experimenter.

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Poster

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Program #/Poster #: PSTR565.10/TT8

Topic: H.05. Working Memory

Title: Induced Cortical Slow Oscillations in medial Prefrontal Cortex Impair the animals Working Memory Function.

Authors: *X. HAN, M. AKUMUO, A. THURMON, M. LORINO, M. GALAZO;
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Abstract: Induced Cortical Slow Oscillations in medial Prefrontal Cortex Impair the animals Working Memory Function.
X. Han[1,2], M. Akumo[1], TN. Abbi[1,2], MG. Lorino[1,2], MJ. Galazo[1,2]. [1]Tulane University, New Orleans, LA; Brain Institute. [2]Tulane University, New Orleans, LA; Dept. Cellular & Molecular Biology During non-REM sleep or Slow Wave Sleep (SWS) cortical neurons alternate between an active "ON" state and silent "OFF" state at low frequencies (0.5-4 Hz). This slow oscillatory activity characterizes the Slow Wave Sleep. During wake slow oscillations only occur sporadically, however, their occurrence progressively increase after prolonged periods of wake and their increase is correlated with impairments in behavioral performance, working memory, and learning (Vladyslav et al., 2011; Coldwell., 2011). It has been proposed that the increased occurrence of cortical "OFF-states" observed in sleep deprived subjects is responsible for memory and cognitive impairments. However, sleep deprivation studies cannot establish a direct causal relationship between cortical "OFF-states" and memory/cognitive performance since sleep deprivation elicits physiological alterations that could also affect memory and cognition. To study the putative direct causal link between the occurrence of cortical "OFF-states" and working memory, we study the effect of inducing "OFF-state like" activity in awake mice via optogenetic manipulation. We induce slow oscillations and "OFF-state like" activity in the medial Prefrontal cortex (mPFC) in awake non-sleep deprived mice, while performing two navigation tasks that required working memory. Induced OFF-states were generated via optogenetic activation of layer 6 corticothalamic neurons (Vaasjo, Han et al., 2021). Working memory performance was assessed in an H-maze navigation task, and delay-non-match to sample T maze task. We find that induction of "OFF-states" during working memory retrieval significantly increased the time to complete the task. The increased time to complete the task is dependent on the frequency of "OFF-states" induced. In addition, induced "OFF-states" increased the frequency of associated with uncertainty in decision or navigation, including pause, head turn, or Vicarious trial and error (VTE). Our preliminary results suggests that induction "OFF-state" during working memory retrieval significantly impair the mice performance, which support the hypothesis that cortical "OFF-states" in mice mPFC impair working memory in non-sleep deprived subjects and provide a direct link of between the "OFF-state" cortical activity to working memory performance, independently of sleep-deprivation status.

Disclosures: **X. Han:** None. **M. Akumuo:** None. **A. Thurmon:** None. **M. Lorino:** None. **M. Galazo:** None.

Poster

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Topic: H.05. Working Memory

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Title: Disentangling the self-initiation modulation on Working Memory: effects of motor control and temporal predictability

Authors: *R. LOYOLA, E. GUERRA, P. DARTNELL;
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Abstract: Self-Initiation has been previously shown to improve accuracy and to speed event related potentials in short-term memory. Yet, previous studies have also proved that Self-Initiation may encompass at least two other mechanisms: motor control and temporal predictability. We aimed to disentangle the contributions of motor control from those of temporal predictability on two self-initiated working memory (WM) tasks, and to assess the role of processing modality (auditory vs. visual).
We invited 64 high schoolers to participate. We obtained signed informed consent from all legal tutors and all teenagers assented to take part of the study. Participants were asked to perform two modified backward digit span experiments with varying initiation styles. Both experiments had a 2x2 repeated measures design, with motor control (Self- vs. Automatically-Initiated) and predictability (Predictable vs. Unpredictable) as factors. In the Self-initiated condition, participants triggered the onset of the stimuli via button pressing, while in the Automatic condition the stimuli started alone. In the Predictable condition, stimuli presentation was fixed to an inter stimulus interval (ISI), while in the Unpredictable condition, the ISI had a range. We presented the experimental conditions in blocks: Automatic-Predictable, Automatic-Unpredictable, Self-Predictable and Self-Unpredictable. We counterbalanced the order of blocks over participants. Finally, Experiment 1 presented the stimulus visually while Experiment 2 did so auditorily.
Experiment 1 results yield higher mean accuracy for the Self-Predictable condition and lower mean accuracy for the Automatic-Predictable one. Generalized Linear Mixed Model (GLMM) results show a significant main effect of the motor control ($p = 0.026$) and a significant interaction effect between motor control and predictability ($p = 0.003$). As for Experiment 2, only the Automatic-Predictable condition shows the lower mean accuracy (vs. other conditions). GLMM results show a significant main effect of the predictability ($p = 0.049$) and a significant

interaction effect ($p = 0.017$).
These results indicate that WM is taxed more strongly when subjects cannot control the onset of stimulus and the ISI is predictable. Thus, temporal predictability cannot alone explain the current and previous performance improvement. Our results also suggest that the influence of Self Initiation on WM is not constant across sensory modalities since modulations appeared different between the visual and auditory domain. Thus, the underlying neurobiological mechanism is probably related to perception rather than to a multimodal process as attention.

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Poster

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Title: Working memory improvement with vibrotactile stimulation of the auricular vagus nerve

Authors: *G. TAN[1,2,3], J. ADAMS[5], K. DONOVANT[3,2], P. DEMAREST[3,2], J. GORLEWICZ[5], P. BRUNNER[2,3], E. C. LEUTHARDT[2,3,4];
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Abstract: Vagus nerve stimulation (VNS) shows promise for individuals with cognitive decline, as recent research suggests its beneficial effects on cognitive function. Noninvasive stimulation of the vagus nerve, known as transcutaneous auricular vagus nerve stimulation (taVNS), has emerged as an alternative method that can potentially induce similar effects to invasive VNS. While transcutaneous auricular VNS has gained popularity, several downsides including auricular discomfort and the requirement of delicate electrode placement limit its feasibility. Working memory is an essential and fundamental part of a wide range of cognitive functions and activities in daily life. In this pilot study, we introduce vibration as a naturalistic and practical modality to stimulate the auricular vagus nerve and investigate its impact on working memory. Seventeen healthy participants were recruited, and a custom-built stimulator, produced by additive manufacturing, was securely attached to the head using a headband. The stimulator delivered precise vibrotactile stimulation through an adjustable contact node to the desired ear

location. Participants completed three sessions of a visual N-back task including a baseline session without stimulation, a session with stimulation at the left cymba concha (Stim), and a session with stimulation at the left earlobe (Sham). Results showed that vibrotactile vagus nerve stimulation (vVNS) improves N-back task performance. Specifically, the hit rate was significantly higher (paired Wilcoxon Rank Sum Test) during the Stim session in comparison to the Sham session; while the false alarm rate and reaction time did not differ across sessions. Additionally, skin conductance and pupil diameter measurements in difficult tasks such as 3-back and 4-back are higher during vVNS compared to baseline. Skin conductance and pupil diameter decreased as the subjects continuously performed. Interestingly, the reduction is less pronounced during the Stim session compared to the sham and baseline. Taken together, we speculate that vVNS counteracts vigilance reduction due to prolonged time and increased task difficulties. Further studies are needed to fully reveal the neural mechanism underlying working memory improvement with vVNS. In conclusion, our findings support the potential of vibrotactile VNS as an effective approach for improving working memory performance.

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Poster

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Topic: H.05. Working Memory

Support: R01-MH114877
R01-AG063775

Title: Age-dependent differences in gating of information flow during selective reactivation of working memory contents

Authors: *H. D. HUANG, R. REINHART;
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Abstract: Regulating working memory (WM) content requires gating the flow of information. Three distinct gating mechanisms have been proposed: input gate that controls access to memory storage, output gate that controls which WM item can influence motor decisions and response gate that controls which motor response gets selected. We hypothesize that switching cost (the difference in reaction time between gate switch and repeat conditions) can be reduced when WM representations are selectively reactivated. Moreover, there is likely a difference in behavioral pattern between young and old adults in light of age-dependent WM deficits. We modified the reference-back-2 task (Rac-Lubashevsky et al., 2021) with two kinds of behavioral cues (neutral, valid) preceding the visual stimuli. We examined switching cost for each of the three gates (input, output and response) during the neutral and valid cue conditions, separately for young and

old adults. For young adults (n=21), we observed switching cost in the neutral cue condition for the input (mean= 70 ms, $p<0.001$) and output gates (mean= 95 ms, $p<0.001$) but not the response gate (mean= 3 ms, $p= 0.84$). We found evidence for our primary hypothesis about mitigation of gate switching cost during selective WM reactivation. While valid cues effectively reduced the behavioral cost for both gate switch and repeat conditions, it had a greater impact on gate switch as switching cost was significantly reduced for all gates. A more detailed analysis reveals that for young adults, switching cost of the input gate is mainly driven by input gate closing, which is reduced under valid cues (average difference= 111 ms, $p<0.001$). We then examined differences between the age groups. Old adults (n=22) showed switching cost in the neutral cue condition for input (average difference= 145 ms, $p<0.001$), output (average difference= 188 ms, $p<0.001$) and response (average difference= 68 ms, $p<0.001$) gates. Like young adults, they also benefited from valid cues which significantly lowered the switching costs for all gates. Interestingly, we observed a trade-off between improving switching cost for input gate opening versus closing. Some old adults (n=7) improved in input gate opening (average difference= 240 ms, $p<0.001$) but sacrificed input gate closing (average difference= -135 ms, $p<0.001$), while others (n=15) exhibited the opposite trend (average difference= -116 ms, $p<0.001$ for input gate opening; average difference= 66 ms, $p<0.001$ for input gate closing). Our results suggest that reactivating WM representations reduces the behavioral cost for multiple aspects of WM regulation, and that such improvement is embedded with an age-dependent difference.

Disclosures: H.D. Huang: None. **R. Reinhart:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

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Program #/Poster #: PSTR565.14/TT12

Topic: H.05. Working Memory

Title: Changes in microsaccade dynamics in spatial working memory and dopamine medication status in Parkinson's disease

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Abstract: Fixational saccadic movements (microsaccades) have been associated with perceptual and cognitive processes, especially in tasks requiring spatial attention and memory. In particular, some studies have shown biased directional microsaccades and changes in rate in relation to visuospatial attentional and task difficulty demand, respectively, although such effects were not always replicated. In one experiment of young adults (N=12, mean age 21.3 years, 7F/5M), we studied the dynamics of microsaccades during a memory-guided saccade task. At the beginning of each trial, two colored dots were briefly presented (0.5s) at various eccentricities and angles

along two concentric ellipses. After a short delay (1.7s or 4.3s), a shift in eye gaze was required to the remembered dot location. The young adults on average showed a dip in microsaccadic rate and then an overshoot during the initial 500 ms of the delay period followed by a return to baseline rate, which is consistent with most of the literature. However, there were no significant differences in other basic microsaccadic characteristics such as amplitude, velocity, and directional bias during working memory in comparison to baseline (initial fixation), and the majority (71%) of participants had microsaccades that were directed within 30 degrees of the horizontal axis, regardless of target location. Using the same study design, we collected similar data from 10 participants with early-stage Parkinson's disease (PD) (mean age 63.2 years, 3F/7M) and 12 healthy controls (OHC) (mean 68.9 years, 5F/5M) to further investigate the potential role of dopamine modulation. Some microsaccade characteristics, such as velocity, duration, and acceleration differed significantly between the two groups (PD off and on medication vs OHC, all p's< .05), but these differences were not affected by task period or target location. PD and OHC both demonstrated preferential horizontally directed microsaccades (92% and 77%, respectively) and a delay period rate pattern consistent with younger participants regardless of disease status, medication status, or age. However, microsaccade rate changes during working memory delay were significantly different between the two groups (PD off and on medication vs OHC, all p's<.001). In sum, these results suggest limited overt involvement of microsaccades in correspondence to visuospatial working memory demand under conditions without any explicit attentional cue but moderate effects of PD-related dopamine deficiency on microsaccadic dynamics.

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Poster

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Program #/Poster #: PSTR565.15/TT13

Topic: H.05. Working Memory

Title: Serial order processing in visuospatial working memory: an eye-tracking study

Authors: *L. JIANG, J. TEPAN, H.-C. LEUNG;
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Abstract: The ability to encode events in succession plays a crucial role in daily functioning from visuomotor to verbal behaviors. Extensive research on verbal short-term memory revealed primacy and recency effects in tasks requiring serial recall of letters, digits, or words. However, the nature of serial-order processing in the visuospatial domain remains underexplored. To address this issue, we recorded eye positions from 44 human participants (mean age = 20.95 yrs, 29F/15M) while performing a serial-order memory-guided saccade task. Each trial starts with a short fixation period, followed by a sequential presentation of four visual images, one in each of the four quadrants. After a short delay, a cue indicates which item is being tested for the trial.

Subjects shifted their eye gaze to the remembered location of the cued item. We conducted 2 experiments that manipulated the testing cue, signaling either the quadrant (quadrant-cue) or the order (order-cue) of to-be-recalled item. We also examined spatiotemporal factors by varying stimulus eccentricity (3 or 5.5 dva) and delay duration (4/6 sec or 8/10 sec). We observed significant serial-order effects on memory-guided saccade performance in the order-cue condition but not in the quadrant-cue condition (Task x Item order on saccade error: $F(3) = 2.39$, $p = 0.076$; saccade latency: $F(3) = 14.35$, $p < 0.001$). For the order-cue condition, saccade errors showed a recency effect (linear, $t(75) = -3.57$, $p < 0.001$), while saccade latency showed both primacy and recency effects (quadratic, $t(75) = -5.90$, $p < 0.001$). Intriguingly, the degree of primacy and recency effects was modulated by stimulus eccentricity and delay duration. These findings suggest a stronger serial-order effect when retaining multiple features (e.g., location and order) than a single feature (e.g., location) in working memory. The dissociation between saccade error and latency indicates that working memory representation is more accessible for the first and last item in a sequence, but it is more precise for recent items with small eccentricity. Further behavior modeling and experimentation on task demands such as explicitness of the order and feature binding are required to pinpoint the exact mechanisms underlying the observed serial-order effects on visuospatial working memory performance.

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Poster

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Program #/Poster #: PSTR565.16/TT14

Topic: H.05. Working Memory

Title: Examining the relationship between pupil size and working memory maintenance precision with pupillometry.

Authors: *A. KHIBOVSKA[1], H.-C. LEUNG[2], L. JIANG[3];
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Abstract: The objective of this study is to explore trial-by-trial pupil variability across high and low visual working memory precision performance trials across maintenance delay using pupillometry. Previously, increases in pupil size while maintaining the object representation in mind were linked to improved working memory performance. Following previous research, we aimed to investigate how pupil trial-to-trial variability influences the recall precision of remembered item locations during a visuospatial working memory task. Secondly, we aimed to explore whether there is a difference in the overall variability of pupil size between trials with high and low recollection precision.We conducted a visuospatial working memory task with 38 participants (M = 20.1 years; 24F). They were asked to remember the precise location of four

sequentially presented items. Pupil data was collected at 2 ms intervals and preprocessed using a custom Matlab script. We developed 5 linear repeated measure models to predict precision error using the average percentage change in pupil size from baseline on a trial-to-trial basis. These models covered the entire delay period (model 1) and its early (model 2), middle (model 3), and late (model 4) phases. Additionally, we analyzed the pupil % change from baseline differences between high and low-precision error trials (model 5). The maintenance delay period was divided into 20 ms increments for this analysis.The first four models did not show a significant relationship between changes in average pupil size and error precision (Model 1: β = -0.002, SE = 0.032, p = .800; Model 2: β = 1.75e-6, SE = 3.14e-6, p = .57; Model 3: β = 6.84e-7, SE = 2.88e-6, p = .81; Model 4: β = 1.42e-6, SE = 2.86e-6, p = .62). This suggests that average pupil size fluctuations may not directly contribute to recall precision in visual working memory.However, across these models, we observed a significant increase in precision errors as the trials progressed, indicating an impact of the duration of the experiment on performance. In the fifth model, analyzing 20 ms time-series increments, we found a significant difference in the % change in pupil size between high and low precision error trials (Model 5: β = 0.69, SE = 0.04, p = .001). Post-hoc analysis revealed that larger changes in pupil size were associated with higher error rates (β = 1.359, SE = 0.001, p = .001). These findings suggest that pupil size variability may serve as a marker reflecting the level of maintenance precision in visual working memory tasks.

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Poster

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Topic: H.01. Attention

Support: R01 MH123687

Title: Anterior Cingulate Cortex Guides Exploration and Supports Working Memory of Object Values during Adaptive Behavior

Authors: *R. TREUTING[1], K. BANAIE BROUJENI[4,2], P. TIESINGA[5], T. WOMELSDORF[2,1,3];
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Abstract: The anterior cingulate cortex (ACC) causally enhances the efficiency of adaptive behavior. This causal importance has been linked to the ACC's role in recognizing unfavorable outcomes, but it could also originate from the ACC predicting which choice options are most valuable to inform future choices. To test this hypothesis, we delivered microstimulation to the

ACC of macaques engaged in learning feature-reward rules, where stimulation occurred during the fixation on objects predicted to be most valuable during learning. We hypothesized that gaze-contingent stimulation interferes with predictive signaling in the ACC and reduces learning efficiency.This hypothesis was tested by transiently microstimulating the ACC during the fixational sampling period of the learning task where animals choose a visual object via maintaining fixation on that object to obtain a reward. Gaze-contingent stimulation was limited to the learning trials of blocks in which animals learned novel feature-reward rules by choosing one of three objects. ACC-stimulation was administered during fixation on either the positively reinforced stimulus (Sr+) or on the negatively reinforced stimulus (Sr-). Sham and Sr+ or Sr- block conditions alternated within each experimental session. Blocks also randomly varied the cognitive demand, and the motivational incentives (gain of 2 or 3 tokens for correct choices) and disincentives (loss of 1 or 3 tokens for incorrect choices), where tokens are exchanged for fluid reward. In two animals, we found that ACC-stimulation impaired learning when cognitive demands were high (objects had three feature dimensions) and motivational contexts were low (gain of 2 or loss of 3 tokens). Stimulation was especially detrimental to learning when it interfered with choosing the positive reinforced stimulus (Sr+). Behavioral modeling confirmed that stimulation affected learning and identified two latent learning processes affected by stimulation. First, ACC-stimulation caused reduced adaptation of exploratory behavior in the trials immediately after stimulation, indicating that errors are used less efficiently to adjust choices during learning. Secondly, ACC-stimulation reduced the use of working memory of recently rewarded objects to guide choices, indicating that learning relied more on slower model-free reinforcement learning. In summary, these findings illustrate that the ACC causally contributes to adjust exploration and working memory for object values during adaptive behavior.

Disclosures: R. Treuting: None. **K. Banaie Broujeni:** None. **P. Tiesinga:** None. **T. Womelsdorf:** None.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR565.18/TT17

Topic: H.01. Attention

Title: Relationship Between Resting Alpha Oscillations and Working Memory Span in Real-World Environments: A Large-Scale Study Using Virtual Reality-Integrated EEG

Authors: Y. LEE, J. KIM, *J. PARK, Y.-W. CHAE;
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Abstract: The Default Mode Network (DMN) is a brain network that is activated when the brain is in a wakeful rest state and not engaged with the external world. Numerous studies revealed its mechanisms and relations with diverse cognitive processes. Specifically, the relationship

between alpha oscillation in the resting state and attention or memory task performance in the attentional state was investigated. However, there remains a gap in knowledge regarding the applicability of DMN research findings, mostly derived from controlled laboratory settings, to real-world contexts.
To bridge this gap, we devised a new device integrating an electroencephalogram (EEG) sensor with a virtual reality (VR), creating a portable device capable of extending research environments from the lab into real-world settings. We examined the relationship between resting-state alpha power and working memory task performance in a large sample of individuals (N=2107; mean age: 52.7 (SD 17.0); 36.9% male, 63.1% female) under real-world conditions. Our results indicated a significant correlation between resting-state alpha power and working memory task performance ($r=0.144$; $R^2=0.021$; $p<0.000$).
This finding supports the existing hypothesis that the connectivity of the default mode network can influence the attentional state memory task. Our study found that previous results that had been investigated in a controlled laboratory environment were also reproduced in a real-world scenario. In addition, through analysis based on EEG data collected for large-scale samples, it was possible to discover correlations that had not been revealed before with a significant level of confidence. It is also noteworthy to mention that the development of an accessible VR platform with integrated EEG sensors has now reached a level of reliability that allows for comprehensive research.

Disclosures: **Y. Lee:** A. Employment/Salary (full or part-time):; Looxid Labs. **J. Kim:** A. Employment/Salary (full or part-time):; Looxid Labs. **J. Park:** A. Employment/Salary (full or part-time):; Looxid Labs. **Y. Chae:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Looxid Labs.

Poster

PSTR565. Working Memory: Behavior and Physiology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR565.19/TT18

Topic: H.05. Working Memory

Title: Cognitive consequences of long-COVID: An event-related potential case study using an EEG-based cognitive assessment

Authors: *R. HANA, J. EL-HABROUK, J. CONNOLLY, K. RUITER;
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Abstract: SARS-CoV-2 infection is known to have effects on multiple organ systems throughout the body, and some patients continue to experience symptoms after infection. For example, patients often report “brain fog,” which can manifest as feeling slow, difficulty thinking or concentrating, confusion, and forgetfulness. Measuring these cognitive symptoms in a way that is objective and quantifiable, however, is difficult with the currently available in-clinic behavioral tools. In the present study, we aimed to quantify the cognitive symptoms of

post-COVID patients across three domains: Attention & Concentration, Information Processing, and Memory. We used VoxNeuro CORE, an EEG-based cognitive assessment that quantifies event-related potentials (ERPs) during traditional neuropsychological assessments and compares a patient's ERPs to a normative database. Multiple studies have shown ERPs to be an objective and quantifiable measure of cognitive function across many domains. EEG recordings were completed in accordance with the 10-20 system, utilizing electrodes at Fz, Cz, Pz, and Oz. Recordings were done as patients completed Auditory Oddball and Continuous Visual Memory (CVMT) tasks. We compared the amplitude of the P300 or N200 ERPs to an age-matched normative database to generate a score of 50 to 150, with lower amplitude sitting closer to 50 and higher amplitude sitting closer to 150. We compared scores from 7 post-COVID patients and 212 normative patients using a Student's t-test to look for significant group differences. We found significant differences between post-COVID patients and normative controls in Working Memory ($t=-0.27$, $p=0.035$), Attention & Concentration ($t=2.98$, $p=0.003$) but no difference in Information Processing ($t=1.51$, $p=0.132$). Changes in Working Memory, Attention & Concentration are consistent with some of the cognitive symptoms many post-COVID patients are experiencing, indicating that ERPs and VoxNeuro CORE are sensitive to the changes in brain responses correlated to these symptoms. Further exploration is needed to validate these findings.

Disclosures: **R. Hana:** A. Employment/Salary (full or part-time):; VoxNeuro. **J. El-Habrouk:** A. Employment/Salary (full or part-time):; VoxNeuro. **J. Connolly:** A. Employment/Salary (full or part-time):; VoxNeuro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VoxNeuro. **K. Ruiter:** A. Employment/Salary (full or part-time):; VoxNeuro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VoxNeuro.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.01/Web Only

Topic: H.07. Long-Term Memory

Support: CONACYT 785509

Title: Effects on behavioral response of male rats at 21 and 90 days of postnatal age by maternal lipopolysaccharide infection.

Authors: *J. J. VIRGEN-GEN[1], M. ALVARADO OLIVAREZ[1], R. I. GUZMÁN-GERÓNIMO[2], A. G. GUTIÉRREZ-GARCÍA[1];
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Abstract: During a maternal infection with lipopolysaccharide (MI-LPS), the neurodevelopment of the offspring is affected due to alterations in the central nervous system (CNS) by the

activation of different proinflammatory mechanisms. We studied the effects of lipopolysaccharide (LPS) infection on the gestational (LPS-G) and gestational-lactation (LPS-GL) periods in male Wistar rats at postnatal days (PND) 21 and PND 90. Three groups were used: CONTROL, LPS-G and LPS-GL (n=8 per group). The CONTROL group was administered with saline (0.9% NaCl) intraperitoneally (IP) on embryonic days (ED) 10,12,14; while the LPS-G and LPS-GL groups received via IP a dose of LPS (50µg/kg) on ED 10,12,14; further, the LPS-GL group received a subcutaneous (SC) injection of LPS (50µg/kg) on PND 7. A battery of behavioral tests was performed for both ages, starting with the least stressful test and a 24h interval for each test. For the PND 21 pups, the descent on different surfaces (DDS) test and the open field (OF) test were performed. In the DDS test, we observed a delay in the response of the hind limbs in the LPS-G and LPS-GL groups compared to the CONTROL group when evaluating total descent time and fall latency. Moreover, the offspring of the LPS-G and LPS-GL showed signs of hyperactivity by increasing their full speed and acceleration compared to the CONTROL group, likewise, at the PND 90 age, only the LPS-GL showed an increase in total acceleration compared to the CONTROL in the OF test. In addition, in the novel object recognition (NOR) test, the LPS-G and LPS-GL groups showed greater fixation on the familiar object compared to the CONTROL in the short-term memory (STM) and long-term memory (LTM) phases. In the elevated plus maze (EPM) test, the LPS-G and LPS-GL groups remained longer in the closed arms in contrast with the CONTROL, evidencing a possible anxiogenic effect. In conclusion, the LPS-G and LPS-GL groups showed damage in the CNS of the offspring, evidencing a delay in the locomotor response also hyperactivity-type behaviors in PND 21 and the LPS-GL group in PND 90. Furthermore, damage to STM and LTM as well as an anxiogenic effect were observed in LPS-G and LPS-GL groups at PND 90. Moreover, among the groups exposed to LPS in PND 21, in DDS test, the LPS-GL group evidenced symptoms of paresis, contrary to the LPS-G group which presented a state of rigidity. Likewise, in PND 90, the LPS-GL group showed greater hyperactivity than LPS-GL, however, in the RON test LPS-G showed greater damage in the LTM phase. According to our findings, maternal exposure to LPS both in gestation and gestation-lactation generated damages in several behaviors, evidencing chronic inflammatory damage in the adult stage.

Disclosures: J.J. Virgen-Gen: None. **M. Alvarado Olivarez:** None. **R.I. Guzmán-Gerónimo:** None. **A.G. Gutiérrez-García:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.02/TT19

Topic: H.07. Long-Term Memory

Support: Agence Nationale Recherche française
China Scholarship Council
INSERM

CNRS
Region Nouvelle Aquitaine

Title: Time-dependent changes in the intrinsic excitability of neocortical neurons underpin the consolidation of long-term memory

Authors: *S. HADZIBEGOVIC[1], L. ZHU[1], M. GINGER[1], R. DE SA[1], M. GUEIDAO COSTA[1], E. COUGOUILLES[1], O. NICOLE[2], B. BONTEMPI[3], A. FRICK[1];
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Abstract: Long-term memories undergo a gradual consolidation process over time. The prefrontal cortex (PFC) is assumed to play a crucial role only for remote memories, although synaptic tagging already occurs early following memory encoding. The nature of this tag as well as its functional role have not been determined yet. Here, we raised the hypothesis that memory encoding triggers plasticity in the neuronal excitability of PFC neuronal populations as part of the early tagging process and that this form of plasticity plays a permissive role for the formation and long-term storage of memories. We therefore examined the nature, dynamics, and role of intrinsic plasticity of putative PFC engram neurons during memory consolidation. To specifically probe putative engram neurons, we used a cFos dependent labeling system combined with viral transduction tools enabling the visualization of PFC neurons specifically engaged in the encoding of contextual fear memory. We used whole-cell recordings from these putative engram neurons in acute PFC brain slices to characterize the learning-specific intrinsic excitability changes and their time course. PFC neurons that were activated during contextual fear conditioning (CFC) endured robust modifications in their intrinsic excitability, pointing to an overall increased excitability. We then probed the duration and functional relevance of this plasticity for long-term memory formation by modulating the excitability of these neurons either during early or remote memory phases. Finally, downregulation of the putative PFC engram neurons' excitability immediately after CFC rescued memory impairment caused by introduction of the memory interference event. Together, by using behavioral, genetic and viral tools combined with cellular and electrophysiological methodologies, we provide novel insights into the neuronal mechanisms underlying the formation and stabilization of enduring associative memories.

Disclosures: S. Hadzibegovic: None. **L. Zhu:** None. **M. Ginger:** None. **R. De Sa:** None. **M. Gueidao Costa:** None. **E. Cougouilles:** None. **O. Nicole:** None. **B. Bontempi:** None. **A. Frick:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.03/TT20

Topic: H.07. Long-Term Memory

Support: NRF 2022R1I1A4063209
NRF 2022R1A2C2005062
NRF-2019M3C1B8090805

Title: Closed-loop reward stimulation at sharp wave ripples for cognitive effect

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Abstract: Recent studies have shown that sharp wave ripples (SWRs) occur during memory consolidation and memory retrieval processes (Hannah R. Joo, 2018). Therefore, these cognitive functions may be enhanced by the induction of SWRs during sleep. In this study, when SWRs were detected, electrical stimulation was delivered to the reward circuit of the medial forebrain bundle (MFB) of rats to promote the SWRs occurrence. Every algorithm is developed with MATLAB (Matlab R2019b, MathWorks, USA) and Sprague-Dawley rats (8 weeks, male) were used for the experiments. Depth electrodes were implanted in the hippocampal CA1 for recording SWRs and MFB for reward stimulation. Surface electrodes were also implanted in the medial prefrontal cortex (mPFC) and neck muscle to monitor the electroencephalogram and electromyogram. To confirm MFB electrode placement and to determine the parameters of the memory-enhancement experiment, intracranial electrical self-stimulation (ICSS) was performed until animals pressed the lever more than 30 times/min. The experiment was conducted for four days in a single week, specifically on the first three days and the last day. As a result, the number of SWRs was increased by MFB stimulation, not only during the first three days of continuous stimulation but also at the last day which is four days apart from the last stimulation day. Thus, the synchronized reward stimulation with the memory consolidation signals during sleep has caused an escalation in the memory consolidation signals. To prove the effect of memory improvement, a Y-maze test was designed and conducted. Therefore, the augmentation of these neural signals may provide a new neuromodulation method for memory improvement.

Disclosures: J. Lee: None. **S. Jun:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.04/TT21

Topic: H.07. Long-Term Memory

Support: 327654276 – SFB 1315

Title: Where do the monkeys live? - An exploratory study on the consolidation of long-term spatial memory

Authors: *D. IGGENA[1,2], T. SCHMELTER[3], P. M. MAIER[4,2], K. REGUIEG[3], C. FINKE[4,2], K. HILDEBRAND[3], C. J. PLONER[4];
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Abstract: Spatial memory is an essential prerequisite for autonomy and survival. Spatial representations are initially acquired through navigational experiences and interactions with the environment. After acquisition, spatial representations may undergo a transformative process of system consolidation, which may consist of a significant rearrangement of hippocampal-neocortical interplay. The temporal properties of this process are however still under debate. Despite the importance of spatial memory for everyday behavior, little is known about consolidation of spatial representations beyond time windows that can be addressed with conventional behavioral experiments, i.e. up to some weeks. Whether consolidation of spatial memories continues at longer delays and whether this process is accompanied by behavioral changes is currently unknown.
Here, we present a virtual reality task called ‘Berlin Zoo task’, where 106 healthy human participants (82 female, 24 male, 14 - 71 years old) were virtually put in a virtual analogue of a Zoo in East Berlin. The principal layout of this environment has remained highly constant during the last decades. Last visit to the zoo ranged from a few days up to three decades. To test egocentric spatial representations, participants were asked to point in the direction of a specific location from a first-person perspective. To test allocentric representations, participants were asked to locate landmarks from a bird's eye view on a 2D-map.
Surprisingly, even participants who had not visited the zoo in the last three decades were able to reliably perform above chance level both in the allocentric and egocentric tasks. Furthermore, we found differential effects of time on allocentric and egocentric spatial memory representations. While performance on the allocentric task deteriorated with increasing delay between the last zoo visit and testing time ($r = 0.48$, $p < 0.001$), performance on the egocentric task was less susceptible to delay ($r = 0.19$ $p = 0.137$).
Our behavioral results suggest that even decades after acquisition, memories continue to transform. This process is not simply a plateau or non-selective decay in performance, but rather a progressive change in the relative contributions of distinct spatial representations. Changes in memory-guided navigation decades after memory acquisition may reflect ongoing systems consolidation.

Disclosures: D. Iggena: None. **T. Schmelter:** None. **P.M. Maier:** None. **K. Reguieg:** None. **C. Finke:** None. **K. Hildebrand:** None. **C.J. Ploner:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.05/Web Only

Topic: H.07. Long-Term Memory

Support: Conacyt :712328

Title: Blue corn tortilla buffers the effects of lipopolysaccharide on memory and hippocampus in offspring of male rats

Authors: *P. F. GONZÁLEZ NIETO[1], M. ALVARADO OLIVAREZ[2], R. I. GUZMÁN-GERÓNIMO[3], J. F. RODRÍGUEZ-LANDA[2], L. T. HERNÁNDEZ-SALAZAR[2];
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Abstract: The prenatal period is a stage susceptible to infections that can affect the health of the fetus, particularly alterations in neurodevelopment that last until adulthood, because conditions cause the presence of proinflammatory cytokines. These cytokines are related to damage in the expression of essential proteins in neuronal signaling, as well as in critical brain structures in memory, such as the hippocampus. However, some foods could mitigate this damage, such as those rich in anthocyanins, due to their anti-inflammatory activity. This project explored the effect of blue corn tortilla on the memory and hippocampus of Wistar rats tested at the age of 120 days (P120) that were exposed to lipopolysaccharide (LPS) during gestation. We experimented on 18 male rats distributed in three groups: control (control), LPS G (offspring of mothers exposed to LPS during pregnancy), and LPS G + TMA (offspring of mothers exposed to LPS during pregnancy and tortilla consumption). LPS (50 µg/kg) during pregnancy was provided on embryonic days 10, 12, and 14. The blue corn tortilla was processed by microwave and added with gallic acid to retain a higher concentration of anthocyanins; it was provided based on the kg/weight ratio, administering 1% of the rat's weight. The novel object recognition test (RON) was performed to assess short and long-term memory, where the interaction time with familiar and novel objects was analyzed. The histological process with Nissl staining was carried out to evaluate the cell density in the hippocampal zones: dentate gyrus (DG), Cornu ammonis 1(CA1), 2 (CA2), and 3(CA3). The results of the RON test show that the administration of LPS alters short and long-term memory by interacting for a short time with the novel object and with the familiar of the LPS G group compared to the control. Whereas, in the LPS G + TMA group, a greater interaction with objects is observed compared to the LPS G group, without showing differences with the control group. On the other hand, in the cell density results, a decrease is observed in the LPS G group compared to the power in the DG, while, in the CA1 and CA2 areas, the LPS G and LPS G + TMA group show a decrease compared with control, presenting more significant damage in CA1, the group of LPS G + TMA, and CA2, the group of LPS G. It is essential to mention that the DG participates in the neurogenesis process and that the other areas of the hippocampus it engages in the trisynaptic circuit, critical to the learning and memory process. Therefore, it can be said that the administration of tortillas processed by microwaves and added with gallic acid dampens the damage in the DG of the hippocampus, generating less memory alteration.

Disclosures: P.F. González Nieto: None. **M. Alvarado Olivarez:** None. **R.I. Guzmán-Gerónimo:** None. **J.F. Rodríguez-Landa:** None. **L.T. Hernández-Salazar:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.06/TT22

Topic: H.07. Long-Term Memory

Support: European Research Council (ERC-2019-COG 866093)
Israel Science Foundation (ISF 526/17)

Title: Behavioral and non-invasive brain stimulation approaches for indirect modulation of visual negative memories

Authors: *S. KOZAK[1], N. HERZ[4], M. TOCKER[5], H. SHARON[2], N. CENSOR[1,3], Y. BAR-HAIM[1,3];
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Abstract: Maladaptive negative memories underlie mental conditions such as posttraumatic stress disorder (PTSD) and fear disorders. This has inspired research geared towards memory modulation by direct targeting of the negative emotional memory. However, direct modulation of emotional memories is often challenging and can cause substantial distress. Given these challenges we propose to modulate negative memories indirectly. We previously demonstrated indirect modulation of neutral visual memory, utilizing behavioral instructions to intentionally remember or forget verbal information, in order to indirectly target an embedded visual memory. In the current research we examined the indirect modulation of negative visual memories. In the first study we applied the indirect modulation paradigm to attenuate short term negative visual memories. In contrast to the neutral visual memory study, negative visual memory showed resistance to indirect modulation, with comparable visual memory strength under instructions to remember or forget the associated verbal information ($F(1,40)=0.529$, $p=0.471$, $\eta^2_p = 0.013$). Therefore, in the second study we used repetitive transcranial magnetic stimulation (rTMS) to indirectly downregulate long term negative visual memory. We hypothesized that rTMS following verbal memory reactivation will directly enhance verbal memory strength and indirectly reduce negative visual memory strength, through a mechanism of memory competition. Prior to the beginning of the experiment, participants completed anatomical and functional MRI scans. On Day1, participants studied neutral words embedded with negative pictures and complete a verbal word memory test. On Day2, participants in the main experimental group underwent a verbal memory reactivation, followed by rTMS over the right PFC to enhance verbal memory strength. On Day3, participants completed an additional set of MRI scans, and verbal and visual memory tests. Preliminary results indicate that rTMS paired with verbal memory reactivation reduced the forgetting of verbal memory on Day3 relative to a Day1 baseline (mean difference -5.0% ± 2.3% SE), compared to verbal memory reactivation without rTMS (-10.0% ± 3.8%). We now proceed to test whether such neuromodulation of verbal memory would indirectly affect long term negative visual memory. This research expands our understanding of the neurobehavioral mechanisms of memory modulation, possibly

uncovering novel approaches to downregulate negative memories and treating psychopathologies such as PTSD.

Disclosures: **S. Kozak:** None. **N. Herz:** None. **M. Tocker:** None. **H. Sharon:** None. **N. Censor:** None. **Y. Bar-Haim:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

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Program #/Poster #: PSTR566.07/Web Only

Topic: H.07. Long-Term Memory

Support: CONAHCYT Ref. no. 277701

Title: Environmental enrichment reinforced long-term memory

Authors: *R. SOLÍS GUILLÉN[1], A. MENESES[2], D. CENTURIÓN[3];
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Abstract: Memory formation involves different stages: acquisition, consolidation, and retrieval. Our study was focus on studying memory retrieval. Retrieval refers to the utilization of learned information. The inability to remember is a recurring issue in patients with memory deficits, where the primary symptom is the ability to learn but the difficulty in retaining information over time. To enhance memory retrieval, we implemented a protocol of environmental enrichment (EE). EE entails enriching a subject's environment by introducing objects that increase sensory, cognitive, and motor stimuli, ultimately improving their biological conditions. To examine the impact of EE on long-term memory retrieval, we designed an experimental protocol that involved evaluating conditioned responses (% CR) after a one-week interruption following autoshaping sessions for memory formation. A decrease in % CR was indicative of memory decay. Male Wistar rats were divided into three groups: A) control, comprising animals in standard laboratory housing conditions; B) animals housed in enriched environments before and during the memory formation and retrieval protocol; and C) animals exclusively housed in an enriched environment during the interruption period (after the 48-hour trial). Our findings revealed that EE prior to and throughout the experimental protocol had no effect on memory recovery. The primary effect of EE was observed when rats experienced EE for 3 or 6 hours during the interruption period (after reaching maximum retention at 48 hours). These results suggest that excessive exposure to EE could contribute to hippocampal-dependent memory loss. Conversely, it is well documented that EE is associated with cellular effects such as synaptic plasticity and neurogenesis in the hippocampal dentate gyrus, indicating that new neurons may play a functional role in enhancing learning. Hence, our results imply that EE also contributes to the maintenance of long-term memory. In conclusion, environmental enrichment enhances long-term memory retrieval.

Disclosures: **R. Solís Guillén:** None. **A. Meneses:** None. **D. Centurión:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.08/TT23

Topic: H.07. Long-Term Memory

Support: Robert Wood Johnson Foundation Amos Program

Title: Persistent effects of early life exercise on hippocampal function and spine density and morphology in adulthood

Authors: B. GOMRINGER[1], ***I. BAGHDASARYAN**[2], I. CHIKEZIE[2], A. VALADEZ[2], A. IVY[1,3];
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Abstract: Introduction: Aerobic exercise in adults enhances cellular and circuit-level plasticity in the murine hippocampus through mechanisms such as neurogenesis, promoting neurotrophic factor expression, and strengthening synapses. These mechanisms converge to support enhanced hippocampus-dependent learning and memory in the adult brain. It is reasonable to consider these mechanisms similarly engaged in the developing brain. Furthermore, aerobic exercise in adults requires maintained exposure to continually access memory-promoting benefits; whether the experience of early life exercise can inform the development and persistent function of neurons and neural circuits is an open question. This study introduces exercise during specific postnatal windows of juvenile hippocampal development to determine whether early exercise exposure confers lasting effects on hippocampal long-term memory, brain volumes, and neuronal structure. **Methods:** Wild-type C57Bl6/J mice were segregated into sedentary cages or cages with access to a running wheel during three postnatal ages: the 4th postnatal week (juvenile EX), the 6th postnatal week (adolescent EX), or the 4th-6th postnatal week (juv-adol EX). To assess adult hippocampal long-term memory, the Object Location Memory (OLM) protocol was used at 2-3 months and 5-6 months of age, and mice were trained for either ten minutes (threshold learning) or three minutes (subthreshold learning), the latter training period previously determined to be insufficient for long-term memory formation. The performance of the OLM task was assessed by a blinded experimenter manually scoring video recordings of object exploration for each mouse and calculating a discrimination index (DI). To assess hippocampal CA1 dendritic spines, brains were either sectioned and stained for PSD-95 or underwent a Golgi neuronal impregnation protocol. The evaluation of dendritic spine morphology via Golgi and PSD-95 labeling offers insight into the presence and quantity of mature synapses. **Results:** All mice, whether exercised or sedentary, preferred a spatially novel object when trained for ten minutes in the OLM task. When exposed to a subthreshold learning stimulus, adult 2-3 month juvenile-exercised female mice demonstrated significant discrimination between a novel and

familiar object placement. Juv-adol EX mice showed a significant increase in the number of dendritic spines in the CA1 region of the hippocampus compared to sedentary mice. These findings suggest that juvenile exercise enables memory function in early adulthood by basal synaptic mechanisms modulated by early-life exercise.

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Poster

PSTR566. Consolidation and Reconsolidation: Behavior

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.09/TT24

Topic: H.07. Long-Term Memory

Title: The interdependence of emotional valence and expectedness in memory

Authors: *A. S. MUKHTAR[1], A. KAFKAS[2];
[1]Human Neurosci., Univ. of Manchester, manchester, United Kingdom; [2]Univ. of Manchester, Univ. of Manchester, Manchester, United Kingdom

Abstract: The interdependence of emotional valence and expectedness in memory
Emotional stimuli usually result in better recognition than neutral stimuli; however, the reason behind the superiority of emotional memory remains poorly understood. In three experiments, we investigated the effect of event distinctiveness and expectedness on memory formation for neutral and emotional stimuli and whether expectation has the same memory effect on mixed lists (including both neutral and emotional stimuli) as compared to purely emotional lists (Experiment 2). We also investigated whether the arousal differences between negative and positive stimuli (Experiment 1) had the same memory effect as a list of matched arousal levels (Experiment 3). To manipulate expectation, participants learned the systematic relationship between 6 symbols and the frame colour accompanying each stimulus in a rule-learning task. At encoding, a new set of stimuli were encoded, the established rules were violated for some stimuli (40%), while it remained intact for the rest. In Experiment 1, the findings showed an interaction between expectation, memory, and emotion. Unexpected stimuli boosted recollection, this was true for negative and neutral stimuli but not for positive ones. This indicates that emotional content modulates the degree to which expectedness affects memory. In Experiment 2, in which emotional but not neutral stimuli were used, there was no significant interaction between emotion, expectation, and memory. On the other hand, in Experiment 3, a significant interaction was found between emotion, expectation, and memory. The results suggest that expectation violation plays an important role in memory enhancement and interacts with emotional content in mixed content, but not in pure content. The findings imply an interaction between memory and emotional neural networks, when events of different level of expectedness and emotional valence are encoded, which is currently investigated using fMRI.

Disclosures: **A.S. Mukhtar:** None. **A. Kafkas:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.10/TT25

Topic: H.07. Long-Term Memory

Support: R01 90091512

Title: Novel two-choice auditory task for reversal learning in mice

Authors: ***F. ZHU**[1], K. KUCHIBHOTLA[1,2,3];
[1]Psychological and Brain Sci., [2]Neurosci., [3]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: The ability to reverse the contingency of a set of previously learned associations is a common example of rule learning in both animals and artificial intelligence - agents can solve novel tasks by applying a simple reversal rule instead of relearning the response-outcome associations when the contingencies are swapped. Consequently, the neural basis of reversal learning has been studied in humans, primates, and rodents. Rodent models offer the advantage of using advanced optogenetic tools to decode the causal role of neuronal activity and application of a reversal rule. Currently, most head-fixed rodent reversal paradigm utilizes the Go/No-Go (GNG) task structure, in which the animal has to learn to inhibit action towards a previously rewarded cue and initiate action for a previously unrewarded cue. However, there are several confounds that arise when interpreting incorrect responses in a GNG task, such as disengagement and purposeful lapses, which makes it challenging to determine when the animal applies a rule during reversal trials. Here, we present a novel two-choice auditory task in which mice complete multiple reversals of learned response-outcome associations. We show that mice can learn this task in a few weeks, and there is a surprising asymmetry in learning to apply the reversal rule to the two actions - animals quickly learn to reverse their response to one stimulus and slowly learn the reversal for the other stimulus. This paradigm provides the basis for future optical interrogation of rule learning in mice models.

Disclosures: **F. Zhu:** None. **K. Kuchibhotla:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.11/TT26

Topic: H.07. Long-Term Memory

Support: IITR/SRIC/2741

Title: Music played during the reconsolidation window blocks the return of fear in humans

Authors: *M. K. ASTHANA[1], A. VERMA[1], S. MITRA[2];
[1]Dept. of Humanities & Social Sci., [2]Dept. of Humanities and Social Sci., Indian Inst. of Technol. Roorkee, Roorkee, India

Abstract: Music played during the reconsolidation window blocks the return of fear in humans Manish Kumar Asthana[1,2*], Ankita Verma[1] and Sharmili Mitra[1]*[1]Department of Humanities & Social Sciences, Indian Institute of Technology Roorkee, India[2]Department of Design, Indian Institute of Technology Roorkee, India*
Blocking the return of fear during the reconsolidation period might offer an effective treatment towards the acquired fear. Several interventions, such as behavioural, pharmacological, cognitive, neurostimulation, etc., have been implemented to block the return of fear. Nevertheless, memory reconsolidation is a complex process that does not undergo a transient state every time upon reactivation. The findings of fear reconsolidation are inconclusive and unclear. Hence, we investigated using pleasant music (e.g., nature's music) to target the fear reconsolidation process. Our results indicate that pleasant music and extinction training during the reconsolidation window prevents the return of fear. The current findings are similar to earlier work. We successfully replicated the reconsolidation study in an Indian context.Moreover, we also report the efficacy of music's effect on memory update. The current findings support the drug-free paradigm targeting the reconsolidation window. Hence, music intervention techniques can offer an effective drug-free paradigm for individuals with post-traumatic stress disorder, specific phobias, etc.
Keywords: Memory reconsolidation, fear memory, reactivation, fear conditioning, drug-free paradigm

Disclosures: M.K. Asthana: None. **A. Verma:** None. **S. Mitra:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.12/TT27

Topic: H.07. Long-Term Memory

Support: IC Postdoctoral Fellowship; ORISE Program between DOE and ODNI
Army Research Office Grant #W911NF-16-1-0274
Army Research Lab Collaborative Agreement #W911NF-21-2-0179

Title: A behavioral benchmark for theories of implicit learning: An extremely high fidelity quantification of the time course of consolidation from a massive behavioral dataset

Authors: *P. COX[1,2,3], C. CALLAHAN-FLINTOFF[4], M. R. KRAMER[5], S. H. MITROFF[2], D. J. KRAVITZ[2];
[1]Psychology, Lehigh Univ., Bethlehem, PA; [2]The George Washington Univ., Washington, DC; [3]Intelligence Community Postdoctoral Res. Fellowship Program, Washington, DC; [4]Army Res. Lab., Aberdeen, MD; [5]Transportation Security Admin., Springfield, VA

Abstract: Human behavior adapts to a given environment with repeated exposures to specific stimuli and/or repeated actions without explicit awareness through implicit learning. The striatum is often pointed to as a critical structure in this type of learning (e.g., Fernandez-Ruiz et al., 2001), but the relative sparing of consolidated implicit memories with striatal damage suggests that they are ultimately stored in cortex, likely via local changes in synaptic strength (see Reber, 2013). Our previous work described a general evidence accumulation function that may underlie such learned adaptations in behavior (Kramer et al., 2022), but questions remain about the time course of the consolidation of this learning. Local synaptic changes in strength can involve short-term changes in membrane conductance (lasting minutes; Zucker & Regehr, 2002) and long-term changes in gene and protein expression (taking hours to arise and lasting days; Kelleher, Govindarajan & Tonegawa, 2004). It is also well known that sleep can play an important role in the consolidation of skill acquisition (Diekelman, Wilhelm, & Born, 2009), and implicit learning is correlated with sleep spindle activation (Stevens et al., 2021). Here we used a massive dataset >15.6 million users, >3.8 billion trials) of human behavioral data from a mobile app to quantify the consolidation of learning in both a visual search task and a categorization task over a range of delays, and to describe the impact of sleep during the consolidation period. The magnitude of this dataset allowed for an extremely high fidelity characterization of the time course of consolidation from seconds to days with a high degree of temporal precision. This high fidelity quantification showed a complex nonlinear effect of time on consolidation and provides much needed temporal constraints on hypotheses about the myriad of neural mechanisms underlying learning and their characteristic time courses. To ensure scientific rigor, 1% of the data was held out and used to establish the analysis pipeline and conduct a power analysis to decide the sizes of the time bins in all analyses.

Disclosures: P. Cox: None. **C. Callahan-Flintoff:** None. **M.R. Kramer:** None. **S.H. Mitroff:** None. **D.J. Kravitz:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.13/TT28

Topic: G.01. Fear and Aversive Learning and Memory

Support: JST Grant Presto 18068712
JST Grant Moonshot 20343198
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JST ERATO JPMJER1801
JSPS KAKENHI JP22H05156
The Innovative Science and Technology Initiative for Security (JPJ004596), ATLA

Title: Time-dependent neural arbitration between cue associative and episodic fear memories

Authors: *A. KOIZUMI[1], A. CORTESE[2], R. OHATA[3], M. ALEMANY[5], N. KITAGAWA[6], H. IMAMIZU[4];
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Abstract: Following traumatic experiences, simple cue-threat associative memories strengthen while episodic memories become fragmented. However, how the brain prioritizes cue associations over episodic coding of traumatic events remains unclear. Here, we developed a novel episodic threat conditioning paradigm in which participants concurrently form two memory representations: cue associations and episodic cue sequences. We revealed that these two distinct memories compete for physiological fear expression in a time-dependent manner. With multivariate fMRI, we tracked inter-area communication of the memory representations to demonstrate neural mechanisms underlying this memory maturation. Critically, this overnight reorganization is altered in individuals with heightened trait anxiety. Time-dependent memory competition may provide a unifying account for memory dysfunctions in posttraumatic stress disorders.

Disclosures: **A. Koizumi:** None. **A. Cortese:** None. **R. Ohata:** None. **M. Alemany:** None. **N. Kitagawa:** None. **H. Imamizu:** None.

Poster

PSTR566. Consolidation and Reconsolidation: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR566.14/UU1

Topic: G.03. Motivation

Support: EU COST TEATIME

Title: TheBehaviourForum.org: an online forum to discuss issues relating to animal behaviour experiments

Authors: *J. E. MCCUTCHEON[1], H. GATES[2], C. LAMY[3], M. RIVALAN[4], D. VIRAG[5];
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Universitätsmedizin Berlin, Berlin, Germany; [5]Pharmacol., Univ. of Zagreb Sch. of Med., Zagreb, Croatia

Abstract: Conducting experiments efficiently has obvious advantages for the effective pursuit of science. This requires robust methods, however, sharing of methods, protocols, and best practice often occurs in a haphazard manner via word-of-mouth, personal communications, and/or coincidental meetings. The Internet has revolutionised the possibilities for disseminating information and best practice in many fields. However, there is still - to our knowledge - no consolidated online forum for discussion and dissemination of methods related to animal behaviour experiments. To fill this gap, we have started an online forum that allows for discussion of animal experimentation techniques. We took inspiration from image.sc, a popular and well-maintained forum focusing on software-oriented aspects of scientific imaging. Our forum, named TheBehaviourForum.org, has been started and is funded by a European COST Action initiative named TEATIME, which focuses on improving home-cage monitoring techniques (www.cost-teatime.org/). While the forum is expected to have many posts relevant to home-cage monitoring, the forum's remit is broader and we envisage it containing material relating to all types of animal behaviour experiment. The forum is divided up into several categories. The Q&A category is expected to contain the bulk of the site's activity giving users the opportunity to ask and discuss any issue relating to hardware, software or experimental design. Other categories include Guides and Tutorials, Community News, Events, Meetings and Training, and Job Opportunities. The forum will include discussion of both open source initiatives and commercial systems. Commercial representatives will be allowed to use the forum but will be identified as such to avoid any conflicts of interest arising. In summary, we believe that use of this forum will improve the quality of scientific outputs, reduce disparities of access to good scientific practice (around the world), and expect it to provide clear benefits to animal welfare by encouraging both refinement and reduction in animal experiments.

Disclosures: J.E. McCutcheon: None. **H. Gates:** None. **C. Lamy:** None. **M. Rivalan:** None. **D. Virag:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.01/UU2

Topic: B.07. Network Interactions

Support: NIH Grant
Marie Curie
Gilmore Award

Title: A robust excitatory network maintains network stability in the hippocampus across 24 hour cycles

Authors: *N. OGNJANOVSKI;
Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: The hippocampus, a region of the brain critical for cognition, shows circadian rhythmicity in both clock-gene expression and synaptic plasticity. There are 24-hour cycles in hippocampus-dependent function; however, it is unknown whether those synaptic/molecular changes correlate with electrophysiological network function that might instantiate the behaviour change. Previous *in vitro* work has pointed to changes in excitatory-inhibitory balance over 24-hour cycles, but fuller characterization of network function *in vivo* has not yet been performed. Since new experimental data is converging towards a critical role of oscillations in connecting activity to sensory processes- such as light perception, we implanted tetrodes into CA1 of C57BL/6J mice to identify circadian patterns in electrical activity in the hippocampus across the day. We continuously recorded neuronal spiking, local field potentials (LFPs), and network connectivity across the 12h:12h light: dark cycle and observe the following findings:I.Daily rhythm of hippocampal neuronal firing shows ultradian inhibitory activity but stable excitatory activity II.Stable excitatory cell firing persists even when controlling for sleep states.III.Excitatory/Inhibitory (E/I) functional connectivity reconfigures on the hourly scale but is stable over 24 hoursIV.Sleep-associated NREM delta (0.5-3Hz) and sharp-wave ripples (SPWRs- 150-250Hz) as well as REM and waking theta (4-10 Hz) are temporally dynamic. Delta and SPWRs were comparatively higher during NREM occurring in the light phase.These results show for the first time the dynamic baseline structure of hippocampal electric activity. Network structure seems to reorganize over time, possibly according to the behavioral demands of the rest-activity cycle. Specifically, stability and E/I balance are shown to be indicative of a critical network state, which may indicate an optimal timing of learning in the hippocampus.

Disclosures: N. Ognjanovski: None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.02/UU3

Topic: B.07. Network Interactions

Support: NIH Grant F060746

Title: The Circadian Dynamics of Neural Criticality in Mouse Hippocampus

Authors: *D. KIRCA, P. J. HALE, D. KIM, B. O. WATSON, N. OGNJANOVSKI;
Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: It is unclear how sleep and wake impact functioning neural networks *in vivo* in the hippocampus and it is even less clear whether circadian rhythms may have a sleep-independent effect on neural dynamics. Recent work suggests neural networks near a state of “criticality” may be particularly able to both take in and out new activity patterns and so the mammalian brain may be tuned to this state. Criticality is a concept that describes the behavior of complex systems as they undergo a phase transition, and distance from criticality is measurable in neural networks by measuring specific known aspects of network function in recordings of multiple sorted neurons. Previous investigations of *in vivo* spiking dynamics revealed that hippocampal dynamics during non-rapid-eye-movement (NREM) sleep are near a critical point and that memory consolidation leads to a shift in markers of criticality. Here we apply models to define criticality states of recorded ensembles of neurons *in vivo* in the hippocampus not only across the circadian day, but also across sleep states: awake, non-rapid eye movement (NREM) sleep, and rapid-eye movement (REM) sleep. We observe that: (I) the hippocampus functions near criticality across the circadian day *in vivo*, implying that criticality may represent a homeostatic set-point. (II) Network criticality seems to be altered over smaller sub-circadian time scales, possibly according to the behavioral demands of the rest-activity cycle. (III) Hippocampal dynamics in NREM sleep are closer to criticality during the light period (when the animal normally sleeps), suggesting that criticality as an evolutionary property is optimized during an animal's rest phase. These findings indicate that the hippocampus operates near criticality at all times but modulates the degree of criticality across the day and sleep state possibly in alignment with ongoing computational needs.

Disclosures: D. Kirca: None. **P.J. Hale:** None. **D. Kim:** None. **B.O. Watson:** None. **N. Ognjanovski:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.03/UU4

Topic: B.07. Network Interactions

Support: Whitehall Foundation: Daniel Fine English 2022-12-93
Simons Foundation: Daniel Fine English 840758

Title: Dynamic coordination of respiration and cortical rhythms in innately rewarding exercise

Authors: *A. SMITH[1], C. BUHLER[1], D. F. ENGLISH[1], J. C. BASSO[2,3];
[1]Sch. of Neurosci., [2]Human Nutrition, Foods & Exercise, Virginia Tech., Blacksburg, VA; [3]Ctr. for Hlth. Behaviors Res., Virginia Tech. Carilion, Roanoke, VA

Abstract: Pranayama, a specific form of cognitively driven rhythmic breathing (CDRB), is one of the only ways to intentionally activate the parasympathetic nervous system and has for thousands of years been used to improve behavioral, emotional, and physical health. CDRB can reduce negative affect while increasing energy, muscle relaxation, and blood pressure stability. Underlying mechanisms have remained enigmatic, though recent reports in rodents have demonstrated that respiration can causally drive neuronal rhythms across a wide range of cortical regions. This finding suggests that breathing may act as a pacemaker, aiding global coordination of neuronal oscillations and potentiating synchronized, coordinated flow of information. Specifically, circuits coordinating hippocampal-cortical dialogue are heavily influenced by respiratory rhythms. Exercise is also known to be a potent driver of both respiratory and brain rhythms, and therefore we tested the hypothesis that exercise enhances the oscillatory coupling of the olfactory bulb (key in respiration) with the prefrontal cortex and hippocampus. We compared local field potential activity in these brain areas in freely behaving mice during sleep, exploration, and exercise (voluntary wheel running). Preliminary results show significant coupling of cortical and olfactory bulb activity with different coupling strengths appearing during distinct behavioral states (e.g., acquisition versus rebound running). Findings from this research will add to our understanding of how respiration and olfactory bulb activity may influence the activity of other downstream brain areas, therefore helping us to understand the biological mechanism underlying the potent effects of breath on behavior.

Disclosures: A. Smith: None. **C. Buhler:** None. **D.F. English:** None. **J.C. Basso:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.04/UU5

Topic: B.07. Network Interactions

Support: NIH Grant R01NS123069
NIH Grant R21EY033080
NSF Grant ECCS-1847436

Title: Tapered Drug delivery, Optical stimulation, and Electrophysiology (T-DOpE) probes reveal the importance of cannabinoid signaling in hippocampal CA1 oscillations in behaving mice

Authors: *J. KIM[1], E. T. GILBERT[1], H. HUANG[1], K. ARNDT[1], D. F. ENGLISH[2], X. JIA[1]; [1]Virginia Tech., Blacksburg, VA; [2]Neurosci., Virginia Tech. Neurosci. PhD Program, Blacksburg, VA

Abstract: Understanding the neural basis of behavior requires monitoring and manipulating combinations of physiological elements and their interactions in behaving animals. Here we developed a thermal tapering process (TTP) which enables the fabrication of novel, low-cost, flexible probes that combine ultrafine features of dense electrodes, optical waveguides, and microfluidic channels. Furthermore, we developed a semi-automated backend connection allowing scalable assembly of the probes. We demonstrate that our T-DOpE (**T**apered **D**rug delivery, **Op**tical stimulation, and **E**lectrophysiology) probe achieves in a single neuron-scale device (1) high-fidelity electrophysiological recording (2) focal drug delivery and (3) optical stimulation. With a tapered geometry, the device tip can be minimized (as small as 50 μm) to ensure minimal tissue damage while the backend is ~20 times larger allowing for direct integration with industrial-scale connectorization. Acute and chronic implantation of the probes in mouse hippocampus CA1 revealed canonical neuronal activity at the level of local field potentials and spiking. Taking advantage of the triple-functionality of the T-DOpE probe, we monitored local field potentials with simultaneous manipulation of endogenous type 1 cannabinoid receptors (CB1R; via microfluidic agonist delivery) and CA1 pyramidal cell membrane potential (optogenetic activation). Electro-pharmacological experiments revealed that focal infusion of CB1R agonist CP-55,940 in dorsal CA1 downregulated theta and sharp wave-ripple oscillations. Furthermore, using the full electro-pharmacological-optical feature set of the T-DOpE probe we found that CB1R activation reduces sharp wave-ripples (SPW-Rs) by impairing the innate SPW-R-generating ability of the CA1 circuit.

Disclosures: **J. Kim:** None. **E.T. Gilbert:** None. **H. Huang:** None. **K. Arndt:** None. **D.F. English:** None. **X. Jia:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.05/UU6

Topic: B.07. Network Interactions

Support: NIH Grant R01NS123069
NIH Grant R21EY033080
NSF Grant ECCS-1847436

Title: A Novel Polymer Multifunctional Probe for High-Fidelity neural recording and manipulation

Authors: *H. HUANG[1], J. KIM[1], E. T. GILBERT[1], K. ARNDT[1], X. JIA[1], D. F. ENGLISH[2]; [1]Virginia Tech., Blacksburg, VA; [2]Neurosci., Virginia Tech. Neurosci. PhD Program, Blacksburg, VA

Abstract: To investigate circuit dynamics, it is necessary to observe and control neurological activities with high fidelities. Here, we have developed a new polymer multifunctional probe using the novel thermal tapering process (TTP) enabling reliable device fabrication and high-resolution single unit recordings. This probe allows simultaneous drug delivery, optical stimulation, and electrophysiological recording. Using this probe, we recorded endogenous neuronal activities from CA1 of hippocampus in wild type mice. We additionally verified the functionality of our probe by optically stimulating the CA1 of transgenic mice. The cells were opto-tagged and observed under the influence of local cannabinoid receptor activation (CB1R; CP-55,940).

Disclosures: H. Huang: None. **J. Kim:** None. **E.T. Gilbert:** None. **K. Arndt:** None. **X. Jia:** None. **D.F. English:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.06/UU7

Topic: B.07. Network Interactions

Support: Whitehall Foundation Grant 2022-12-93
Simons Foundation Grant 840758

Title: Axo-axonic cell influence on pyramidal cell activity in CA1 sharp wave-ripples

Authors: *E. T. GILBERT[1], K. C. ARNDT[1], J. KIM[1], J. C. BASSO[2], S. A. MCKENZIE[3], G. BUZSAKI[4], D. F. ENGLISH[5];
[2]Human Nutrition, Foods & Exercise, [1]Virginia Tech., Blacksburg, VA; [3]UNM HSC, Albuquerque, NM; [4]New York University, Langone Med. Ctr., New York, NY; [5]Neurosci., Virginia Tech. Neurosci. PhD Program, Blacksburg, VA

Abstract: Memory-guided behaviors require the coordinated spike timing of hippocampal CA1 pyramidal neurons (PYR) during sharp wave-ripples (SPW-Rs). PYR spike timing is controlled by a diverse group of GABAergic interneurons (INTs). Axo-axonic cells (AACs) are thought to have precise control over PYR spike timing because they hyperpolarize the axon initial segment and can veto spike initiation. About ~50% of AACs robustly participate in SPW-R events, and we found that participation is driven by pre-synaptically paired PYRs. Together, these findings suggest that AACs may be important for controlling CA1 network activity in SPW-Rs. To

address this question, we optogenetically silenced AACs during ensemble recordings of CA1 neurons in behaving mice. Using these data, we quantified AAC activity and interactions with local PYR. We found that PYR activity during SPW-Rs is modulated when AACs are inhibited. We found that both the firing rate gain and percentage participation of PYRs in SPW-Rs increases when AACs are silenced. These results suggest that CA1 AACs are involved in lateral inhibition controlling the activity of PYRs in SPW-Rs.

Disclosures: E.T. Gilbert: None. **K.C. Arndt:** None. **J. Kim:** None. **J.C. Basso:** None. **S.A. McKenzie:** None. **G. Buzsaki:** None. **D.F. English:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.07

Topic: B.07. Network Interactions

Support: The Whitehall Foundation: Daniel Fine English 2022-12-93

Title: Extracting spikes from the noisy brain: quantifying the effects of neuronal dynamics on spike sorting quality

Authors: *K. C. ARNDT[1], E. T. GILBERT[1], L. M. KLAVER[2], E. AKBAR[5], C. BUHLER[3], J. C. BASSO[4], S. A. MCKENZIE[6], D. F. ENGLISH[7];
[1]Sch. of Neurosci., [2]Sch. of Neuroscience, English lab, [3]English Lab., [4]Human Nutrition, Foods & Exercise, Virginia Tech., Blacksburg, VA; [5]Neurosci., Virginia Polytechnic Inst. and State Univ., Blacksburg, VA; [6]Neurosciences, UNM HSC, Albuquerque, NM; [7]Neurosci., Virginia Tech. Neurosci. PhD Program, Blacksburg, VA

Abstract: The ability to simultaneously record more neurons in electrical local field potential recordings is rapidly expanding as new devices are developed and technological advances are made (tetrodes to silicon probes to Neuropixels). Spike sorting algorithms responsible for extracting action potential wave-forms from extracellular electrical signals have made similar strides to those in the devices field. However, the rigor and reproducibility of the quality and accuracy of neurons extracted by these sorting algorithms are negatively impacted by the following features. (1) Accuracy of spike detection and clustering spanning multiple behavioral states coinciding with key LFP oscillations (EMG, ripples, theta) is poorly understood. (2) Action potential wave-forms markedly change over the course of single unit and population bursts allowing for potential commission and omission errors in cluster assigned spikes. (3) the first two issues are altered by the cell types and brain regions of interest (cortex vs thalamus vs hippocampus) which these algorithms do not take into account. These issues can be attributed, in some part, to the lack of sufficiently long ‘ground truth’ data that meets statistical power requirements. Ground truth data for spike sorting consist of extracellular recordings in which a subset of neurons is also recorded with a second, unambiguous technique, such as a glass

electrode: a significant technical challenge. Ground truth data are essential for quantifying spike sorting performance and developing automatic spike sorters to replace manual methods which are time-consuming and error prone. Here we use silicon-juxtacellular hybrid microelectrode probes to obtain ground truth data in awake head-fixed mice. We found spike waveforms significantly change over the course of a burst in a non-linear fashion. This resulted in a significant depreciation of the signal to noise over the course of bursts, corresponding in an increase in omission errors. These effects were compounded by large amplitude local field potential oscillations, specifically EMG noise and sharp-wave ripples in our hippocampus recordings. Our data support the need for a growing ground truth data set across brain regions in all brain states, to accurately tune spike sorting algorithms to eventually be fully autonomous.

Disclosures: K.C. Arndt: None. **E.T. Gilbert:** None. **L.M. Klaver:** None. **E. Akbar:** None. **C. Buhler:** None. **J.C. Basso:** None. **S.A. McKenzie:** None. **D.F. English:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.08/UU8

Topic: B.07. Network Interactions

Support: Simons Foundation: 840758
Whitehall Foundation 2022-12-93
iTHRIV Scholars Program:which is supported in part by the National Center for Advancing Translational Sciences of the NIH : Julia C Basso
UL1TR003015 - KL2TR003016

Title: Hippocampal sharp wave-ripple dynamics in NREM sleep encode motivation for anticipated physical activity

Authors: *C. BUHLER[1], J. C. BASSO[1,2,3], D. F. ENGLISH[1];
[1]Sch. of Neurosci., [2]Dept. of Human Nutrition, Foods, and Exercise, Virginia Tech., Blacksburg, VA; [3]Ctr. for Hlth. Behaviors Res., Fralin Biomed. Res. Inst. at VTC, Blacksburg, VA

Abstract: Physical activity is an integral part of every mammal's daily life, and as a driver of Darwinian fitness, required coordinated evolution of the body and brain. Coordinated evolution of neuronal and somatic physiology enabled bidirectional communication between the body and brain, enhancing success in foraging and reproduction. The decision to engage in physical activity is driven either by survival needs or by motivation for the rewarding qualities of physical activity itself. Rodents exhibit innate and learned motivation for voluntary wheel running, and over time run longer and farther, reflecting increased incentive salience and motivation for this consummatory behavior. Dynamic coordination of neural and somatic physiology are necessary to ensure the ability to perform behaviors that are motivationally variable. Yet the neural substrates of internally generated motivation driving body-brain coordination are not understood.

Hippocampal sharp wave-ripples (SWRs) have evolved both cognitive and metabolic functions, which in modern mammals may facilitate body-brain coordination. To determine if SWRs encode aspects of exercise motivation we monitored hippocampal CA1 SWRs and running behaviors in adult mice, while manipulating the incentive salience of the running experience. During non-REM (NREM) sleep, the duration of SWRs before (but not after)running positively correlated with future running duration, and larger pyramidal cell assemblies were activated in longer SWRs, suggesting that the CA1 network encodes exercise motivation at the level of neuronal spiking dynamics. Inter-Ripple-intervals (IRI)before but not after running were negatively correlated with running duration, reflecting more SWR bursting, which increases with learning. In contrast, SWR rates before and after running were positively correlated with running duration, potentially reflecting a tuning of metabolic demand for that day's anticipated and actual energy expenditure rather than motivation. Furthermore, the duration of ripples with and without sharp waves had both distinct distributions and relationships to anticipated and actual physical activity, indicating distinctions in CA3 drive to CA1. These results suggest a novel role for CA1 in exercise behaviors and specifically that cell assembly activity during SWRs encodes motivation for anticipated physical activity.

Disclosures: **C. Buhler:** None. **J.C. Basso:** None. **D.F. English:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.09/UU9

Topic: B.07. Network Interactions

Support: Department of Atomic Energy, Government of India, under Project Identification No. RTI 4006
Science and Engineering Research Board, Government of India (SERB)

Title: Pattern Learning in CA1 Pyramidal Cells of Mouse Hippocampus

Authors: *A. KANNAMKULATH SHAHUL HAMEED, U. S. BHALLA;
Bhalla-Lab, NCBS-TIFR, Tata Inst. of Fundamental Res., Bangalore, India

Abstract: During context learning in rodents, the CA1 pyramidal cells (PC) in the hippocampus selectively respond to a learned context. The animal's current context will decide the pattern of synaptic input that a CA1 PC receives. This raises the question of how a CA1 PC displays a selective response to certain input patterns over others.
We address this question with the help of whole-cell patch clamp recordings made from CA1 PCs on a mouse hippocampal slice. To deliver the presynaptic input to CA1 PC we stimulate the CA3 PCs with the help of optogenetics. Past work in the lab has shown that the inputs from the CA3 PCs reach CA1 PC directly as excitatory inputs as well as via interneurons as Inhibitory inputs. We have found that with this technique, we can induce plasticity in a select set of

synapses at CA1 PCs. We select one optical pattern out of three for 'training', which is done by associating only that pattern with postsynaptic depolarization. Thus the neurons become trained to respond to this specific pattern of input as compared to other patterns.
Based on our observations, we find that the 'trained' pattern elicits a sustained potentiation, compared to other patterns which have no overlap with the trained pattern. We compared the excitatory postsynaptic potentials(EPSP) amplitudes at the soma of CA1 PCs while we presented individual points in a pattern and the pattern as a whole. This data reveals that the pattern as a whole elicits a sublinear response compared to its individual points summed up. This observation suggests that plasticity strengthens inhibitory inputs more in the microcircuit. To support this, we see the plasticity of excitatory and Inhibitory inputs on the CA1 PCs. In most cases we see the inhibitory plasticity overpowering the excitatory plasticity. We hypothesize the inhibitory inputs lead to the tuning of the CA1 PC receptive field from the CA3 PC population.

Disclosures: A. Kannamkulath Shahul Hameed: None. **U.S. Bhalla:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.10/UU10

Topic: B.07. Network Interactions

Support: ERC HOPE 951330

Title: How single GABAergic neurons shape local circuit dynamics: an all-optical approach

Authors: M. BOCCHIO[1], ***A. VOROBYEV**[2], S. SADEH[3], S. BRUSTLEIN[2], S. REICHINNEK[2], V. EMILIANI[5], C. CLOPATH[4], R. COSSART[6];
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Abstract: Inhibitory interneurons are a critical component of cortical circuits. Beyond providing inhibition, they have been proposed to coordinate the firing of excitatory neurons within cell assemblies. This function is thought to be rooted in their diversity, with a specialized role for each interneuron class. However, interneuron networks could also orchestrate cell assemblies at the population level. While many studies have dissected the function of specific interneuron subtypes, the spatiotemporal activity of groups of interneurons and its relationship to cell assemblies in vivo remain unclear. To address this question, we have developed a custom optical setup allowing us to combine 2-photon calcium imaging and holographic optogenetic stimulation at single-cell resolution. We used soma-targeted ChroME opsins to excite specific GABAergic cells with high spatiotemporal precision. Using this all-optical approach we simultaneously

recorded hippocampal interneurons and pyramidal cells in adult CA1 hippocampus in mice, and test the network influence of single interneurons with optogenetic stimulation.

Disclosures: **M. Bocchio:** None. **A. Vorobyev:** None. **S. Sadeh:** None. **S. Brustlein:** None. **S. Reichinnek:** None. **V. Emiliani:** None. **C. Clopath:** None. **R. Cossart:** None.

Poster

PSTR567. Hippocampal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR567.11/UU11

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI JP 20H03547
JSPS KAKENHI JP 20K20862
JSPS KAKENHI JP 21H00961
JSPS KAKENHI JP 22K18645
JSPS KAKENHI JP 23H01056
JST FOREST Program JPMJFR2040 (MO)
JST FOREST Program 22KJ1765 (TK)

Title: Basal forebrain-hippocampus cholinergic signaling facilitates associative learning for behavioral adjustment upon task state changes

Authors: *H. MUKOHIRA[1,2], S. ISHINO[1,2], T. KAMADA[1,2], M. OGAWA[1,2];
[2]Grad. Sch. of Med., [1]Kyoto Univ., Kyoto, Japan

Abstract: How efficiently animals adjust their behavior to changing environments is critical for survival. For example, in the face of multiple times of the absence of expected reward, animals need to learn to withhold their behavior to obtain the reward. The hippocampus (HPC) has been shown to be important for associative learning (e.g., Biane et al., 2023). However, the neural substrates that regulate HPC-mediated associative learning remain unclear. Acetylcholine (ACh) neurons in the basal forebrain have been implicated in attention and learning, and send strong projections to the HPC. We hypothesized that the ACh projections to the HPC might be critical for associative learning. To address the potential role, we trained head-restrained rats to push forward a lever to trigger presentation of an auditory cue and then pull back the lever toward their mouth to obtain a probabilistic reward from the tip of the lever (Ishino et al., 2023). One of three auditory cues was presented on each trial, associated with 100, 50, or 0% probability of reward. As rats learned the contingency, they tended to pull the lever slowly in response to cues predicting 0% reward. We recorded ACh transients in the HPC and basolateral amygdala (BLA) using an ACh fluorescent sensor $GRAB_{Ach}$ (Jing et al., 2020). We found that ACh levels in HPC increased in response to reward omission, whereas those in BLA decreased. ACh levels in HPC positively correlated with the amount of time rats kept pulling the lever in the face of reward omission. Furthermore, the correlation was stronger in the earlier stage of learning. Next, we

recorded activity of ACh neurons with single-cell level calcium imaging. Most of ACh neurons in the medial septum (MS), which mainly project to HPC, showed the similar activity patterns as observed in ACh levels in HPC. Subsequently, we recorded ACh transients in the HPC and BLA in rats performing a typical Pavlovian task, in which rats passively process reward omission and just wait for the next reward. We found that reward receipt induced higher ACh levels than reward omission in HPC. Finally, we examined the causality of the optogenetic stimulation of MS to HPC cholinergic pathway. The stimulation of cholinergic axon terminals in HPC at the time of the absence of reward facilitated the behavioral adjustment to repetitive reward omissions. These results demonstrate that MS-HPC cholinergic signaling facilitates the processing of unexpected outcomes and associative learning for behavioral adjustment upon task state changes.

Disclosures: **H. Mukohira:** None. **S. Ishino:** None. **T. Kamada:** None. **M. Ogawa:** None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.01/UU12

Topic: H.08. Learning and Memory

Title: Modeling training speed, memory capacity, energy efficiency, loss of learning and degradation in biological time-delay neural networks

Authors: *Y. TIRAT-GEFEN;
Maxwave Res. LLC, Rockville, MD

Abstract: This work discusses the modeling of biological time-delay neural networks to evaluate their learning capabilities and rate of learning compared to other neural networks without delay. We attempted to find the expected level of state observability in such biological neural nets in the presence of background random noise due to thermal and cellular noise. We go a step further by considering the effect of unlearning due to strength decay of synapses, and random and localized damage found in aging, brain trauma and injury, or degenerative diseases such as dementia and Parkinson's disease. We modeled in silico a large network of thousands of spiking neurons connected by time-delayed synapses using waveform relaxation simulation techniques originally developed for simulation of large nonlinear electrical networks, providing a more detailed modeling capability than event simulation approaches. The neurons were placed in bidimensional and tridimensional lattices. We deployed cost functions that gave preference to synapses between neighboring neurons. Time-delays due to synapse length and neuron metabolism were considered. Our models incorporated the presence of biological noise such as cellular and thermal noise. We compared multiple learning approaches used in artificial neural nets (e.g. backpropagation and gradient descent) and evaluate the training speed of different neuron configurations and learning algorithms, as well as memory capacity, and expected energy consumption. We modeled neural degradation as occurring in strokes, brain trauma, Parkinson's

disease and dementia as a mixture of random and localized loss of neurons and synapses in the lattice. We considered the effect of creating new synapses and limited creation of new neurons on degraded neural networks. We evaluated the loss of learning due to neural degradation. The loss of memory capacity, lowered training speed in degraded neural lattices were found to be dependent on the training approach and spatial configuration of the neurons. The expected energy consumption in degraded networks increased. The waveform relaxation simulation techniques were found to be effective and scalable for modeling large networks of biological time-delay neural networks.

Disclosures: Y. Tirat-Gefen: None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.02/UU13

Topic: H.08. Learning and Memory

Title: The role of groupI metabotropic glutamate receptors in dorsal striatum of rats in temporal memory formation.

Authors: *J. TANABE, R. OSAKADA, T. HATA;
psychology, Doshisha Univ., Kyoutanabe, Kyouto, Japan

Abstract: Title The role of groupImGlu receptors in dorsal striatum of rats in temporal memory formation.**Authors**Jo Tanabe[1], Ryugo Osakada[1], Toshimichi Hata [11]Doshisha University, Japan**Abstract**Time perception of a few seconds to minutes is called interval timing. Although interval timing and memorizing the duration of perceived time are thought to be necessary for adaptive behavior in animals, the neural basis of these functions remain unclear. In the striatal beat frequency (SBF) model (Matell, & Meck, 2000; 2004), glutamatergic inputs to medium spiny neurons (MSNs) in the dorsal striatum (DS) are thought to represent the duration of elapsed time. Moreover, some studies indicate that temporal memory formation requires synaptic plasticity in the DS (Dallérac et al., 2017; Macdonald, Cheng & Meck., 2012; Nishioka et al., 2022). However, a study showed that ionotropic NMDA receptors, known as a trigger of synaptic plasticities in the DS, blockade has no effect on temporal memory formation (Nishioka, 2022). Therefore, the present study conducted three experiments to examine whether metabotropic glutamate (mGlu) receptors are involved in temporal memory formation. In Exp.1, we compared the effect of three doses (0, 0.77, 1.55 µg/side) of 1-aminoindan-1,5-dicarboxylic acid (AIDA), a group I mGlu receptors antagonist, injection into bilateral DS of rats on memory formation of duration using peak interval procedure. The result showed that 1.55µg group failed to acquire short-term temporal memory. The Exp.2, however, could not replicate the result of Exp.1, showing no effect of AIDA. While rats were required to memorize 40s in the two experiments, we used differential reinforcement of response duration (DRRD) task to investigate the memorization of a shorter interval, 1.5s in Exp.3. The result again showed that AIDA

(2.54mg/kg, i.p.) has no effect on memory formation of the shorter duration. To sum up, we concluded blockade of group I mGlu receptors by AIDA in DS dose not impair temporal memory formation. Having said that, other experiments using other drugs, doses or other time scales are still needed.

Disclosures: J. Tanabe: None. **R. Osakada:** None. **T. Hata:** None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.03/UU14

Topic: H.08. Learning and Memory

Title: Multiregional neural computation and memory underlying flexible motor control in zebrafish

Authors: *E. YANG[1], J. E. FITZGERALD[1], M. B. AHRENS[2], M. ZWART[3], M. RUBINOV[4]; [1]HHMI Janelia Res. Campus, Ashburn, VA; [2]Janelia Res. Campus / HHMI, Ashburn, VA; [3]Univ. of St. Andrews, St. Andrews, United Kingdom; [4]Vanderbilt Univ., Nashville, TN

Abstract: Memory, learning, and other components of flexible behavior engage communication across brain regions with a multitude of functions. These include regions specialized in sensation and motor production, as well as networks implementing a plethora of 'intermediate' functions such as sensory integration, motor preparation, planning, and learning. We used larval zebrafish to investigate neural mechanisms of behavioral flexibility as they adapt their motor control strategies to experimental manipulations of the motosensory gain. We show that these manipulations lead to short- and long-term memories of past behavior and action-outcomes, and we find that zebrafish can adapt to nontrivial manipulations of action-outcomes. These memories are represented in distributed networks in multiple brain regions representing a variety of timescales of retention. We parse these representations in the context of sensory-to-motor transformations and assign specific brain sub-networks to specific stages along the control algorithm.

Disclosures: E. Yang: None. **J.E. Fitzgerald:** None. **M.B. Ahrens:** None. **M. Zwart:** None. **M. Rubinov:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.04/UU15

Topic: H.08. Learning and Memory

Support: ANR-17-CE37-0014 Time-Memory
LABEX CORTEX

Title: Parallel absolute and relative codes for time in the striatum, and context-dependent retiming in the hippocampus

Authors: *F. ROLANDO[1], T. W. KONONOWICZ[1,2], J.-R. DUHAMEL[1], V. DOYÈRE[2], S. WIRTH[1];
[1]Inst. des Sci. Cognitives Marc Jeannerod UMR5229, CNRS, Bron, France; [2]Inst. des Neurosciences Paris-Saclay (NeuroPSI), Univ. Paris-Saclay, CNRS, Saclay, France

Abstract: The brain tracks time to anticipate a future event, or to estimate elapsing time since a past one. How time perception is supported in the two cases, "time to an event" and "time since an event", is unknown. On one hand, it was shown that when an interval (*time*) is extended or shortened (*retime*), the neural signals allowing to track time adjusted their activity in a relative manner for the two durations with identical patterns when computed on a normalized scale. This relative code for time, adjusted as a function of expectancy, is found in many brain areas, including the striatum and the hippocampus. On the other hand, absolute codes for time, which are neural signals that do not adjust their activity between a *time* and a *retime* conditions, have rarely been reported. Here, we asked: 1) do absolute codes exist in the hippocampus or the striatum? and 2) can absolute and relative codes be found in parallel within the same brain area? To test this, we recorded single-cells activity in these two areas in two macaques as they performed a time categorisation task across different ranges. First, monkeys were tested in a "*time*" condition, where they had to discriminate 0.5, 1 and 2s. Then, we tested them in three "*retiming*" conditions: either halving the intervals (0.25, 0.5 and 1s), or multiplying them by two (1, 2 and 4s) or by four (2, 4 and 8s). We asked whether cells displayed codes relative to each range, thereby rescaling across conditions, or whether cells maintained the same pattern since the beginning of the interval regardless of the range. In the hippocampus, neurons' activity changed between *time* and *retime* conditions in a way that did not match relative nor absolute patterns, but rather in a context-dependent manner by completely altering their firing rates. In the striatum, we found two distinct sub-populations of cells. The first, relative, adjusted its activity across conditions. The second, absolute, did not adapt its activity across ranges, suggesting that they were recruited by a past event, triggering a feedforward process unrelated to the upcoming interval. When all cells were grouped together, the first second of "*retiming*" conditions could be decoded based on the activity of the first second of "*time*" condition. This suggests, for the first time, the presence of an absolute code for time in the striatum which may support a perception of elapsed time since events; complementary of a relative code allowing a prospective control of behaviour.

Disclosures: F. Rolando: None. **T.W. Kononowicz:** None. **J. Duhamel:** None. **V. Doyère:** None. **S. Wirth:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.05/UU16

Topic: H.08. Learning and Memory

Support: Brain and Behavior Research Foundation Young Investigator Grant: 2021-1-15
NIH/NIMH: 1 DP2 MH129958-01
NSF CAREER Award : IOS-2145814

Title: Examining the role of the dorsal hippocampus in context-dependent interval timing behavior

Authors: *E. BIGUS, J. HEYS;
Univ. of Utah, Salt Lake City, UT

Abstract: Animals rely on the ability to form memories of temporal durations to guide a range of adaptive behaviors. The medial temporal lobe (MTL) memory system, in particular, tracks how events unfold over seconds to minutes (i.e. interval timing), allowing animals to form memories of temporal relationships between stimuli. Still, it remains unclear how MTL structures learn and encode temporal relationships into memory. We recently found that the medial entorhinal cortex (MEC) is required to learn context-dependent temporal contingencies, demonstrating MEC plays a key role in interval timing within the MTL memory system. However, it remains unclear whether MEC is specialized in this function, or whether the downstream hippocampus is also required for timing behavior. Numerous prior studies have identified “time cells” in the dorsal hippocampus (dHPC), which fire sequentially to span a timed duration and “retime” in distinct behavioral contexts. Though dHPC time cells seem suited to track time across contexts, it remains unknown whether dHPC activity is required for context-dependent timing behavior. We are testing the hypothesis that dHPC is required to learn context-dependent temporal relationships by training mice to learn a temporal Delayed Non-Match to Sample (tDNMS) task previously shown to require MEC. To determine the necessity of dHPC, we are using DREADDs to inhibit dHPC during tDNMS learning. Our preliminary results (n = 9 control mice, n = 9 DREADD mice; male and female C57BL/6 mice) suggest that dHPC is not required for tDNMS learning, suggesting that upstream computations in MEC are sufficient for timing behavior. In a parallel series of experiments, we are using cellular-resolution Ca2+ imaging to observe the neural dynamics in CA1 neurons as mice learn and perform the tDNMS task. Based upon prior work, we expect to observe an increase in time cells and emergence of context-dependent time cell sequences with tDNMS learning. However, we anticipate that contextual time cell sequences in CA1 may not be predictive of task performance or timing behavior. Such findings would suggest that dHPC time cells serve a distinct purpose than guiding temporal judgements. Together, these experiments will provide insight into whether dHPC is required for interval timing and will inform models of how interval time is tracked and encoded within the MTL memory system.

Disclosures: E. Bigus: None. **J. Heys:** None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.06/UU17

Topic: H.08. Learning and Memory

Support: NIH Grant 3R01MH068073

Title: What does dopamine track during associative learning tasks and how does this relate to striatal firing?

Authors: *B. DECORTE[1], C. TATHAM[3], C. UPRETI[2], E. H. SIMPSON[2], P. D. BALSAM[4]; [1]Psychiatry, [2]Columbia Univ., New York, NY; [3]Psychology, Barnard Col., New York, NY; [4]Barnard Coll Columbia Univ., New York, NY

Abstract: Striatal dopamine plays a key role in reward-learning. Nonetheless, we know little regarding the variables striatal dopamine tracks or how it modulates striatal neurons to ultimately guide behavior. To address this, we developed an operant task for mice that, unlike many protocols, unconfounds the rate, probability, and number of rewards presented during an associative cue. To simultaneously measure striatal dopamine-levels and single-neuron firing during the task, we combined dopamine-sensor fiber photometry with tetrode electrophysiology in the ventral striatum—designing a novel commutator system that allows mice to move freely for long time-periods. We report three key results. First, the magnitude of striatal dopamine transients (e.g., to reward-delivery and/or cue-onset) reflects a cue's reward-rate, rather than its reward-probability or the overall number of cue-reward pairings. Second, this reward-rate coding emerges rapidly—long before mice show behavioral sensitivity to reward-rate differences. Third, dopamine-transients exert a robust effect on the firing rates of striatal neurons. Notably, this relationship is often nonlinear. Specifically, on a within-neuron basis, some striatal units show firing elevation following large dopamine transients and suppression following small transients (or vice versa). These data provide key insight into the variables that accumbal dopamine tracks during learning tasks. The results support timing-based theories that emphasize the importance of reward-rate over other factors in guiding behavior. Furthermore, our results provide crucial in vivo data addressing how dopamine modulates striatal firing, revealing the relationship between the two is more complex than often assumed.

Disclosures: B. Decorte: None. **C. Tatham:** None. **C. Upreti:** None. **E.H. Simpson:** None. **P.D. Balsam:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.07/UU18

Topic: H.08. Learning and Memory

Support: Simons Global Brain Grant

Title: A neural clock underlying the temporal dynamics of an auditory memory

Authors: *A. H. BAHLE, M. S. FEE;
Brain & Cognitive Sci., MIT, Cambridge, MA

Abstract: Songbirds acquire their songs through an imitation process reminiscent of speech acquisition in humans; juvenile songbirds form a memory of the song of an adult male tutor and gradually refine their own vocalizations to produce a mature song closely matched to the tutor song. In previous work Hahnloser et al., showed that neurons in the pre-motor nucleus HVC produce a sequence of ultra-sparse activity during adult song. Long et al., subsequently demonstrated that the timing of song is controlled by dynamics within HVC, by observing that HVC cooling slows the production of adult song. However, the key processes of tutor memory formation and recall that support imitation are poorly understood. Recent work showed that disruption of HVC or its auditory inputs and outputs during tutoring impairs birds' ability to imitate, suggesting a role for HVC in tutor memory formation and recall. Here we propose a specific model by which neural dynamics in HVC form during tutoring and act as a neural clock, storing temporal information and recalling it to guide subsequent imitation. Motivated by this hypothesis, we first recorded from neurons in HVC during tutoring and found that auditory evoked activity formed sparse sequences reminiscent of adult motor activity. Next, we tested a critical prediction of our theory: that cooling of HVC during tutoring leads to a sped-up tutor song memory, analogous to slowing the motor of a tape recorder during recording and then playing the recording back at normal speed. To test this, we designed a new modular thermoelectric cooling device and found that transient cooling during tutoring caused birds to produce faster imitations consistent with our theory. This work gives insight into the mechanism by which tutor song memories are formed and recalled and shows how memory content can be systematically manipulated during memory formation.

Disclosures: A.H. Bahle: None. **M.S. Fee:** None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.08/UU19

Topic: H.08. Learning and Memory

Support: Grant-in-Aid for JSPS Fellows (22J20301)
Grant-in-Aid for Scientific Research(C:22K03201)

Title: Cholinergic interneurons in dorsal striatum play an important role in an acquisition of new duration-memory.

Authors: *M. NISHIOKA[1,2], T. HATA[3];
[1]Psychology, Grad. of Doshisha Univ., Kyotanabe, Japan; [2]JSPS, Tokyo, Japan; [3]Psychology, Doshisha Univ., Kyotanabe, Japan

Abstract: Formation of duration memory is important for optimizing timing of their behavior based on their experiences, but complete picture of this neural mechanism is still unclear. Our previous work revealed that muscarinic acetylcholine 1 receptors (M1R) in dorsal striatum play a role in consolidation of duration memory in rat (Nishioka et al., 2022). ChIs may be also involved in formation of duration memory, because M1R receptors are activated by cholinergic interneurons (ChIs) in dorsal striatum. Thus, we investigated the effect of immunotoxic ChIs lesion in dorsal striatum on formation of duration memory. We trained rats using peak interval (PI) 20 s procedure in an operant chamber with a lever and food magazine. This procedure consisted of food trials and empty trials: The formers were fixed interval (FI) -20 s schedule (i.e., an initial lever-press response after 20 s from the starting of a trial was reinforced), the latters were terminated after 80 s with no reinforcement. In the last session of PI-20 s training, mean response rate curves as a function of elapsed time of empty trials had a peak around 20 s. They then received injection of ChAT-Saporin, a neurotoxin of ChIs, or aCSF into dorsal striatum. To make the rats acquire new-duration memory, we conducted a PI-40 s session (i.e., food trials were FI-40 s schedule) 72 hours after infusion. This is why cell death would be induced sufficiently within 72 hours. In that session, the peak of the response curve of aCSF group located around 30 s, whereas around 20 s in ChIs-lesioned group. As PI-40 s trainings progressed, both groups' response curves overlapped each other, having peaks around 40 s. In following re-shift sessions to PI 20 s, both groups of response curves moved leftward in the same way. In another cohort, the response curves overlapped between pre- and post-ChIs lesion in PI 20 s training. Together, these results suggest that ChIs lesion impaired only an acquisition of new duration memory, but not an adjusting their behavior depending on changing reinforcement schedule of PI-training and temporal perception itself.

Disclosures: **M. Nishioka:** None. **T. Hata:** None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.09/UU20

Topic: H.08. Learning and Memory

Support: NSERC

Title: Modeling temporal duration sequence memory acquisition in rodents and humans

Authors: *Z. WU, S. PROMPIENGCHAI, A. LEE, R. ITO;
Psychology, Univ. of Toronto, Scarborough, ON, Canada

Abstract: Rodent research has played a significant role in shaping our understanding of how the human brain processes temporal information in the service of memory. Yet, limited work has explored how temporal memory compares behaviourally between these two species. In the current study, we developed a novel cross-species behavioural task to examine the qualitative and quantitative similarities and differences between rat and human temporal duration sequence learning. Across a number of training days, Long Evans rats (n = 16; 8 female) learned to identify, via a left/right lever press, two distinct auditory sequences, each comprised of a pure tone and white noise of differing durations (Sequence A: Tone [1s]- Noise [4s]; Sequence B: Tone [4s]- Noise [1s]). In parallel, human subjects (n = 38; female =21; age = 18-45 years) learned over a series of trial blocks to identify, via a key press, two groups of two different sequences (Sequence 1A: Tone [0.5s]- Noise [4.5s]; Sequence 1B: Tone [3.5s]- Noise [1.5s]; Sequence 2A: Tone [4.5s]- Noise [0.5s], Sequence 2B: Tone [1.5s]- Noise [3.5s]). We then developed a computational model to characterize the temporal learning dynamics of each participant and to identify the source of inter-participant variability. This model comprised of two components: (1) Bias, in which a cubic spline function captured the extent to which a participant's responding is biased towards one lever/key in the early phases of learning; and (2) Learning, in which a sigmoid function was implemented to provide insight into each participant's learning process, including learning onset, speed and outcome. We found that although both rats and humans were able to successfully learn the different temporal duration sequences, there were significant cross-species differences. The majority of human participants acquired all sequences equally throughout learning (i.e., minimal preference) while rats were biased towards one sequence over the other early on. Moreover, rats had a longer learning latency and slower learning speed compared to humans. Our findings shed light on how rodents and humans acquire temporal duration memories and lay the foundation for future work to investigate the neural mechanisms of temporal duration sequence learning.

Disclosures: Z. Wu: None. **S. Prompiengchai:** None. **A. Lee:** None. **R. Ito:** None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.10/UU21

Topic: H.08. Learning and Memory

Support: Brain and Behavior Research Foundation Young Investigator Grant: 2021-1-15
NIH/NIMH: 1 DP2 MH129958-01
NSF CAREER Award : IOS-2145814

Title: Neural network underlying time cells in medial entorhinal cortex in flexible timing task

Authors: *H.-W. LEE[1], C. E. MARTINEZ-NAVARRO[2], J. G. HEYS[1];
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Abstract: The medial entorhinal cortex (MEC) is well known for its role in spatial memory, due to the presence of various spatially modulated cell types, including grid cells, head direction cells, and border cells. Additionally, a growing body of evidence suggests the involvement of MEC in interval timing behavior. Perturbation of MEC impairs the behavior performance when associating elapsed time with a specific response, and time cells showing firing at a specific moment during an interval are observed in MEC. However, neural mechanisms responsible for generating time cell activity in MEC remain unclear. Accumulating computational and empirical evidence indicates that a continuous attractor network (CAN) is responsible for generating grid cell activity. This network model allows populations of grid cells to estimate the travelled distance by integrating speed and head direction inputs into the local recurrent network (i.e., path integration). By providing a constant input instead of speed and directional input, the same network can be used to estimate elapsed time. Thus, we hypothesize that both time cells and grid cells emerge from the CAN. To test the hypothesis, we developed a timing task by modifying a delayed-non-match-to-sample (DNMS) task where mice are required to report whether pairs of sequentially presented odor stimuli are match or mismatch in duration. We confirmed the necessity of MEC in learning this temporal DNMS task by chemogenetically silencing MEC (n = 15 DREADD; n=16 control). Using two photon calcium imaging to measure neural dynamics in the tDNMS task we found that 41% of cells were identified as time cells that fire selectively at particular moments during each trial. As an initial test of the CAN model, we measured signal and noise correlations as mice performed the tDNMS task. We found that the correlation structure of pairs of MEC time cells measured during the tDNMS task was coherent during non-task relevant periods, such as the inter-trial interval. This correlation structure suggests that the activity of MEC time cells is governed by a hard-wired recurrent network, and is consistent with predictions of a CAN model. In our on-going experiments we have used single unit electrophysiological recordings with Neuropixels in order to test whether MEC grid cells and MEC time cells arise from similar recurrent network topology. Together, this work will reveal the circuit mechanism underlying the sequential activity of MEC time cells and determine whether distance and duration could be computed using the same local recurrent CAN.

Disclosures: H. Lee: None. **C.E. Martinez-Navarro:** None. **J.G. Heys:** None.

Poster

PSTR568. Timing and Temporal Processing II

Location: WCC Halls A-C

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Program #/Poster #: PSTR568.11/UU22

Topic: H.08. Learning and Memory

Title: Exploring the Role of Stress Hormone Concentration Changes in Episodic Memory Segmentation.

Authors: *M. NAMBU, K. Z. TANAKA;
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Abstract: Episodic memory is a type of memory that involves past subjective experiences and events. This type of memory is linked to our sense of time and the ability to place events in a temporal context (Tulving, 1972). However, the underlying mechanisms by which each piece of episodic memory is tagged with temporal information are not well understood.
One of the possible neuronal substrates for such temporal segmentation of episodic memory is representational drift. Ca^{2+} imaging for several weeks from the hippocampal pyramidal cells of freely moving mice revealed their receptive fields (place fields) showed higher correlation when two episodes are close in temporal distance compared to those remote in time (Ziv et al., 2013; Robing et al.,2015). These observations suggest that the degrees of drifting serve as timestamps for representations of episodic experiences, but 1) whether this phenomenon is a passive decay of the representation or an active remapping of place fields and 2) whether the drift of spatial map is causally linked to temporal segmentation of the episodic memories remain to be determined. In the present study, we experimentally test the idea that circadian fluctuations of stress hormones produce representational drift over days in the hippocampus. Past studies demonstrated that aversive experiences cause remapping in the hippocampal place cells (e.g., Moita et al., 2004; Wang et al., 2012). Moreover, an increase in stress hormones substantially impacts the hippocampal physiology and dendritic structures, leading to reduced stability of place cells (Chattarji et al., 2015; Tomar & McHugh, 2022; Park et al., 2015). We hypothesize that cortisol awaking response (CAR), an elevated stress hormone immediately after waking, causes representational drift over days and serves as timestamps for episodic memories. To test this idea, we examine the stability of the hippocampal place cells while monitoring the blood corticosterone concentrations through non-invasive automated blood collection. Through the simultaneous monitoring of stress hormone and hippocampal representation, we determine if an awake-associated increase of stress hormone produces hippocampal remapping and behavioral discrimination of temporally segregated episodes. Together, these experiments will determine the physiological role of representational drift and provide a novel mechanism underlying the temporal segmentation of episodic memory.

Disclosures: M. Nambu: None. **K.Z. Tanaka:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR568.12/UU23

Topic: H.08. Learning and Memory

Support: start up funds

Title: Multimodal Temporal Pattern Discrimination in a Mouse Model of Fragile X Syndrome

Authors: *M. E. LEE[1], W. MOL[1], S. POST[2], A. GOEL[3];
[1]Univ. of California, Riverside, Riverside, CA; [2]UC Riverside, Riverside, CA; [3]Psychology, UCR, Riverside, CA

Abstract: Timing is a critical ability across species and behaviors: prey must feint predators at precisely the right moment, drivers must judge whether or not to go through a yellow light, and language users must produce sequences of syllables in a temporally structured manner. Therefore, an important hallmark of learning, is being sensitive to and remembering the temporal structure of events, so that we can make expectations and guide our future decisions. As a result, it's not surprising that disruptions in timing and timed performance are associated with a number of neurological disorders such as Parkinson's, Schizophrenia, and Autism. Specifically, disorders of speech perception in Autism and Fragile X Syndrome (FXS) are linked to temporal processing deficits. Currently mechanisms that allow neurons in the brain to represent and learn temporal structure of events, specifically in the sub-second range, is unclear. To examine neural mechanisms that contribute to subsecond timing, we designed a go/no-go timing task using patterns of subsecond audio-visual stimuli in mice. Our data shows that wild-type (WT) mice learn to preferentially lick to the preferred pattern and withhold licking for the non-preferred pattern, reflected in a dprime>1.5 and pattern-specific changes in licking profile. Combining 2-photon calcium imaging in awake-behaving mice performing the timing task, we found that, while naive sessions showed overlap in neural trajectories, learned sessions showed trajectories that indexed stimulus type and trial outcome, suggesting that distinct functional neural populations developed with learning. Compared to WT mice, Fmr1 KO mice (a well-established mouse model of FXS) exhibit impaired and delayed temporal discrimination learning. Our data suggests that local modification of cortical dynamics (likely by changes in E-I balance) contributes to learning the task. An imbalance in E-I is recognized as a central defect that contributes to the symptoms of FXS and ASD. Therefore, we are currently examining disruption in E-I that contributes to impaired learning on the timing task in Fmr1 KO mice. By combining cutting-edge tools with simple behavior, we will not only provide fundamental information about neural mechanisms of timing but also guide future therapies.

Disclosures: M.E. Lee: None. **W. Mol:** None. **S. Post:** None. **A. Goel:** A. Employment/Salary (full or part-time):; UCR.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.13/UU24

Topic: H.08. Learning and Memory

Support: CONACYT: A1-S-8430
UNAM-DGAPA-PAPIIT IN201721

Title: Amodal population clock in the primate medial premotor system for rhythmic tapping

Authors: *A. BETANCOURT-VERA[1], O. PEREZ[2], J. GAMEZ[3], G. MENDOZA[1], H. MERCHANT[1];
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Abstract: The neural substrate for beat extraction and response entrainment to rhythms is still unknown. Here we analyzed the population activity of hundreds of medial premotor neurons of monkeys performing an isochronous tapping guided by brief flashing stimuli or auditory tones. The animals showed a strong bias towards visual than auditory metronomes, with rhythmic tapping that was more precise and accurate on the former. The population dynamics shared the following properties across modalities: the circular dynamics of the neural trajectories formed a regenerating loop for every produced interval; the trajectories converged in similar state space at tapping times resetting the clock; the tempo of the synchronized tapping was encoded in the trajectories by a combination of amplitude modulation and temporal scaling. Notably, the modality induced a displacement in the neural trajectories in auditory and visual subspaces without greatly altering time keeping mechanism. These results suggest that the interaction between the MPC amodal internal representation of pulse and a modality specific external input generates a neural rhythmic clock whose dynamics governs rhythmic tapping execution across senses.

Disclosures: A. Betancourt-Vera: None. **O. Perez:** None. **J. Gamez:** None. **G. Mendoza:** None. **H. Merchant:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.14/UU25

Topic: H.08. Learning and Memory

Support: NIH R01MH116043
NIH R01NS120987

Title: External Globus Pallidal neurons encode temporal information

Authors: *V. KHANDELWAL[1], A. BOVA[1], K. SIVAKUMAR[1], J. KRYCA[1], N. S. NARAYANAN[2];
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Abstract: Timing refers to the choice of *when* to act. Timing is fundamental to behaviors such as obtaining food, capturing prey, and evading predators, as well as to human activities such as communication, driving, cooking, and crossing the street. Although time-based decisions are important for higher-order cognitive functions and are impaired in many brain diseases, the basic mechanisms of timing are unclear. Prior work by our group and others have shown that Medium

Spiny Neurons (MSNs) in the dorsomedial striatum encode temporal information through ramping, a monotonic change in firing rate over time. Manipulating both MSNs expressed D1-type dopamine receptors (D1-MSNs) or D2-type dopamine receptors (D2-MSNs) powerfully affects performance of interval timing task, in which mice estimate the passage of time by making motor responses. Critically, both D1-MSNs and D2-MSNs project to the external globus pallidus (GPe), an area hypothesized to play an important regulatory role in basal ganglia motor output. We sought to characterize the role of the GPe in interval timing. In mice, we recorded from neuronal ensembles in the GPe using microwire arrays during interval timing. . We found that neurons in the GPe encode temporal information through ramping, similar to striatal MSNs. We then asked if disrupting MSN input to the GPe would attenuate ramping activity and timing behavior. We optogenetically inhibited D2-MSNs terminals in the GPe while recording from GPe ensembles. We investigated how manipulation of striatal inputs disrupts temporal encoding in GPe ensembles. We will further explore GPe populations and projections. Our work may lead to a better understanding of basal ganglia circuits in elementary cognitive processing and help guide novel therapeutic interventions for disorders of dopamine like Parkinson's disease.

Disclosures: V. Khandelwal: None. **A. Bova:** None. **K. Sivakumar:** None. **J. Kryca:** None. **N.S. Narayanan:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.15/UU26

Topic: H.08. Learning and Memory

Support: R01NS120987
R01MH116043

Title: Efficacy of Terazosin on ameliorating behavioral deficits related to Alzheimer's Disease in amyloid (5XFAD) mice

Authors: *B. KIRKPATRICK[1], Q. ZHANG[1], N. S. NARAYANAN[2];
[1]Iowa Neurosci. Inst., Iowa City, IA; [2]Neurol., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA

Abstract: Energy deficits are a major associated feature with Alzheimer's Disease (AD). Terazosin is an alpha-1 blocker and is widely used to treat benign prostatic hyperplasia and hypertension. Terazosin was recently found to act as an activator of phosphoglycerate kinase-1 (PGK-1), the first ATP-generating enzyme in glycolysis. Based on analysis of 2 large retrospective clinical datasets (the Truven/IBM Medicare claim dataset and the Alzheimer's disease neuroimaging initiative (ADNI) dataset), Terazosin could not only enhance glycolysis/ATP generation in preclinical models of AD, but also potentially slow the progression of AD in humans. 5XFAD mice (knockout gene mice that express 5 genes linked with AD) were

used as a model for AD due to the many AD-related phenotypes associated with the transgenic mice such as inability to remember tasks as well as other wild-type (WT) mice or capacity to perform instinctual tasks such as nesting during later stages of life compared to WT mice. 5XFAD transgenic mice were given 1.2mg/kg/day of Terazosin through water starting at approximately 5 weeks of age and were trained on a switch-time task at around 6 months of age. 10 mice (5 males and 5 females) were assigned to each group (Transgenic mice with Terazosin, Transgenic mice without Terazosin, Non-transgenic mice with Terazosin, and Non-transgenic mice without Terazosin). After approximately 6 months of data collection from the switch-time task, the mice were perfused with PBS and their brains dissected to remove and stain the hippocampus with APP antibody 6E10 to highlight amyloid proteins in the region. Those stains were then blindly scored for comparison. Terazosin treatment for the transgenic mice not only improved switch-time task performance compared to transgenic mice without treatment, but also was associated with a lower associated CA1 scoring of the hippocampus. Our research is exploratory, but it appears to indicate that enhancing glycolysis through Terazosin could potentially have preventative effects for Alzheimer's disease

Disclosures: B. Kirkpatrick: None. **Q. Zhang:** None. **N.S. Narayanan:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.16/UU27

Topic: H.08. Learning and Memory

Support: R01NS120987
R01MH116043

Title: Substantia nigra dopamine and temporal encoding activity of striatal neurons

Authors: *R. VOLKMAN[1], A. BOVA[2], V. KHANDELWAL[2], K. SIVAKUMAR[1], N. S. NARAYANAN[3];
[2]Univ. of Iowa, [1]Univ. of Iowa, Iowa City, IA; [3]Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA

Abstract: Time-based decision making requires nigrostriatal dopamine signaling. Temporal control of action is critical for daily behavior and is disrupted in Parkinson's Disease (PD), a neurodegenerative disease that disrupts dopamine neurons in the substantia nigra pars compacta (SNc). Prior work has shown that disrupting striatal dopamine disrupts interval timing, a task that requires working memory for temporal rules and attention to the passage of time. SNc neurons send large dopaminergic projections to the striatum that is largely comprised of Medium Spiny Neurons (MSNs). These MSNs are critical for encoding temporal intervals and exhibit time-related 'ramping' activity throughout temporal intervals, which is defined as a monotonic change in neuronal firing rate over time. However, it is unknown how SNc dopamine signaling

modulates MSN temporal activity. To address this question, we optogenetically inhibited or stimulated SNc dopamine cell bodies while recording MSN ensemble activity as mice performed an interval timing task. Preliminary data suggest that optogenetic manipulation of SNc dopamine cell bodies modulates timing behavior. Additionally, ongoing work is investigating how optogenetic inhibition of SNc dopamine cell bodies modulates striatal MSN activity during timed intervals. These results will help us understand the role of SNc dopamine on striatal projection neuron activity and have implications for PD-related deficits in timing and cognition.

Disclosures: R. Volkman: None. **A. Bova:** None. **V. Khandelwal:** None. **K. Sivakumar:** None. **N.S. Narayanan:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.17/UU28

Topic: H.08. Learning and Memory

Support: DOD Grant PD20037

Title: Deploying intersectional strategies to investigate prefrontal D1-interneurons and pyramidal neurons

Authors: *A. OPPMAN[1], M. OYA[1], N. S. NARAYANAN[2], Y. KIM[1];
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Abstract: The mesocortical dopamine pathway is implicated in cognitive processes like working memory, attention, and interval processing. Previous research suggests that prefrontal D1-neurons are essential to cognitive functions such as interval timing and working memory performance. D1-type dopamine receptors (D1DRs) are found on both interneurons and pyramidal neurons, however the roles of each subpopulation in executive function remains unknown. We leveraged viral intersectional genetic approaches to manipulate prefrontal D1-interneurons. In line with our past work, we found that cell-type-specific optogenetic inhibition of prefrontal D1-interneurons alters interval timing performance. Additionally, we developed and partially characterized a D1-flp transgenic mouse who exhibits site-specific expression of flp recombinase within D1-containing neurons, which presents a new opportunity for targeting D1-pyramidal neurons. We performed stereotaxic surgical injections with conditional AAV vectors, employed immunohistochemistry techniques, and implemented cross breeding strategies to investigate the co-localization of D1DRs with flp. Together, these results advance our understanding of the roles of subpopulations of D1 neurons in executive function, which is relevant to Parkinson's disease and other neurodegenerative diseases.

Disclosures: A. Oppman: None. **M. Oya:** None. **N.S. Narayanan:** None. **Y. Kim:** None.

Poster

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Program #/Poster #: PSTR568.18/VV1

Topic: H.08. Learning and Memory

Support: NIH Grant NS 120987
NIH Grant MH 116043

Title: Medial prefrontal projections in interval timing

Authors: *X. DING[1], M. A. WEBER[2], A. BOVA[2], E. TABAKOVIC[2], N. S. NARAYANAN[2]; [1]Neurol., Univ. of Iowa, Iowa city, IA; [2]Univ. of Iowa, Iowa City, IA

Abstract: Medial prefrontal projections in interval timingAuthors Xin Ding, Matthew A. Weber, Alexandra S. Bova, Ervina E. Tabakovic, Nandakumar S.Narayanan**Abstract**Cognitive impairments are a common symptom of many neurological diseases, includingAlzheimer's disease (AD) and Parkinson's disease (PD). The medial prefrontal cortex (mPFC) iscrucial for normal cognitive control and impaired activity in this brain region may contribute tocognitive symptoms related to different neurological diseases. Interval timing is a cognitive controlprocess of tracking the passage of time over seconds-to-minutes that is commonly impaired inpatients with neurodegenerative disease. Our lab has shown that neurons in both the mPFC andthe dorsomedial striatum (DMS) display similar patterns of time-related ramping activity – ormonotonic changes in firing rate – during a timed interval. We aimed to study interval timingrelated patterns of activity in two specific mPFC projections: 1) mPFC to DMS projections and 2)mPFC to mediodorsal thalamus (MD) projections. To investigate these projections, we used fiberphotometry to record calcium dynamics in projection-specific neuronal cell bodies. First, inseparate cohorts of C57BL/6 mice, we unilaterally injected retrograde-Cre in either the DMS orthe MD. We then injected Cre-dependent GCaMP6s and implanted optic fibers in the mPFC. Micewere then trained in an interval timing task in which they much switch from one response port toanother based on the internal representation of time. Mice well-trained in this task are thencoupled to fiber photometry LEDs via a commutator to record calcium dynamics from mPFCneurons that project to either the DMS or the MD. My preliminary data suggest that calciumdynamics in mPFC-DMS neurons were distinct from mPFC-MD neurons during interval timing.mPFC-DMS neurons were modulated at trial start and display strong negative modulation atreward delivery, while mPFC-MD neurons displayed a strong positive reward-related signal. Ourfindings provide anatomical and functional evidence that mPFC-DMS neuronal activity maypredict when animals respond in time while mPFC-MD neurons may be related to goal-directedbehaviors during the interval timing task. Together, these findings inform the function of mPFCprojections during interval timing, which will provide a new fundamental understanding of time-based cognitive processes. These findings could enhance our understanding of the pathologicalmechanisms of cognitive dysfunction in disease and is essential for the development ofneuromodulation therapies for neurological disease.

Disclosures: **X. Ding:** None. **M.A. Weber:** None. **A. Bova:** None. **E. Tabakovic:** None. **N.S. Narayanan:** None.

Poster

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Topic: H.08. Learning and Memory

Support: NIH Grant 1 T35 HL 166206-1

Title: Role of mediodorsal thalamic projections in temporal processing

Authors: *E. TABAKOVIC[1], M. A. WEBER[2], N. S. NARAYANAN[3];
[1]Iowa Neurosci. Inst., Iowa City, IA; [2]Dept. of Neurol., Univ. of Iowa, Iowa City, IA; [3]Univ. of Iowa Hosp. and Clinics, Iowa City, IA

Abstract: Parkinson's disease (PD) can present challenging cognitive symptoms that dramatically impact quality of life. Cognitive deficits are observed in the majority of PD patients but remain understudied with few treatment options. Interval timing, or the estimation of time over seconds-to-minutes, recruits multiple modalities of cognitive control such as attention and working memory. This cognitive function is vital for guiding everyday human behavior and is dysfunctional in many PD patients. Our lab has extensively studied the rodent medial prefrontal cortex (mPFC) which is crucial for normal cognitive processes in the context of interval timing. However, we have not yet studied how circuits projecting to the mPFC are involved. Mediodorsal (MD) thalamic projections to the mPFC may contribute to interval timing due to its involvement in both the basal ganglia pathway and mesocorticolimbic system, linking circuits with primary roles in movement and motivation, respectively. We will record MD thalamic neuronal ensembles to investigate how MD thalamus neurons and field potential encode time. Next, we will inactivate MD thalamic projections to the prefrontal cortex and investigate how inactivating these projections impact interval-timing behavior as well as temporal processing within the mPFC. Overall, these experiments will inform our understanding of temporal processing in MD thalamic projections to the mPFC. We will interpret these data in the context of normal cognitive control and how this circuit may contribute to cognitive symptoms in neurodegenerative disease.

Disclosures: **E. Tabakovic:** None. **M.A. Weber:** None. **N.S. Narayanan:** None.

Poster

PSTR568. Timing and Temporal Processing II

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Program #/Poster #: PSTR568.20/VV3

Topic: H.08. Learning and Memory

Support: R01MH116043
R01NS120987

Title: Frontal cortex RNA sequencing in a Parkinsonian mouse model

Authors: *K. SIVAKUMAR[1], M. EVANS[2], M. A. WEBER[1], B. HING[2], H. E. STEVENS[2], R. HULTMAN[3], N. S. NARAYANAN[1];
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Abstract: Parkinson's disease (PD) patients experience cognitive symptoms that significantly reduce their quality of life. Although deep brain stimulation alleviates motor symptoms, there are only a few minimally effective treatments for PD-related cognition. Interval timing, or the ability to estimate time over seconds, is commonly impaired in PD, and lower cognitive scores are associated with greater interval timing variability. Our prior work has shown that depleting dopamine in the rodent ventral tegmental area (VTA) increases interval timing variability, and optogenetically stimulating the frontal cortex ameliorates interval timing deficits. However, the frontal cortex cell types that have the most potential for reducing timing variability remain unclear. A recent human study showed that 40 genes, notably *STAT1*, *CALB2*, and *FOXP2*, are differentially expressed in the frontal cortex between living PD patients and controls with essential tremor. Interestingly, decreased *STAT1* expression correlated with decreased low-frequency frontal cortical brain rhythms, which has been shown to predict cognitive dysfunction in PD. However, it is unknown whether changes in cortical gene expression are related to pathological dopamine loss in the midbrain. Exploring the specific cell types that undergo these genetic changes can inform future studies aimed at relieving cognitive symptoms of PD. I performed single-cell RNA sequencing in the frontal cortex of a 6-hydroxydopamine (6-OHDA) Parkinsonian mouse model. We bilaterally depleted mesocortical dopamine in the rodent VTA using 1 µg/µL 6-OHDA. Control mice received equivalent volumes of saline injected in the VTA. Each group contained four mice that were 16 weeks old and counterbalanced across sex. Six weeks after saline or 6-OHDA injection, we obtained single-cell suspensions of the frontal cortex and cryopreserved them in a Neurostore freezing medium while histologically confirming the depletion of tyrosine hydroxylase positive neurons in the VTA. We utilized Trypan Blue staining to estimate cell counts and processed the samples using the 10X Genomics Single Cell protocol. We interpreted these data in the context of PD-related cognitive symptoms, which may also inform other neurological and psychiatric illnesses characterized by frontal cortex dysfunction. These findings are particularly useful in mapping the genetic landscape that underlies PD-related cognition and informing future behavioral paradigms that utilize cell-type specific manipulations.

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Poster

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Topic: H.08. Learning and Memory

Support: R01: NIH/NIDCD R01 DC-018561

Title: Transcriptional changes within the adult auditory system contribute to lasting experience dependent neuroplasticity of auditory temporal processing.

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Abstract: Temporal cues in speech are prominent perceptual cues for understanding language sounds. The ability to appreciate spectrotemporally rich speech sounds relies on learning-dependent processes in the cortex. Auditory cortical neuroplasticity can establish memories for the language-relevant acoustic cues that help the auditory system to "tune in" to salient timing cues in the auditory soundscape by enhancing sound-evoked temporal processing for learned salient cues. Recent work has demonstrated that blocking an epigenetic regulator of neuroplasticity, histone deacetylase 3 (HDAC3) promotes long-term memory formation for highly specific temporal features of acoustic cues in animals learning an amplitude-modulation (AM) discrimination task (Rotondo & Bieszczad 2021). The mechanism of HDAC3 action is thought to be on de novo gene expression events that are required for memory. Thus, we capitalized on an opportunity to determine genes and biological pathways that may be important for efficient and high-fidelity temporal cue learning and processing in the auditory cortex (AC). We performed bulk RNA-sequencing (RNA-seq) on adult auditory cortical samples in trained rats treated with an HDAC3 inhibitor (vs. a group of vehicle-treated trained rats) learning the same established AM rate discrimination task. We further utilized single-nucleus RNA-sequencing to determine cell-type specific patterns of experience-induced transcription with a particular focus to uncover transcriptional contributions in non-neuronal cell types such as astrocytes, which are a critical component of the tripartite synapse and have been implicated in disorders which have auditory processing difficulties such as autism spectrum disorder. The present work demonstrates that auditory associative learning results in changes to the adult auditory transcriptome that, combined with prior work in the lab, is now known to be task-dependent (Graham et al., bioRxiv). There are 28 unique genes that were differentially expressed (vs. vehicle). These gene sets include *Homer1* and *Shank2*, which regulate excitatory synapse morphogenesis and play a critical role in synaptic plasticity. Bioinformatic analysis was used to determine gene network analysis and protein-protein interactions. This analysis revealed three functional clusters in learning-induced auditory cortical genes: glutamate receptor binding, cellular response to interferon alfa, and amine ligand-binding receptors. To our knowledge, this is the first snRNA-seq dataset in adult AC. Revealing gene targets for temporal processing informs mechanisms of speech sound impairment or developmental language disorders.

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Poster

PSTR569. Cortico-Hippocampal Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR569.01/VV5

Topic: H.09. Spatial Navigation

Support: IBS-R015-D1

Title: Spatial learning enhances hippocampal and medial entorhinal whole-brain connectivity measured by optogenetic fMRI

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Abstract: Recent research has revealed that the visual cortex and parietal cortex show spatial representations that are influenced by experience (Diamanti et al. 2021, Sato et al., 2016). These findings suggest that the neocortices may play a significant role in spatial memory. However, how the hippocampus (HPC) and the medial entorhinal cortex (MEC), which are well-established as crucial brain regions for processing spatial information, interact with multiple neocortical regions to support spatial learning remains poorly understood.In this study, we used optogenetics with functional magnetic resonance imaging (ofMRI) to investigate the evoked connectivity (EC) of HPC and MEC in the whole brain. Wild-type C57BL/6 mice (male, n = 12) were injected with a retrograde AAV (AAV-hSyn-hChR2(H134R)-eYFP) in the right HPC (AP: -2.2 mm ML: 1.6 mm, DV: 1.3 - 2 mm) and implanted with optic fibers (diameter: 200 mm, NA = 0.37) in the left HPC and the right MEC (AP: -4.2 mm, ML: 3.4 mm, DV: 3.2 mm). After six weeks of recovery period, the first ofMRI scan (TR/TE = 1000/11.5 ms, 0.132 X 0.132 mm^2, 18 0.5-mm slices) was conducted in the 15.2T MRI scanner, where optogenetic stimulation (3-5mW, 30 Hz, 20% duty cycle) of the HPC or MEC was given in a block design (20s stimulation, 60s recovery, repeated twice per trial, 10 trials per condition) followed by a single ten-minute resting-state trial. After the scan, mice belonging to the training group (n=8) were trained for 2-3 weeks on the active place avoidance task (APAT) using a custom-built maze (Lee et al., 2022), while the control group mice were left untrained. On each day, two 30-minute trials were conducted with a 90-minute break between them. In each trial, the animal was placed inside a rotating maze, and if it entered the predetermined shock zone, a foot shock (500ms, 0.2mA) was given. The shock zone was relocated once the mouse had undergone a minimum of four sessions and received fewer than five shocks in the last three 5-minute intervals. Once the mice had learned four different shock zones, the second ofMRI scan was conducted in an identical manner to the first scan.Based on our data, initial HPC EC was stronger with sensory and parietal cortices while MEC EC was stronger with higher-order cortices (e.g. mPFC, ACC). Compared to the pre-training responses, post-training responses to HPC stimulation were significantly

increased in the contralateral hemisphere within the HPC. The MEC EC showed significant increases in post-training responses in subregions within the medial temporal lobe (DG, CA1, subiculum, lateral entorhinal cortex, and perirhinal cortex), as well as the retrosplenial cortex, anterior cingulate cortex, and the medial and lateral septum.

Disclosures: C. Lee: None. **T. You:** None. **G. Im:** None. **S. Kim:** None.

Poster

PSTR569. Cortico-Hippocampal Interactions

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Title: A place with a view - Organizing space through saccades and fixations between primate posterior parietal cortex and hippocampus

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Abstract: Human primarily use vision to explore and guide actions in space. It is known that posterior parietal cortex (PPC) provides a map of visual space to guide saccades to salient cues, while the hippocampus provides a memory-based cognitive map of the environment. How does the visual map interface with the cognitive map during navigation? To probe the link between view and place, we compared neural activity in the intraparietal sulcus, and hippocampus of macaques navigating in a virtual maze. When analyzed as a function of animal's position in the virtual environment, more neurons displayed spatial selectivity in the PPC than in the hippocampus. We hypothesize that PPC would support self-position, along with the hippocampus, via the processing of environmental visual cues through explorative saccades and fixations. Indeed, neural selectivity to "place" in both regions appeared to result from saccades and fixations directed to salient landmarks and routes during virtual navigation. First, we observed a population of parietal cells whose saccade-related responses differed according to monkeys' position into the maze. However, we showed that position-selectivity did not solely correlate with simple oculomotor dynamics. Instead, it would rather be driven by sensori-motor contexts of the task. Second, parietal cells and, to a lesser extent, hippocampal ones, were driven by viewing behavior, and divided into two populations that preferentially responded to direct

fixation of landmarks, or of maze paths (i.e. landmarks in the periphery of the visual field). The parietal landmarks cells displayed a higher activity when the monkey's eyes directly fixated a landmark, while the hippocampus ones were less impacted by the precise position of the visual cue on the fovea. Thirdly, we demonstrated that spatial selectivities arose from this recruitment of the cells encoding specific visual cues, providing a task-relevant segmentation of the maze. Finally, a great part of PPC and hippocampus cells responded to the appearance of the landmarks on screen, and some even expressed selectivity to features such as their side of appearance or identity. At the population level, both regions showed an anticipation of the landmarks appearance, suggesting the existence of a cognitive map of the spatial layout, and an active part in memory-directed visual exploration. Overall, our results support a dynamic flow of activity between the parietal cortex and the hippocampus, organised along directed saccades and fixations towards anticipated landmarks, at strategic positions, leading to a contextual, task-based processing that ultimately links action and objects in space and memory.

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Poster

PSTR569. Cortico-Hippocampal Interactions

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Program #/Poster #: PSTR569.03/VV7

Topic: H.09. Spatial Navigation

Title: Diverse, state-dependent coupling between cortical activity patterns and the activity of hippocampal and thalamic neurons

Authors: *C. LEWIS[1], A. HOFFMANN[2], S. BERRY[2], L. MEIENBERG[2], T. YASAR[3], M. YANIK[3], F. HELMCHEN[2];
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Abstract: Adaptive behavior is enabled by the dynamic coordination of diverse signals across spatial and temporal scales. We combined extracellular recording of subcortical activity using flexible multi-electrode arrays with wide-field or 2-photon calcium imaging of cortex to reveal aspects of large-scale dynamics invisible to standard, single-modality approaches. We investigated the relationship between fast dynamics recorded with chronically implanted multi-electrode arrays with simultaneously acquired cortical activity patterns monitored via the expression of GCaMP6f in transgenic animals. We find diverse state-dependent patterns of coupling between concurrently acquired hippocampal and thalamic spiking activity and calcium dynamics across dorsal cortex. The repertoire of activity patterns in single hippocampal and thalamic neurons is stable across days. However, within a recording session the activity of subcortical neurons exhibits distinct patterns of brain-wide coupling dependent on changes in behavioral state, as well as ongoing, intrinsic variations in brain state. The topographical patterns of coupling between the activity of subcortical neurons and cortex activation are anatomically

specific and fluctuate over long time scales (10s of seconds to minutes) in a frequency-dependent manner. We believe that these diverse, dynamic activity patterns reflect shifts in functional connectivity that underlie distinct functional modes of brain-wide coordination and communication. The combined application of electrical and optical methodologies provides a powerful tool with which to monitor and perturb brain-wide activity patterns. We believe the refinement of combined approaches will continue to reveal previously inaccessible and under-appreciated aspects of coordinated dynamics in the brain.

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Poster

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Title: Strategy associated oscillatory coupling in the mPFC-HPC and mPFC-PPC axis during spatial memory formation

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Abstract: Spatial memory formation is manifested as the progression from less-to-moreefficient navigation strategies across memory acquisition. This process requires the gradual integration of several sets of information represented in distributed neural networks as the hippocampus (HPC), the parietal cortex (PPC) and the prefrontal cortex (PFC). It is suggested that large-scale integration of information in the brain is supported by oscillatory coupling. We aim to investigate the frequency-dependent coupling of mPFC, HPC and PPC in a rodent model during a spatial memory formation task. We observed that during goal-directed navigation, animals implemented sequentially two well discriminated behaviors: searching the goal and exploring of the arena. Interestingly, mice gradually incremented their efficiency during the searching stage. During learning, mPFC-HPC coupling was dominated by theta band (6-12Hz), whereas mPFC-PPC was observed mainly in the delta band (2-5 Hz). Furthermore, prefrontal high-gamma activity (60-120 Hz) was comodulated by both HPC and PPC at their respective low-frequency bands. Interestingly, we observed that both delta and theta prefrontal HPC/PPC coupling was

strongest during the searching stage, which was gradually incremented according to efficiency of navigation strategy. These results suggest navigation strategy associated differential and complementary frequency-dependent modulation among brain regions involved in spatial memory formation.

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Poster

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Topic: H.09. Spatial Navigation

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Title: Predictive Sequence Learning in the Hippocampal Formation

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Abstract: The hippocampus of rodents receives sequences of sensory inputs from the cortex during exploration and then encodes the sequences with millisecond precision despite inter-regional transmission delays. Our study linked such temporal precision to the cognitive functions of hippocampus in a self-supervised recurrent neural network that was trained to predict its next input. The model exhibited localized place cells and experimentally observed features such as one-shot learning, replay and phase precession. We tested and confirmed the assumption that area CA3 is a predictive recurrent autoencoder by analyzing the spike coupling between simultaneously recorded neurons in hippocampal subregions. These results imply that the place field activity of neurons in area CA1 report temporal prediction error, which decays with familiarity.

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Poster

PSTR569. Cortico-Hippocampal Interactions

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Title: The role of the direct entorhinal-hippocampal pathway to CA1 in the emergence of a novel spatial representation

Authors: *O. M. T. CHADNEY[1], M. GUARDAMAGNA[1,2], F. STELLA[2], L. A. L. DESCAMPS[1], F. P. BATTAGLIA[2], C. G. KENTROS[1];
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Abstract: The entorhinal cortex (EC) is the interface between a wide diversity of cortical regions and the hippocampus (HPC). The EC processes spatial information used to shape a stable representation of self-location in downstream hippocampal place cells, enabling accurate navigation. In response to environment change, firing patterns of place cell ensembles reorganise orthogonally, a phenomenon called remapping, thus storing unique neural representations of space (Muller and Kubie, 1987). The EC is thought to trigger hippocampal remapping and grid-place transformational models provide insight regarding how global hippocampal remapping may arise. Recent studies propose that the direct projection from EC layer III (ECLIII) neurons to CA1 drives dendritic plateau potentials, essential for nonlinear integration of both CA3 and EC inputs, leading to the formation of place fields (Bittner et al., 2015). How populations of CA1 place cells, the output region of the HPC, rapidly remap and form a representation of a novel environment remains unclear. To empirically examine the role of the ECLIII-CA1 projection in the formation of a novel environment representation, we performed population recordings of CA1 place cells combined with specific optogenetic inhibition of local ECLIII presynaptic terminals during periods of exploration, leaving firing patterns of cell bodies in the EC and the trisynaptic pathway intact. CA1 activity was recorded while mice explored a familiar environment without (F1) then with (F2) inhibition of ECLIII input. Next, mice were exposed to a novel environment (N1) while direct entorhinal inputs were inhibited, before being placed back into the familiar (F3) and novel (N2) environments with intact entorhinal input. Interestingly, our results reveal that remapping of place cells upon exposure to the novel environment (N1) was impaired. Indeed, a subset of place cells did not change their preferred firing location, in contrast, control animals that did not express the inhibitory opsin, exhibited global remapping. Conversely, spatial coding in the familiar environment (F2) was largely unaffected by our

manipulation. In addition, place fields in the novel environment (N1-N2) tended to be less stable without ECLIII input. Our findings suggest that the generation of novel spatial memory representations in CA1 relies on the ability to integrate and compare incoming inputs of previously stored representations (via CA3) with updated environmental information (via ECLIII). The direct entorhinal input may provide a novelty-related signal driving the necessary plasticity for the stable emergence of a novel environment representation in CA1.

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Poster

PSTR569. Cortico-Hippocampal Interactions

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Contrat Doctoral FIRE Doctoral School

Title: Hippocampus-cortex communication and global brain hemodynamics during hippocampal ripples observed with functional ultrasound imaging

Authors: *A. BERGEL[1,2], M. MATEI[3], S. PEZET[3], A. PEYRACHE[1], K. BENCHENANE[2], M. TANTER[3];
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Abstract: Episodic memories are thought to be first encoded in the hippocampus and progressively consolidated in the neocortex. During sleep and rest periods, neuronal traces are reactivated at the time of hippocampal sharp-wave ripples (SWRs) which are thought to mediate memory consolidation (Girardeau et al 2009). Though SWRs have been widely studied within the hippocampus, the activity in the rest of the brain during hippocampal reactivations remains elusive. Observing the global brain activity during these events is technically challenging: electrophysiology cannot easily resolve the whole brain and optical recording techniques have limited access to brain tissue. In a 2012 elegant study, Logothetis and colleagues used event-triggered functional magnetic resonance imaging to demonstrate that, during SWRs, hippocampal-cortex interactions occur over a background of subcortical silence. We used the emerging modality functional ultrasound (fUS) imaging to monitor brain activity during NREM sleep in rats, over a series of coronal and sagittal slices, spanning more than 2/3 of total rat brain volume. These recordings reveal the precise spatiotemporal (150 microns, 200 milliseconds)

dynamics of brain cerebral blood volume before, during and after SWRs in both cortical and subcortical structures. We confirm that SWRs are consistently followed by robust vascular activations in the dorsal hippocampus and association cortices, particularly in the retrosplenial and prefrontal cortices, peaking 1.5 to 2 seconds after peak ripple time, which is consistent with the delays of neurovascular coupling. We did not observe significant activations in subcortical structures. Analyzing the diversity of SWRs events revealed that the degree of hippocampal-cortex coupling was stronger for longest ripples and largest ripples (though more moderately), but not for faster ones. Interestingly, SWRs occurred at specific of rhythmic low-frequency (0.15 Hz) vascular activity suggesting that brain hemodynamics could modulate the probability of occurrence of SWRs. Taken together, our findings confirm that SWRs correspond to episodes of increased hippocampal-cortex interaction in rats and provide a detailed view of their global spatiotemporal dynamics and variability at unprecedented resolution.

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Poster

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Title: Generation of position correlated activity in primary sensory cortices requires bottom-up inputs

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Abstract: Semantic memory and abstraction of knowledge from experience are thought to involve communication between the hippocampus and neocortex. The hippocampus encodes novel episodes using sparse and orthogonal neural representations, which form and stabilize in ~10 minutes of exploration of a novel environment. Recent studies have shown that several neocortical areas, including the superficial layers of retrosplenial cortex (RSC), primary somatosensory cortex (S1), and primary visual cortex (V1) contain sparse neural coding

correlated to spatial location in one-dimensional environments. This spatial coding depends on an intact hippocampus, at the time of learning of a new environment, but can be expressed in familiar environments without hippocampal influence. We recently found that RSC pyramidal cells, like hippocampal neurons, rapidly formed spatial representations in novel (virtual) environments, but only in the presence of stable visual cues. This suggests that a correspondence between bottom-up sensory and spatial (top down) signals is required to form spatially correlated activity in both hippocampus and neocortex. In contrast, in some instances, distance correlated sequences form in the hippocampus in the absence of any changing external cues (e.g. when running on a stationary treadmill). Therefore, we asked whether cortical areas receiving different bottom-up inputs would be equally capable of forming position-correlated activity in the presence of visual cues alone. We used 2-photon imaging to simultaneously study activity in RSC, V1, and S1. We predicted that V1, which receives inputs from the optic nerve via the thalamus, and RSC, which is directly connected to V1, would be able to form an association between visual cues and top-down spatial inputs, and thus express consistent spatial activity, while S1 would not. We found that indeed the activity of S1 neurons could not decode position in a familiar visual environment as well as simultaneously recorded V1 and RSC neurons. Therefore, we conclude that the expression of position-linked memory indexes in neocortex requires a consistent correspondence between unique modality-specific cues and spatial information from hippocampus during learning.

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Poster

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Title: Accelerated new item learning and altered population coding after exposure to multiple prior experiences

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Abstract: Humans continually acquire new knowledge and skills throughout life without forgetting what they've previously learned (catastrophic forgetting). Complementary learning systems theory suggests that our brain employs complementary systems to achieve this. Hippocampus (HC) enables rapid acquisition of new knowledge, while neocortex (NC) supports the assimilation of context-free structured knowledge (schemas) over longer periods. During

sleep or awake rest, HC triggers the replay of recent experiences in NC. Simultaneously, NC retrieves and interleaves prior knowledge, and this joint replay allows learning new items without forgetting. Previous work with deep neural networks showed that replaying highly similar old items with new items significantly speeds up learning (Saxena et al., 2022). Additionally, learning speed increases as the distance between old item representations grows, requiring fewer old items to be replayed. Building on this, we hypothesized that a knowledge-rich brain with orthogonal item representations would exhibit faster new item learning, better performance, and sparser, higher-dimensional population activity. We used environmental enrichment as a proxy for a knowledge-rich brain. The animals were made to run on either a control (CT) or enrichment track (ET) for 10 weeks. The ET animals were exposed to a unique configuration of objects daily, allowing for a rich repertoire of experiences. After 10 weeks, both groups of animals were injected with a virus with Cre-dependent expression of hM3Dq receptor in Parafacial Zone (PZ) GABAergic neurons, which allowed rapid induction of Slow-wave sleep (SWS). We recorded single-neuron spontaneous activity for atleast 2.5 hr of awake period and 16 hrs of induced SWS from the hippocampus (CA1, DG) and retrosplenial and secondary motor cortex using 256-channel Silicon probe arrays. We found an increase in population sparsity and dimensionality in both cortices but not in CA1 of enriched animals. Interestingly, we noticed a decrease in the number of incoming and outgoing excitatory-excitatory (E-E) connections per excitatory neurons and an increase in outgoing and incoming inhibitory (I-E) connections per interneuron for enriched animals compared to control. These results suggest that exposure to complex experiences produces much more orthogonal, sparse, and high-dimensional population coding, perhaps due to altered functional synaptic coupling (increased number of inhibitory connections). The higher-dimensional population coding allows for more orthogonal item representation, enhancing discrimination and enabling rapid learning of new items.

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Poster

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Topic: H.09. Spatial Navigation

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Title: Enriched Training in Adult Mice Develops a Long-lasting Memory for Objects

Authors: *E. GHANBARIAN, B. L. MCNAUGHTON;
Univ. of California Irvine, Irvine, CA

Abstract: Enriched training in adult mice develops a long-lasting memory for objects
Authors*E. Ghanbarian, B.L. McNaughton; Dept. of Neurobiology and Behavior & Center for Neurobiology of Learning and Memory, University of California Irvine, CA, USA**Disclosures**

E. Ghanbarian: None, **B.L. McNaughton**: None.**Abstract**Cognitive reserve is known as the main protective factor against age-related cognitive decline. To study cognitive reserve in animals, we have developed a new model of environmental enrichment that has shown superior and longer-lasting effects on several cortical and hippocampal memory tests compared to the standard home cage enrichment. One group of adult male mice (6-8 months, n=5) ran on the enrichment track, which was a square track, loaded with several complex objects made with different textures and materials. The control group (n=5) ran on the control track, which was similar to the enrichment track but loaded with simple, repetitive ramp-shaped objects. We trained the mice on the enrichment/control tracks for one hour per day for three months. When the subjects were middle-aged (13-15 mo) and old (22-24 mo) we imaged CA1 neural activity using head-mounted one-photon miniscopes during free exploration of a familiar and a novel environment. We found that fraction of active cells and firing rate (calcium event rate) of cells in the enriched mice were lower in both familiar and novel environments. We also tested their memory performance with a quick schema memory retention task, in which they had to explore familiar or new objects to reach a reward zone. The results showed that the enriched group finished the task faster than the control group both with familiar (4.28 ± 1.92 *vs* 14.12 ± 2.2 min, respectively; $p<0.001$) and novel objects (41.84 ± 10.60 *vs.* 75.11 ± 8.65 min, respectively; $p= 0.03$). These findings indicate that our enrichment model, when started in adult mice, develops a memory for objects (schemas) that lasts into older ages.

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Poster

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Title: Closed-loop gain control of visual landmarks reveals that pre/parasubiculum neurons are more closely bound to visual input than medial entorhinal cortex neurons

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Abstract: Animals combine environmental landmarks and self-motion inputs to navigate their surroundings. The process of using self-motion inputs to derive an estimate of position is called

path integration (PI) . PI is a noisy computation that accumulates errors, which can be corrected by landmarks. Recent studies in CA1 place cells have shown that landmarks can act as a teaching signal to recalibrate the gain of the PI system when the system is confronted with sustained conflicts with landmarks (Jayakumar, Madhav et al., 2019). The medial entorhinal cortex (MEC) is believed to be the locus of the PI computation in the medial temporal lobe (McNaughton et al., 2006). However, it is unknown whether and where landmark information exists in the medial temporal lobe to correct for PI errors. We performed simultaneous tetrode recordings from MEC and neighboring medial temporal lobe regions, presubiculum (PrS) and parasubiculum (PaS), in Long-Evans rats (n=3) under conditions of persistent conflict between landmarks and self-motion inputs. This conflict was produced by gradually rotating an array of landmarks in a virtual reality environment as a function of the rat's speed, producing an illusion that the rat was moving slower or faster than its actual speed. In a subset of sessions where a large conflict was introduced, a striking dissociation between MEC and PrS/PaS neurons was observed: the firing fields of all MEC neurons broke away from landmarks while the firing fields of all PrS/PaS neurons remained strongly tied to the landmarks (4 sessions, 21 PrS/PaS neurons, 98 MEC cells). This strong control of landmarks over PrS/PaS neurons persisted even under extreme landmark manipulations, such as sudden jumps in the closed-loop gain controlling landmark movement. Furthermore, the firing rates of these PrS/PaS neurons were strongly correlated to the brightness of the landmarks, and consequently about ~30% of these cells stopped firing in the absence of landmarks. In open-field recordings, ~75% of these cells had single/multi-lobed head-direction tuning curves. These results show that the MEC neurons reflected a combination of landmarks and self-motion inputs , whereas PrS/PaS neuronal responses were solely driven by landmarks. It is known from prior work that PrS/PaS neurons provide extensive monosynaptic synaptic inputs to MEC (Canto et al., 2012), and lesions of dorsal PrS results in degradation of landmark control over head direction cells in the anterior dorsal thalamic nucleus (Goodridge et al., 1997). Taken together with these prior studies, our findings point to the possibility of PrS/PaS neurons carrying the landmark information to the MEC to enable the correction of PI errors.

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Poster

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Title: Three-axis adjustable Neuropixels microdrive for semi-chronic multilocation recordings in freely moving animals

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Abstract: Multi-channel silicon probes, such as Neuropixels, offer a significant advantage over traditional electrophysiology techniques, providing high density recordings over a large depth range. For chronic recordings, these probes are typically inserted and cemented in place with a single use for each probe. For some brain regions, this approach results in high-quality single unit recordings over weeks to months. However, such signal quality and stability are not consistent across all brain regions. In the lateral entorhinal cortex (LEC) of rats, for example, electrical signal-to-noise degrades rapidly (hours to days), resulting in difficulty isolating single unit activity after the normal surgical recovery period. To circumvent these challenges and maximize the ability to perform high-quality and high-yield recordings, we developed a 3D-printed implantation system that allows the repeated insertion and extraction of silicon probes for chronic freely moving behavioral recordings while also providing the ability to adjust the position of the silicon probe along three axes. A post is cemented onto the skull onto which a microdrive with a 12.7mm range at 0-20° relative to the DV axis is securely attached at positions that can be adjusted 600 µm in AP and 1.4 mm in ML axes. For comparison, prior studies (Hargreaves, 2005; Deshmukh, 2011; Wang, 2018) targeting the LEC with independently movable tetrodes that were slowly advanced over the course of several weeks typically yielded a total of 30-40 well-isolated single units with manual spike sorting across the deep and superficial layers of LEC (~1.5 mm). This relatively low yield was the result of the small extracellular spikes typical of LEC neurons, compared to the much larger spikes of the hippocampus (Hargreaves, 2005). We have successfully implanted 8 animals with a total of 31 insertions of Neuropixels 1.0 probes in the LEC, with one animal successfully and stably implanted in 11 separate locations along the ML and AP axes. On average, each probe insertion resulted in a yield of manually sorted, well-isolated units in a single session that was comparable to the tetrode recordings performed over many weeks. The ability to remove, refurbish, and reinsert the probes allowed for many more neurons and behavioral sessions to be recorded than with chronically implanted Neuropixels probes. Thus, this method vastly increases the yield of high-quality data from an individual animal by allowing multi-location sampling and greatly increases the efficiency of data collection in single animals.

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Poster

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Title: Automated vs. manual spike sorting: Assessing the quality of automated spike sorting using grid cells

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Abstract: Spike sorting is crucial to isolate ‘clusters', putative individual neurons, from extracellular recordings that comprise noisy signals from many neurons. Traditionally, this process has relied on manual procedures. Recently, there has been a notable shift towards automated approaches. Automated spike sorting reduces the human intervention, but nevertheless requires manual curation of the sorted output that involves making categorical decisions, such as accepting, rejecting, or merging clusters. This pipeline of automated sorting followed by manual curation yields more clusters in less time than manual sorting. However, due to the unsupervised nature of the spike sorting, it is challenging to determine how automated sorting compares to manual sorting in terms of accurate identification of true single neurons and the degree of contamination present in the clusters.
To address this challenge, we took advantage of the distinctive firing pattern of grid cells in the medial entorhinal cortex (MEC), neurons whose rate maps form a hexagonal lattice with regular spacing and orientation as a rat moves through an environment. By employing both manual sorting (WinClust, a custom, in-house spike sorting program) and a popular automated sorter (MountainSort4, Chung et al., 2017), we identified grid cells in tetrode recordings from two Long-Evans rats. Automated sorting found more grid cells than manual sorting (n = 31 vs 21 in two sessions). We then compared rate maps and spike waveforms of a specific subset of automatically and manually identified cluster pairs (n = 19), presumed to belong to the same grid cells. We found that rate maps of automatically identified clusters were more contaminated ($p = 0.051$), as measured by the relative firing inside and outside the hexagonal firing fields of grid cells. The increase in the out-field firing of automatically identified clusters was strongly correlated with greater variability in their spike waveforms ($r2 = 0.81$), indicating contamination of the underlying grid cells from other neurons or recording noise (Pouzat et al., 2002). To mitigate the variability in spike waveforms, we removed outlier spikes relative to the mean waveform of each cluster, as suggested by Hill et al. (2011). This additional semi-automated curation step improved the automated sorting in terms of both waveform and behavioral contamination to levels comparable with the manual sorting. Our results suggest that, although automated sorting yields more clusters, they might suffer from higher contamination compared to manual sorting. Thus, meticulous curation is essential, particularly when characterizing single neuron responses.

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Poster

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Title: A non-conventional form of landmark control over path integration: N-to-M entrainment of CA1 place cells to virtual cues in a 1D circular track

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Abstract: Hippocampal place cells use idiothetic cues (i.e., path-integration) and landmark cues to create and update a representation of the animal's environment and its location in it. Prior work shows that the cues are integrated when the error between the two is small, with landmarks being weighted more. When the error grows larger, the influence of landmarks decreases until the map appears to be driven purely by path integration (McNaughton et al, 1996, Knierim et al. 1998). The mechanisms underlying the interaction between path integration and landmarks are not well understood. To examine this interaction, we recorded CA1 cells from Long Evans rats as they ran unidirectional laps on a circular track in a virtual reality apparatus (Jayakumar*, Madhav* et al., 2019; Madhav et al., 2022). Visual landmark cues projected on the periphery of the track were moved in the same or opposite direction as the animal as a function of its movement. In most sessions, the place field map was anchored by landmarks, always maintaining a 1:1 periodicity relative to the cues. In other words, the location in the internal map was updated by one lap for every lap the animal completed relative to the landmarks. However, in sessions where the place field map broke away from this 1:1 relationship (i.e., the map drifted relative to the landmarks), it often exhibited a range of complex yet consistent relationships with the landmarks, maintaining precise rational N:M periodicities relative to the visual cues for extended durations. This rational relationship means that the location of the animal in its internal map was updated by N laps for every M laps the animal completed relative to the landmarks. Notably, all but two of the observed rational ratios fit the pattern of N being the number of landmarks and M being a low integer: three landmarks (13 sessions) exhibited ratios of 3:6, 3:5, 3:4, 3:2, 3:1, and 2:1; four landmarks (2 sessions) exhibited 4:5, 4:2; two landmarks (1 session) exhibited 3:2. We analyzed this phenomenon in the framework of a ring attractor network and

found that the experimentally observed N:M periodicities are indeed theoretically possible but require spatially inhomogeneous feedback from landmarks, such as if the strength of visual drive is modulated by the animal's proximity to landmarks. Assuming such proximity-dependent visual drive, we found that, like the patterns seen in the data, the network exhibits stable regimes at different periodicities depending on the number of landmarks. These results show a non-conventional anchoring of CA1 place cells by visual cues beyond the common situation of a one-to-one mapping, pointing to a complex interaction between path integration and landmarks.

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Poster

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Title: Responses of retrosplenial cortex head direction cells to dynamic visual cue conflicts

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Abstract: Integrating external sensory inputs into cognitive representations of the environment is essential for animals to navigate through the environment. Head direction (HD) cells function as an internal compass by firing persistently when animals face a specific allocentric direction. The achievement of stable HD representations can be explained by a theoretical HD "ring attractor" model, in which neurons are conceptualized as forming a ring architecture that can sustain a stable, bump-like activity pattern through recurrent circuitry. External inputs, such as stable visual landmarks, can reset the location of the HD bump when the rat re-enters a familiar environment and can keep the bump of the HD ring attractor aligned with the external world by dynamically correcting errors caused by angular path integration. However, experimental data related to how landmark information forces the attractor bump of the HD ring to reset to a new location are limited. Here we performed visual cue manipulations to create continuous conflict between the location of a high-precision landmark (a vertically oriented bar with sharp luminance edges) and a low-precision landmark (a diffuse, gaussian-modulated luminance profile with no sharp edges) under dynamic, feedback control when the rat moved freely in a virtual reality apparatus (called "the Dome"). We introduced dynamic cue conflicts by moving the landmarks relative to each other at different speeds based on the animal's movement along a

circular track in the Dome. We recorded from retrosplenial cortex from 2 Long-Evans rats. Analysis of 166 neurons from one rat over 10 sessions revealed that 28 of these cells were head direction cells in a prior, open-field recording session. When the high-resolution and low-resolution landmarks were placed in conflict in subsequent session in the Dome, 10 cells were controlled by one or the other cue, whereas 18 cells were controlled by the midpoint between the two cues. Thus, retrosplenial head direction cells appear to act heterogeneously, as some cells are controlled by the specific landmarks while other cells are controlled by the center of mass of the two landmarks. This result contradicts a simple Bayesian framework, which predicts that the high-precision cue should dominate over the low-precision cue. However, consideration of such variables as differential motion parallax between the two cues may suggest that the putative "high-precision" cue is actually less reliable compared to the "low-precision" cue for purposes of orienting the HD cell system, requiring a more sophisticated model of visual cue processing than is typically present in standard HD ring attractor models.

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Poster

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Title: Predictive firing of place cells before gap-crossing behaviors

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Abstract: Place cells are neurons in the hippocampus that fire when an animal occupies a particular space within its environment. A subset of place cells, called splitter cells, encode the same location differently depending on the route taken to or from that location (Wood et al. 2000). As shown previously (Lashkari, Woronowicz et al., SFN 2022), individual cells show splitter-like phenomena by differentiating 3D trajectories during a gap-crossing behavior. However, the predictive nature of place cell activity during this biomechanically challenging task remains unknown.
To investigate the firing properties of place cells during such behaviors, we studied Long-Evans rats (n = 3 male) as they ran across a linear track with an adjustable gap in the middle. The

animals crossed the gap back and forth to get to the reward locations at each end of the track. When crossing, rats had to decide to either jump over the gap ("jumping") or leap into and out of the gap ("ditching"), choices that generated distinct 3D trajectories but similar 2D projections onto the horizontal plane. The reward was not contingent upon the animals' current or prior decisions.

We used Neuropixels 2.0 probes to record the neural activity of CA1 and CA3 cells in sessions with both jumping and ditching behavior and found that some place cells encoded each of these trajectories differently. For example, in one session (n = 50 CA1/CA3 cells), 16 cells had firing field locations in the gap region, of which 12 had differing firing rates during jumping versus ditching. We used a Bayesian decoder to determine whether the population of place cells contained predictive information about whether the rat was about to jump or ditch. We trained the decoder using 80% of the data, labeled as either jump or ditch based on the final decision in each trial. The decoder utilized the average firing rates of the place cells, within the time interval from 3 to 0.5 seconds before takeoff, as its input to classify whether the animal jumped or ditched on a given trial. It was then validated on the remaining 20% of the data to predict the animal's actions using Bayesian inference. The decoder had accuracies ranging from 65% to 87% for different sessions and/or animals, and the accuracies were significantly higher ($p = 0.02$) than chance.

In conclusion, our study provides evidence of predictive firing in place cells before gap-crossing behaviors. Using a trained Bayesian decoder, we predicted the animal's behavior solely based solely on place cells' average firing rates prior to takeoff, despite the rat being in the same location before jumping or ditching. These findings suggest that place cells encode predictive information before complex locomotor behaviors.

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Poster

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Title: Conflicting external and internal positional cues distort theta phase precession

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Abstract: Theta phase precession by hippocampal place cells is one of the most well-studied forms of phase coding. As an animal traverses through a place field, the place cell fires at progressively earlier phases of the LFP theta oscillation. Place cells can also exhibit phase procession towards the end of the field (Wang et al., 2020). However, the underlying mechanisms of phase coding remain unknown. In this study, we investigated how two types of spatial cues, allothetic landmarks and idiothetic cues, influence the spike timing within a theta cycle. Using a planetarium-style VR environment (the "Dome"), in which an array of visual landmarks projected onto the wall of the dome was rotated as a function of the rat's movement, we created a persistent conflict between the two types of spatial cues. The motion of the visual landmarks was controlled by an experimental gain, G, which specified the ratio of the animal's speed in the landmark frame to its speed in the lab frame. In G > 1 sessions, landmarks moved in the direction opposite to the rat's movement, causing an illusion that the rat was moving faster than it actually was; the reverse was true in G < 1 sessions. We monitored the activity of place cells (n = 261) from hippocampal CA1 as 5 male, Long-Evans rats ran around a circular track inside the Dome. In 40/51 sessions, place fields were stable in the rotating landmark frame; consequently, the fields enlarged or shrank in the lab frame under conditions of G < 1 and G > 1, respectively (Jayakumar, Madhav et al., 2019). Averaged over all laps, phase precession was maintained in the landmark frame under all gain conditions, even though the size of the fields in the lab frame varied greatly. We then investigated whether phase coding was maintained in single traversals through the fields. The slopes of single-trial precession were calculated through circular-linear regression and showed a bimodal distribution with modes at -160 and +150 [°/ field size]; negative slope trials often exhibited precession throughout the traversal, while positive slope trials often showed an initial phase precession in the later phases of theta followed by phase precession or procession in the earlier phases, thus producing a two-lobed average phase precession plot. The second lobe disappeared as G deviated from 1 in either direction, which increased the error between the spatial inputs provided by the allothetic and idiothetic cues. We hypothesize that phase precession in the later phases of theta is robust to conflicts between the two spatial inputs, whereas coherence of the two is required for spiking in the earlier phases of theta as the rat progresses through the latter part of the place field.

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Poster

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Title: Medial prefrontal cortex neuronal representations and ensemble patterns in sequence memory

Authors: *V. ROLDAN[1], A. VASALLO VELIZ[1], S. LEYVA[1], M. JAYACHANDRAN[2], T. A. ALLEN[1];
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Abstract: The medial prefrontal cortex (mPFC) provides top-down control of memories for sequences of events, which are otherwise acquired and stored via the hippocampus (HC) (Dolleman-van der Weel et al., 2019). Importantly, mPFC and HC are critical to sequence memory (Allen et al., 2020; Jayachandran et al., 2019), and are highly synchronized during and after retrieval events (Jayachandran*, Viena* et al., 2022). While it is known that hippocampal CA1 neurons represent nonspatial sequential contexts and have feature-conjunctive properties (Allen et al., 2016; Shahbaba et al., 2022), less is known about the mPFC whose neurons represent outcome monitoring, decision making, and prediction in other tasks (Alexander & Brown, 2011; Luk & Wallis, 2009; Moorman & Aston-Jones, 2015). Here, we recorded single-unit activity from the prelimbic region of mPFC in rats performing a nonspatial sequence memory task that included well learned, switched, and novel odor sequences. We first investigated mPFC neuronal representations with respect to odors, positions, sequential contexts, and trial accuracy across three task phases: before, during, and after the odor sampling/memory demand period. The results show that a subset of mPFC neurons coded for odors and positions during and after memory demands (G-test p's < 0.05), but we did not observe a significant number of mPFC neurons that differentiated sequential contexts (i.e., in or out of sequence) as reported in CA1. Instead, we found that mPFC neurons coded for trial accuracy most commonly after a decision (G-test p's < 0.001). Interestingly, most mPFC neurons were conjunctive during this time window and reflected information across all task domains. We also found more mPFC neurons were active during well-learned, compared to new, odor sequences. Next, we organized mPFC neurons into ensemble matrices and performed representational similarity analyses across odors, positions, and across different odor sets. We found that different populations of mPFC neurons were active before, during, and after a memory trial, and that ensembles coded for specific odor-position relationships with a great degree of fidelity. However, unlike reports in CA1, mPFC ensemble representations generalized extensively to other odor-position conjunctions and odor sets. These results suggest mPFC provides a complementary set of representations to HC supporting memory for sequences of events and may be particularly geared toward that generalization and feedback.

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Poster

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Title: High frequency ripple activity in the rodent medial prefrontal cortex and hippocampus during a non-spatial sequence memory task

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Abstract: High frequency ripple activity in the brain represents synchronized multi-unit synaptic activity and is often studied in rodents in the form of sharp-wave ripples (SWRs). SWRs are restricted to the CA1 region of the hippocampus and occur during sleep, decision making and reward. Spiking in SWRs contains information about past and future events in a time compressed manner and are thought to provide a rapid retrieval mechanism contributing to real-time decision making or feedback in cortex. However, it is unknown when and how ripple activity outside of the CA1 relates to rapid memory retrieval or performance feedback in memory-based decision making. Here, we examined ripple activity from the medial prefrontal cortex (mPFC) and hippocampus (HC) in a non-spatial sequence memory task that drives mPFC-HC interactions (Jayachandran et al., 2019). Briefly, rats were trained on an odor sequence task that required them to demonstrate sequence memory by correctly identifying in sequence (InSeq) and out of sequence odors (OutSeq). Rats indicated that odors were InSeq by holding their noses in the odor port for one second, or indicated odors as OutSeq by withdrawing in less than a second. After training, we recorded local field potentials (LFPs) and single-unit activity from mPFC and HC while rats perform the sequence task (Jayachandran*, Viena* et al., 2023). We found ripple activity (100-250Hz) occurred reliably in both mPFC and HC precisely timed to the offset of beta bursts (1) just before a decision and (2) just after poke out. Ripples occurred at a similar latency in the mPFC and HC before decision making but at different latency after poke out. Ripples in the mPFC had greater average amplitudes for correct OutSeq trials than ripples in the HC, suggesting a key role of the mPFC in executing a memory-based decision. During correct InSeq trials, ripples in the HC had greater average amplitudes compared to ripples in the mPFC, suggesting a role of the HC in sequence memory. In addition, ripple activity increased only after beta burst activity ceded, suggesting a model by which beta bursts enable ripple activity.

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Poster

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Title: The Role of the Nucleus Reuniens of the Thalamus in Interval Timing

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Abstract: The capacity for accurate time estimation is vital to memory and behavior. Dysfunction in this faculty is implicated in numerous neurological disorders, including Alzheimer's disease, schizophrenia, and ADHD, underscoring the need for an in-depth understanding of the neural mechanisms underlying timing abilities (Allman & Meck, 2012; Coull et al., 2011). While prior research has largely centered on the role of corticostriatal circuits in interval timing, less attention has been given to the medial temporal lobe circuitry known to be involved in temporal contexts (Buhusi & Meck, 2005; Matell & Meck, 2004; Jayachandran & Allen, 2023). Here, we examined the role of the nucleus reuniens of the thalamus (RE) in interval timing using a fixed-interval timing task. Briefly, rats were water restricted and trained to nose poke for a water reward at 10- and 40-sec fixed intervals following the onset of a white noise cue. After a few weeks, peak poking behavior was well-timed to the training interval. Rats were then implanted with a 3D-printed RatHat that included a guide cannula targeting RE. Following recovery, we inactivated RE on random sessions using pretesting infusions of the $GABA_A$-agonist fluorophore-conjugated muscimol TMR-X (fMusc). We observed that muscimol inactivations of RE led to an increase in trial-to-trial poking variability, including (1) an increase in poking behaviors between trials suggesting a decrease in the use of cued memory, and (2) a decrease in temporal precision during cues (less ramping) suggesting a loss of timing in memory. These results demonstrate that RE has a critical role in interval timing. Going forward, it will be important to distinguish the role of specific RE circuits in interval timing. For example, RE projections to the medial temporal lobe may relate more to memory retrieval, and RE projections to the medial prefrontal cortex relate more to timed behavior.

Disclosures: **M. Schlecht:** None. **G. Howland:** None. **A. Castillo:** None. **T.A. Allen:** None.

Poster

PSTR569. Cortico-Hippocampal Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR569.21/VV25

Topic: H.09. Spatial Navigation

Support: NIH MH113626
NIH NS128718

Title: The influence of thalamic reuniens projections on prefrontal-hippocampal network activity during NREM and REM states

Authors: *T. D. VIENA[1], **A. VASALLO-VELIZ**[2], T. A. ALLEN[1];
[1]Psychology, [2]CCF, Florida Intl. Univ., Miami, FL

Abstract: The nucleus reuniens of the thalamus (RE) supports the coordinated activity of the prefrontal-hippocampal (mPFC-HC) system in the delta (1-4Hz), theta (6-12Hz) and beta (15-30Hz) bands during memory-based decision making, memory consolidation and in preclinical models of schizophrenia and epilepsy (Dolleman-van der Weel et al., 2019). Anatomically, RE contains distinct populations of glutamatergic neurons that monosynaptically project to mPFC or HC, as well as dual projecting RE neurons that send collaterals to both regions (Viena et al., 2021). Based on this unique anatomy, these pathways represent potential candidates in the modulation of mPFC-HC interactions and neuronal spiking within the network. We recently showed that, in awake rats, RE drives beta synchrony in mPFC-HC system in support of memory (Jayachandran*, Viena* et al., 2023). However, the specific role of RE during sleep (NREM/REM states) remains under investigation. Here, we recorded rats in their home cage during reversed light conditions (5-9AM) as they naturally slept. Subjects were never sleep-deprived. We recorded local field potentials (LFPs) from mPFC, dorsal/ventral HC and mPFC single-unit activity in rats using single-wire electrodes. Dorsal HC LFP recordings were used to classify sleep/wake states using an automated sleep scoring script (modified from Costa-Miserachs et al. 2003) and high-resolution body tracking via DeepLabCut software (Nath et al., 2019). We selectively stimulated RE neurons that project to mPFC or ventral HC optogenetically (retrograde AAV-channelrhodopsin) using 10Hz pulsed stimulation (60 msec pulse width, 20 sec total duration) during REM or NREM periods. Results showed that stimulating RE-mPFC neurons led to large increases in delta, theta, and beta power during NREM and REM periods in mPFC, but weaker responses in vHC. A subset of mPFC neurons showed firing activity preference for NREM, REM or both sleep states during blue light stimulations. On the other hand, stimulating RE-vHC neurons only showed changes in delta power. Overall, our findings demonstrate RE neurons are capable of differentially modulating delta, theta and beta LFP rhythms, as well as single unit activity, in the mPFC-HC system across sleep states. This is consistent with RE's role in entraining the mPFC-HC system in order to support memory processes during waking and sleeping states.

Disclosures: T.D. Viena: None. **A. Vasallo-Veliz:** None. **T.A. Allen:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.01/VV26

Topic: H.12. Aging and Development

Support: NIH R56

Title: High Control Working Memory Training in Healthy Aging Results in Greater Deactivation of the Default Mode Network for Trained and Transfer Task

Authors: *C. BASAK[1], S. QIN[2], P. SKOLASINSKA[3], E. SMITH[1];
[1]Univ. of Texas at Dallas, Dallas, TX; [2]Univ. of Texas At Dallas, Dallas, TX; [3]The Univ. of Texas at Dallas, Addison, TX

Abstract: Cognitive training, esp. executive control training, is effective for enhancement of broad cognition in older adults (Basak et al., 2020), however the underlying neural mechanism of these enhancements are unclear as the findings regarding training related plasticity in brain activations have been mixed and vary by duration of training and imaging task Recent research has emphasized the importance of large-scale networks on cognition, with focus on task-induced activations in the fronto-parietal network (FPN) that is associated with cognitive control and working memory (Bresslor & Menon, 2010). However, our recent research implicates that age-related deficits in unpredictable n-back, that makes high demands on executive control and working memory, are not in FPN engagement but with disengagement of default mode network (DMN) regions (Qin & Basak, 2020). Task-related disengagement of DMN is thought to facilitate externally directed cognition In this clinical trial (NCT03988829), we determined whether a closed-loop adaptive working memory updating training requiring higher cognitive control (unpredictable switching, High-C), compared to lower cognitive control (predictable switching, Low-C), results in patterns of brain activations similar to that of young adults, i.e., greater engagement of FPN and disengagement of DMN Of 51 older adults who were randomized, 30 participants ($N_{High\text{-}C}$=15; $N_{Low\text{-}C}$=15) completed training and provided all neuroimaging and cognitive data. Task-related fMRI activations in old were assessed at pre- and post-training during a random n-back task with trained (bird) and untrained (digits) stimuli, and compared to fMRI data on 24 young adults. The random n-back fMRI task consisted of 3 runs of six 40 s task blocks interleaved with seven fixation blocks of 6 s. The blocks had memory loads of 0-back, 2-back or 3-back. The stimuli were either pictures of birds (trained) or digits (untrained). fMRI images were acquired using a slice accelerated multiband EPI sequence with TR/TE = 500/30 ms. Whole-brain Group (High-C vs low-C) x Time (Pre vs Post Training) interactions were conducted for Digits>Bird and 2+3-back>0-back contrasts with cluster threshold at Z>3.1, p<.01. Two significant clusters resulted for Digits>Birds contrast (Left Frontal Pole and bilateral intracalcarine/lingual gyrus). where post-training deactivations were observed for High-C group, but not in Low-C group, in both trained and untrained stimuli. For frontal pole, the deactivation in High-C matched that of younger controls, suggesting that High-C training can induce transfer to trained and untrained stimuli by suppressing endogenously driven DMN.

Disclosures: C. Basak: None. **S. Qin:** None. **P. Skolasinska:** None. **E. Smith:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.02/VV27

Topic: H.12. Aging and Development

Title: Increased Neural Differentiation and Executive Function after a Single Session of Aerobic Exercise in Old Age

Authors: *J. PURCELL[1], R. WILEY[2], J. WON[3], D. CALLOW[4], L. R. WEISS[1], A. ALFINI[5], Y. WEI[1], J. SMITH[1];
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Abstract: Aging is associated with decreased cognitive function. One theory posits that this decline is in part due to multiple neural systems becoming de-differentiated in old age. Exercise is known to improve cognition in old age - even after only a single session. We hypothesized that one mechanism of improvement is a re-differentiation of neural systems affected by old age. We used a within-subject, cross-over design involving two sessions: either 30-minutes of aerobic Exercise or 30-minutes of seated Rest (n=32; ages 55-81 years). Both fMRI and Stroop performance were acquired soon after Exercise and Rest. We quantified local neural differentiation via General Heterogeneity Regression and employed Bayesian Multilevel Modeling to quantify effects of exercise. There were three prominent findings following exercise. First, participants were better at reducing Stroop interference. Second, there was a gradient of neural differentiation differences from posterior to anterior in the brain - while there was greater neural differentiation within more posterior brain regions including the temporal lobe and cerebellum, there was lower neural differentiation within frontal cortices (Figure). Third, the greater neural differentiation in the cerebellum and temporal lobe were more pronounced in the older ages. These data indicate that exercise can induce both greater and reduced neural differentiation in healthy aging, and that it may contribute to counteracting ageing dependent neural de-differentiation.

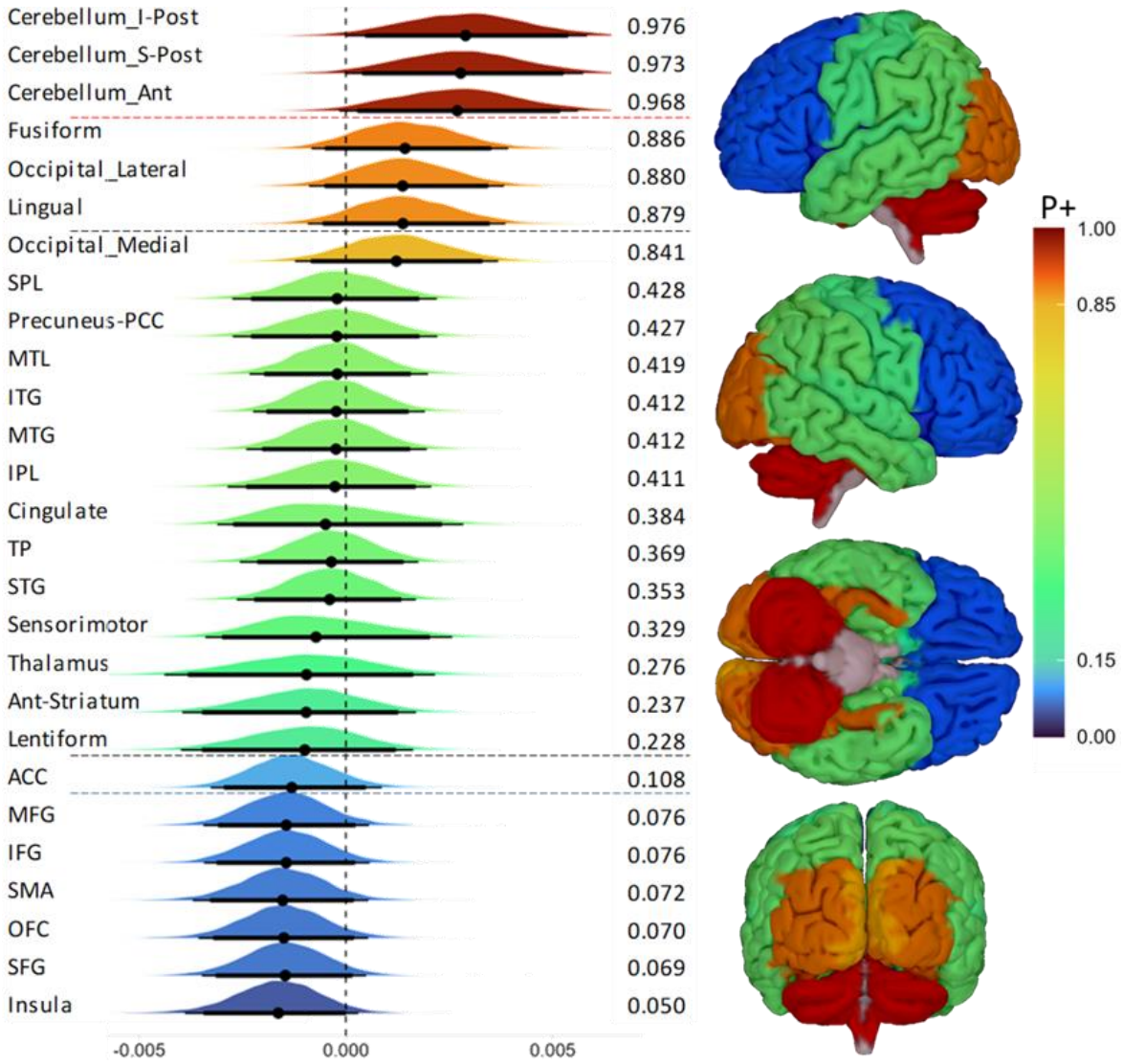

Figure: Neural differentiation differences due to Exercise or Rest. These results use the average Hreg values across all of the voxels in each ROI. Marginal posterior distributions from each Level 1 region of interest results. The area under the curve greater than 0 (a null effect; vertical dotted line) indicates the P+ of the effect of exercise. For each parcel, High P+ (red/orange colors) indicates greater mean Hreg (neural differentiation) due to Exercise minus Rest. Low P+ (blue colors) indicate lower mean Hreg due to Exercise minus Rest. There is no traditional thresholding, and all of the effect posterior distributions are plotted. Horizontal dotted lines depict thresholds for reportable ROI effects as follows: weak = .85-90 or .15-.1, medium = .9-.95 or .1-.05, strong = .95-1 or .05-0. On the right are brain renderings of the colors of the posterior distributions with the associated P+ color scale. TP=temporal pole; I/M/STG=inferior/middle/superior temporal gyrus; S/I-post = superior/inferior posterior; MTL=medial temporal lobe; S/IPL= superior/inferior parietal lobule; A/PCC=anterior/posterior cingulate cortex; Ant=anterior; I/M/SFG=inferior/middle/superior frontal gyrus; SMA=supplementary motor area.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.03/VV28

Topic: H.12. Aging and Development

Support: NIH Grant R37 AG025667
Center for Nutrition Learning and Memory, UIUC (2012-04673)

Title: Physical activity-related individual differences in functional human connectome are linked to fluid intelligence in older adults

Authors: *D. M. PINDUS[1], M. AI[4], L. CHADDOCK-HEYMAN[2], A. Z. BURZYNSKA[8], N. P. GOTHE[5], E. S. SALERNO[9], J. FANNING[10], S. A. ANTERAPER[11], A. N. CASTANON[5], S. WHITFIELD-GABRIELI[6], C. H. HILLMAN[6], E. MCAULEY[3], A. F. KRAMER[7];
[1]Dept. of Kinesiology and Community Hlth., [2]Beckman Inst. for Advanced Sci. and Technol., [3]Kinesiology and Community Hlth., Univ. of Illinois Urbana-Champaign, Urbana, IL; [4]Northeastern Univ., Northeastern Univ., Brookline, MA; [6]Dept. of Psychology, [7]Psychology, [5]Northeastern Univ., Boston, MA; [8]Col. of Hlth. and Human Sci., Colorado State Univ., Fort Collins, CO; [9]Sch. of Med., Washington Univ., St. Louise, MO; [10]Dept. of Hlth. and Exercise Sci., wake Forest Univ., Winston-Salem, NC; [11]Carle Fndn. Hosp., Urbana, IL

Abstract: Functional connectivity (FC) in brain networks critical to complex cognition decreases during adult aging. Physical activity may help optimize FC in these brain networks while sitting may reduce it, leading to decrements in complex cognition such a fluid intelligence. The relationship between sitting, FC and complex cognition may also depend on sedentary behavior type. However, the relationship between these physical behaviors, resting state FC and complex cognition during aging remains poorly understood. We evaluated the associations between moderate-to-vigorous physical activity (MVPA), sedentary time (ST), TV viewing and computer use-related FC patterns and complex cognition in 119 seniors (Mage = 64.8 ± 4.3 yrs, 84 females) using baseline data from the Fit & Active Seniors Trial. MVPA and ST (min/d) were measured with an accelerometer, time viewing TV and using a computer was self-reported. We identified latent cognitive constructs (fluid intelligence, episodic memory, processing speed and executive functions) based on a comprehensive cognitive battery. We hypothesized positive correlations between MVPA, and computer use-related seeds in default mode (DMN), frontoparietal (FPN), dorsal attention (DAN) and ventral attention (VAN) networks with voxels located in these networks. Conversely, we predicted that ST and TV viewing-related seeds in FPN, DAN, and DMN would show fewer correlations within networks critical to top-down control (FPN, DAN and VAN), and more correlations outside these networks. Finally, we explored the associations between physical behavior-related FC patterns of significant seeds and

the four latent cognitive constructs. Multivariate pattern analyses were used to determine which FC patterns explained the main results, using clusters with >50 voxels, related to each behavior, as seeds in the post hoc seed-to-voxel analyses. Controlling for device wear time, age, sex, education, aerobic fitness, and ST, MVPA was related to a cluster in left superior frontal gyrus (SFG) in FPN and VAN, and a cluster in right precentral (PrG) and postcentral gyri (PoG) in the somatosensory network. A correlation between the left SFG seed and a cluster spanning DMN, DAN, FPN and VIS was linked to higher fluid intelligence, as was FC between the right PrG/PoG seed and a cluster in VIS. No significant FC patterns associated with ST, TV viewing or computer use were found. Our findings suggest that MVPA might enhance cognitive reserve in seniors by promoting FC patterns of network integration. Furthermore, our results raise the possibility that increasing MVPA might reduce, or reverse de-dedifferentiation of neural networks often observed with aging.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.04/VV29

Topic: H.12. Aging and Development

Support: Alzheimer's Research and Prevention Foundation
AT009198

Title: Yoga for prevention of cognitive decline in older women at risk for Alzheimer's disease.

Authors: *H. LAVRETSKY;
UCLA, Los Angeles, CA

Abstract: Background: Yoga may produce positive effects on cognitive functions in older adults at risk for cognitivedecline. In this study (NCT03503669), we investigated neural and peripheral biomarkers therapeutic response in older women with subjective cognitive decline (SCD) and cerebrovascular risk factors (CVRFs)following three months of yoga compared to memory enhancement training (MET).
Method: We conducted a randomized, controlled trial to assess the efficacy of Kundalini yoga (YOGA) andmemory enhancement training (MET) on mood and cognitive functioning in a group of older women with CVRFs and SCD. RNA sequencing and cytokine/chemokine assays were analyzed as well as a multimodal MRI (Siemens 3T Prisma scanner).
Result: A total of 79 patients (YOGA=40; MET=39) were randomized and 63 completed the 24-weekfollow-up (Mean age=66.5 years and mean MMSE was 28.4). At 12-weeks and 24-weeks,

both interventions demonstrated improvement in frequency of forgetting (MFQ-Factor 1) (F(1, 76) = 0.2, p=0.7).At 24-weeks, YOGA participants demonstrated between- and within-groups improvements in seriousness of forgetting/MFQ-Factor 2 (effect size (95% confidence interval) = -0.73 (-1.26, -0.19)). Compared to MET,at 12- and 24-weeks follow-up, YOGA uniquely modulated targets related to interferon signaling and innate and adaptive immunity. Compared to YOGA, MET participants displayed higher Eotaxin-1 levels (F(2,67) =3.94, p=0.02). On sMRI-Compared to KY + KK, MET showed reductions in GMV in left prefrontal, pre- and post-central, supramarginal, superior temporal and pericalcarine cortices, right paracentral, postcentral,superior and inferior parietal cortices. Right hippocampal volume increased after yoga. rs-fMRI analysis showed a left anterior hippocampal subregion assigned to the default mode network (DMN) with greater increases in connectivity with largely ventral visual stream regions with YOGA than with MET (p<.001), in associations with lower stress (p<.05). Conclusion: At 24-weeks follow-up, YOGA yielded a significant, large effect size improvement insubjective cognitive impairment, and a robust mediation of inflammatory-immune pathways, including suppression of pro-inflammatory molecules. YOGA also offered neuroprotective effects compared to MET. These results suggest clinical, neural and biological benefits to YOGA for SCD in post-menopausal womenat risk for AD.

Disclosures: H. Lavretsky: None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.05

Topic: H.12. Aging and Development

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National Natural Science Foundation of China [grant number 31700997]
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State Key Program of National Natural Science of China [grant number 81430100]

Title: Spatial variation of silent lacunar infarction with aging and its influence on multiple cognitive functions in community-dwelling older adults

Authors: *S. LONG, S. ZHAO, M. DANG, Y. CHEN, Z. ZHANG;
Beijing Normal Univ., Beijing, China

Abstract: Background: Silent lacunar infarction (SLI) have received a lot of attention in recent years. Previous studies have shown that silent lacunar infarction may be associated with

cognitive decline in the general population. But the spatial pattern of SLI during aging and the role of SLI in cognitive decline remains unknown. **Methods:** 319 subjects with SLI (aged 67.58±7.07, 166 females) and 627 cognitively normal controls (aged 67.43±6.13, 166 females) independently underwent T1-weighted MRI scans and comprehensive cognition assessments. All participants completed a battery of neuropsychological tests, including the MMSE and five cognition domains (memory, visuospatial processing, language ability, attention and executive function). the SLI were manually segmented by two trained observers. Lesion location and volume of SLI in each subject were identified and calculated after we aligned all brain images in standard space. Piecewise linear regression, two-sample t-tests and general linear regression were used to explore the relationship between Lesion location and volume of SLI, age and cognition. **Results:** Compared with the control group, the SLI group showed significant cognitive decline in visuospatial processing ($t=4.347$, $p<0.001$), language ability ($t=2.709$, $p=0.007$), attention ($t=3.986$, $p<0.001$), and executive function ($t=2.508$, $p=0.012$). The total lesion volume of SLI did not change significantly with age before 65 years old (95%CI of slope [-14.60, 11.51]), and increased significantly with age after that (95%CI of slope [0.69, 17.64]). There was no significant relationship between the lesion volume in white matter and age (95%CI of slope [-0.81, 4.15]). The lesion volume in basal ganglia region increased significantly with age (95%CI of slope [0.75, 6.13]), and there was no significant inflection point. The lesion volume in thalamus increased significantly with age before 79 years (95%CI of slope [0.06, 9.10]), but did not change significantly after that (95%CI of slope [-91.03, 61.70]). The results of general linear regression showed that the presence of SLI in the thalamic is associated with poorer attention ($\beta=-0.107$, $p=0.041$), and the lesion volume in white matter is associated with poorer language ability ($\beta=-0.166$, $p=0.007$) and poorer memory ($\beta=-0.195$, $p=0.001$). **Conclusions:** Subjects with SLI showed significant cognitive decline in multiple cognitive functions. Our data also suggested that specific spatial distribution pattern of SLI may contribute to multi-domain cognitive decline. These results may enhance our understanding of the pathogenesis and prevention of silent lacunar infarction.

Disclosures: S. long: None. **S. Zhao:** None. **M. Dang:** None. **Y. Chen:** None. **Z. Zhang:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.06/VV30

Topic: H.12. Aging and Development

Title: Age-related enhancement of the association between episodic memory and gray matter volume in medial temporal and frontal lobes

Authors: *S. ZHAO, S. LONG, D. WANG, X. LI, Z. ZHANG;
State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China

Abstract: Episodic memory (EM) deteriorates as a result of normal aging as well as Alzheimer's disease. The neural underpinnings of such aging-related memory decline are not well-understood. The present study utilized a sliding window approach to examine how the association between EM and gray matter structure changed with age in a sample of 926 Chinese older adults. We found that both verbal EM (VEM) and spatial EM (SEM) exhibited positive correlations with gray matter volumes (GMV) in extensive areas primarily in the temporal and frontal lobes and that these correlations typically became stronger with age. Moreover, some age-related changes in the strength of the association between EM and GMV varied by sex and type of EM. Specifically, the association between VEM and GMVs in the insula and parietal regions became stronger with age for females but not for males, whereas the association between SEM and GMVs in the parietal and occipital regions became stronger for males but not for females. At the brain-system level, for males, there were no age-related changes in the correlations between EM (either VEM or SEM) and the GMV of either the anterior temporal (AT) system or the posterior medial (PM) system. For females, however, both VEM's and SEM's association with GMV became stronger with age in the AT system, but SEM's association with GMV became weaker with age in the PM system. By examining the age-related changes in the association between EM and GMV, our study provides novel insights for our understanding of the impact of structural brain degradation on memory decline during aging.

Disclosures: S. Zhao: None. **S. Long:** None. **D. Wang:** None. **X. Li:** None. **Z. Zhang:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.07/VV31

Topic: H.12. Aging and Development

Title: Cerebellar purkinje cell proteins but not number correlate with spatial memory in a rodent model of normal cognitive aging

Authors: *L. CHENG, C. P. COOPER, J. BHATTI, E. L. R. MELENDEZ, E. PEREZ, J. M. LONG, P. R. RAPP;
Lab. of Behavioral Neurosci., Natl. Inst. on Aging, Baltimore, MD

Abstract: In addition to its well-established role in sensorimotor function, the cerebellum is now associated with higher-order cognitive functions. The cerebellum is also vulnerable to age-related degeneration; however, it is unknown if cerebellar degeneration contributes to age-related cognitive decline. Purkinje cells (PC) are the sole neuronal output of the cerebellum and loss of PCs results in cognitive deficits. As a first-pass analysis, we evaluated whether PC integrity is associated with individual differences in a rodent model of normal cognitive aging. Young 6-month-old and aged 24-month-old male Long-Evans rats were trained in a water maze task. Subsequent learning index scores for spatial learning and memory grouped aged rats into aged-impaired (AI) or aged-unimpaired (AU) compared to young rats. Cerebellar tissue samples were

then collected for either western blot analyses or exhaustive PC counts. Western blots of PC-specific proteins, calbindin-D28k and pcp-2, showed significant correlations with learning index scores in young and aged rats (r^2=0.179, p=0.041; r^2=0.348, p=0.005; respectively), such that lower protein expression was associated with worse memory. To determine whether loss of PC-specific protein co-occurred with frank neuron loss, we conducted immunohistochemical staining for calbindin-D28k in young (n=9), AI (n=9), and AU (n=8) rats for exhaustive PC counts in an evenly spaced 1-in-8 series of histological sections, with experimenters blinded to learning index scores. Cerebellar volume was measured using Cavalieri estimation, and PC density was calculated. We found no significant differences in overall cerebellar PC number, or among vermis lobules and hemisphere regions between young, AI, and AU rats. Concurrently, there were no significant differences in cerebellar volume or PC density by age or cognitive status. In conclusion, we demonstrate that reductions in PC-specific proteins but not PC loss is associated with age-related cognitive decline. Our results highlight PC involvement in the aging brain and point towards expanded investigation elucidating the cerebellum's role in higher-order cognition and its changes with age.

Disclosures: L. Cheng: None. **C.P. Cooper:** None. **J. Bhatti:** None. **E.L.R. Melendez:** None. **E. Perez:** None. **J.M. Long:** None. **P.R. Rapp:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.08/VV32

Topic: H.12. Aging and Development

Title: Age-related vulnerability in dopamine-glutamate neurons projecting to the lateral entorhinal cortex

Authors: J. N. TOMAIO[1], S. FLEURY[1], J. NACIMBA[1], A. BILDER[1], Y. KIM[2], L. E. FENNO[2], C. RAMAKRISHNAN[2], K. DEISSEROTH[2], ***S. MINGOTE**[1];
[1]Neurosci., CUNY Advanced Sci. Res. Ctr., New York, NY; [2]Stanford, Stanford, CA

Abstract: Aging is an established risk factor for cognitive decline, particularly in terms of memory impairment. Within the memory system, the lateral entorhinal cortex (LEC) is the first region to exhibit age-related atrophy and a decline in activity. Dysfunction in the LEC due to aging is associated with difficulties in discriminating novelty. Given that the LEC depends on dopamine (DA) inputs from the ventral tegmental area (VTA) to signal novelty and facilitate memory formation, our objective is to investigate whether impairments in this system contribute to LEC dysfunction. To test this hypothesis, we examined how DAergic innervation of the LEC changes with age. Our previous studies have found that LEC-projecting DA neurons are capable of co-releasing glutamate (GLU). Therefore, we used an intersectional approach to selectively label neurons that exclusively release DA (DA-only neurons) and neurons that release both DA and GLU. Our findings revealed a significant reduction in the density of VTA DA-GLU neurons

in old-aged mice (24 months old). In the LEC of young animals, we observed that 85% of DA projections to the LEC originated from DA-GLU neurons. In old mice, these DA-GLU projections to LEC and labeled by the INTRSECT virus decreased by 70% in both 14 and 24 months old mice. Further investigations unveiled that this decrease is not attributable to a loss of terminals, but rather to a decline in the expression of tyrosine hydroxylase and the vesicular glutamate transporter 2. Considering that both the LEC and DA are implicated in learning and memory processes, the predicted decreases in DA and GLU cotransmission from LEC-projecting DA neurons in aged mice may contribute to cognitive deficits such as novelty discrimination.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.09/VV33

Topic: H.12. Aging and Development

Title: Aging and the Role of Prior Knowledge in Neural Discrimination of Scene Images

Authors: *K. KIMURA, Y. HONG, C. R. BOWMAN;
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Abstract: It is well established that the detail and specificity of episodic memory declines in older age, which can make it more difficult for older adults to distinguish between old and new information when they share overlapping elements. Semantic memory - general world knowledge - declines less substantially with age. Prior work has shown that using prior semantic knowledge can sometimes help older adults successfully encode new information. However, relying on semantic knowledge can also sometimes lead to increases in false recognition. It has become increasingly common in memory and aging research to use neural pattern information as a window into the contents of memory, but it is not known how prior knowledge of to-be-learned stimuli affects the discriminability of neural patterns in older adults. The present study investigated how using images of famous versus non-famous locations affected the ability to decode a scene category during perception and during memory. In this experiment, both young (18-30 years old) and older adults (60-80 years old) viewed a set of scene images while undergoing fMRI. Some scenes were well-known landmarks and others were less well-known. Half of the images contained manmade buildings and half were natural landscapes. After viewing those scenes, participants were asked to recall them also while undergoing fMRI. To assess neural discriminability, we trained and tested a multivariate classifier to distinguish manmade from natural scenes separately based either only on the famous locations or only the non-famous locations. We also did this separately for perception/memory encoding and memory recall. Preliminary results revealed that patterns of brain activations were more distinct for

famous landmarks compared to non-famous landmarks, particularly in perception when the scene label was also on the screen. This effect emerged despite similar behavioral memory performance for each scene type and was also present in our aging sample.

Disclosures: K. Kimura: None. **Y. Hong:** None. **C.R. Bowman:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR570.10/VV34

Topic: H.12. Aging and Development

Title: Hemodynamic response variability and its relationships to BOLD signal and behavior in young and older adults

Authors: *M. TAYLOR[1], M. TURNER[4], K. WEST[2], D. ABDELKARIM[6], Y. ZHAO[3], J. SPENCE[4], B. RYPMA[5];
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Abstract: The blood-oxygen-level-dependent (BOLD) signal yields a characteristic "hemodynamic response function" (HRF) that reflects a combination of blood-flow and oxygen-hyperperfusion changes that follow neural activity. In healthy aging, multiple components of the HRF (e.g., rise time, peak amplitude, and fall time) are susceptible to the mediating effects of age-related cerebrovascular alterations and underlying processes. Additionally, previous studies from our lab have demonstrated that neurovascular coupling differences adults are mirrored in HRF differences. These differences include an increased time-to-peak and a decreased peak amplitude of the HRF. To further explore these phenomena, the current study utilized the publicly available Cambridge Center for Aging and Neuroscience (CamCAN) dataset to estimate variability in HRF amplitude in a visual-auditory task in 80 younger (18-30 years old; 44 Female/36 Male) and 212 older adults (54-74 years old; 100 Female/112 Male). Linear mixed models were used to assess individual and age-related differences in HRF features. My results showed that HRF feature variability exhibits region- and task-dependent differences that need to be accounted for when performing age-group comparisons, the latter-half of the HRF evolution and underlying mechanisms are potential sources of additional variability in older adults, and the difference between HRF features in the precentral cortex and other sensory cortices may serve a mediatory role between age and processing speed ability.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

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Program #/Poster #: PSTR570.11/VV35

Topic: H.12. Aging and Development

Support: NIH Grant AG05290302

Title: Age differences in reactivation of neural representations in encoding and retrieval of modified associations

Authors: O. A. DIGIOVANNI[1], B. C. FOWLIN[2], S. J. MILLER[3], W. H. CARLTON[4], A. ABDOULRAZIG[5], N. A. DENNIS[6], J. D. W. STEPHENS[5], *A. A. OVERMAN[3]; [1]Biochem. Dept., [2]Publ. Hlth. Studies Dept., [3]Psychology Dept., [4]Biol. Dept., Elon Univ., Elon, NC; [5]Psychology Dept., North Carolina A&T State Univ., Greensboro, NC; [6]Psychology Dept., The Pennsylvania State Univ., State College, PA

Abstract: Older adults have been shown to have a specific deficit in forming associations (Naveh-Benjamin, 2000). This deficit also includes difficulty with modifying existing associations (Wahlheim, 2014), which may be due to age differences in reactivation of neural representations. Specifically, older adults are hypothesized not to effectively reinstate patterns of neural activity representing previously-encoded associations when encoding and retrieving new or updated associations. The current study tested this hypothesis in an fMRI experiment (N = 51) in which young and older adults memorized and recalled pairings of low-imageability words with pictures of faces, scenes, and objects (similar to the procedure of Richter et al., 2016). Each of the five scanner runs included three phases: 1) initial learning of word-picture pairs; 2) cued recall of initial pictures and learning of new pictures; and 3) cued recall of new pictures. At post-test outside the scanner, participants identified the "new" picture for each word, from a set of three that included the new picture for that word, the initial picture for that word, and an unstudied picture. In order to quantify activation of neural representations, MVPA classifiers were trained to classify picture type on each trial using fMRI patterns obtained during the initial encoding phase, and then tested on the patterns obtained during subsequent phases. For both age groups, MVPA successfully classified neural patterns by picture type during the encoding phases. For neural patterns obtained during recall, classification was better for young than older adults, consistent with better reactivation of encoded information during recall. At post-test, older adults were significantly more likely than young adults to mis-identify the initial picture for each word. Critically, post-test performance was linked to reactivation of neural representations in the scanner. For both age groups, correctly identified "new" pictures at post-test were associated with greater reactivation of neural patterns for initial pictures during encoding of the new picture. The findings lend support to the hypothesis that older adult memory may be improved by encouraging reinstatement of prior associations when learning new or modified associations.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Topic: H.12. Aging and Development

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Title: Cerebroarterial reactivity changes underlie processing speed decline in healthy aging; Support for a neurovascular uncoupling hypothesis

Authors: *G. H. SHERARD[1,2], D. ABDELKARIM[6], M. TURNER[3], Y. ZHAO[4], P. LIU[7], J. SPENCE[2], B. THOMAS[4], H. LU[8], B. RYPMA[5];
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Abstract: As the proportion of the population who are aged over 55 years is steadily rising, it is increasingly urgent to examine and understand interrelations between age-related neurological and cognitive degeneration. Although numerous studies have found that older adults demonstrate higher neural activity, as measured by cerebral metabolic rate of oxygen ($CMRO_2$), previous studies from our lab and others have demonstrated age-equivalence in cerebral blood flow (CBF), despite a higher neural demand for metabolic resources from vasculature. Such results suggest age-related decline in neurovascular coupling (NVC). Indices of NVC, cerebroarterial reactivity (CAR), and cerebrovascular reactivity (CVR), are known to decline with age; we hypothesize that these deteriorations are associated with decline of fundamental cognitive abilities, especially processing speed and working memory. In the current study, 25 Older Adults and 31 Younger Adults completed dual-echo calibrated functional magnetic resonance imaging (cfMRI) scans, under both normocapnic and hypercapnic conditions. Participants also completed an attention-controlled visual stimulation task during a cfMRI scan. The utility of dual-echo cfMRI optimizes the near-simultaneous measurement of both CBF and Blood-Oxygen-Level-Dependent (BOLD) signal, while the inclusion of a hypercapnic condition facilitates measurement of CVR. In addition, all participants completed an out-of-scanner cognitive assessment battery, including processing speed and working memory tasks. Age group x CAR interactions were significant for a composite measure of processing speed (comprised of the

Digit-Symbol, Box Completion, and Number Comparison tasks), indicating that cerebroarterial reactivity had a differential relationship with processing speed between Younger and Older Adults (where CAR was predictive of processing speed particularly in Older Adults); no such results were observed for CVR. These results support the hypothesis that age-related decline in the NVC unit underlies age-related changes in processing speed.

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Poster

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Program #/Poster #: PSTR570.13/VV37

Topic: H.12. Aging and Development

Support: Margaret Milam McDermott Foundation

Title: Alterations to erythropoietin in aged mouse hippocampus

Authors: *B. SNYDER[1], H.-K. WU[1], S. WAZIR[1,2], T. F. FLOYD[1];
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Abstract: Previous studies demonstrate exogenous erythropoietin (EPO) enhances cognition by modulating neural transmission through the EPO receptor (EPOR), but little is known about the cellular source and role of endogenous hippocampal EPO in synaptic plasticity, its role in aging, or interactions with hypoxia. The hippocampus is composed of highly structured heterogeneous cell-types within subregions (DG, CA1, & CA3) and is sensitive to hypoxia. Astrocytic EPO is necessary to support memory under mild-moderate hypoxia, likely by targeting neuronal EPOR (Leiton, et al., 2018). Expression of hypoxia-inducible factors (HIF1a and HIF2a) and altered gene transcription is present in aged mouse hippocampus, suggesting the presence of chronic hypoxia (Snyder et al., 2021). Therefore, aged hippocampus may be susceptible to acute hypoxia exhibit and exhibit region and cell specific perturbations in the HIF-EPO pathway. Hippocampi were rapidly collected from wildtype male and female C57Bl6/J mice of various ages (3mo - 24mo) exposed to either 21% or 8% oxygen for 3 hours. Protein and mRNA expression was assessed by immunostaining (IHC) using fluorescently labeled antibodies for HIF1a, HIF2a, EPO, GFAP, or NeuN or *in situ* hybridization (fISH) of probes for *Epo* mRNA on 10um sequential coronal slices containing the dorsal hippocampus. Subregion regions of interest (ROIs) were drawn in FIJI by a technician blinded to treatment to assess pixel intensity of antibodies and the number of fISH spots/um^2 within each ROI. Gene expression was validated using PCR of total hippocampi or hippocampal astrocytes isolated using magnetic cell sorting (MACS). Mean expression was analyzed by ANOVA. Aging is associated with increased astrocyte transcription of *Epo* from 3 mo to 18 mo and expression of HIF, indicating chronic

hypoxia. At 24 months Epo transcription is substantially elevated. These changes were also apparent in hippocampal subsection analysis. Chronic hypoxia in the aging hippocampus is likely to induce cell- and layer-specific impairments of the HIF-EPO pathway, preventing protective actions of EPO under acute hypoxia. Further experiments will focus on determining mechanisms which cause dysregulated HIF in aged hippocampus and how those mechanisms impact synaptic plasticity and cognitive outcomes during aging.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Program #/Poster #: PSTR570.14/VV38

Topic: H.12. Aging and Development

Support: ANID 2020 folio 21200451

Title: Impaired mPFC-HPC oscillatory coupling in aged mice during spatial learning

Authors: *L. CHACANA-VÉLIZ, I. NEGRÓN-OYARZO;
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Abstract: In aging, the learning and memory of locations important for survival, known as Spatial Reference Memory (SRM), deteriorates. The coordination of several brain areas supports this memory, including the Hippocampus (HPC) and the medial Prefrontal Cortex (mPFC), which synchronize the patterns of oscillatory brain activity. Recent research using local field potential recordings in freely moving rodents showed that spectral coherence, a measure of oscillatory activity synchronization, gradually increases in the mPFC-HPC network during the acquisition of SRM. However, it is unknown whether aging involves an alteration in synchronization between mPFC-HPC axis that could explain the decline in goal-oriented spatial memory in elderly mice. To address this question, chronic electrodes were implanted in the mPFC and HPC of adult and aged C57BL/6 mice. Subsequently, in both groups, the LFP and neural activity were recorded during the SRM in the Barnes maze task. The results evidence a decrease in the synchronization between the phase theta band of HPC and amplitude gamma band mPFC in aged mice during the SRM, which suggests that the decreased ability of neural circuits in aged mice to synchronize their oscillatory patterns may contribute to the impaired goal-directed memory acquisition in aged mice. These findings may have important implications for the development of therapies aimed at improving or preserving cognitive function in aging.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Topic: H.12. Aging and Development

Support: NIH Grant R01 AG033546

Title: Factors contributing to biological sex related differences in aerobic exercise benefits in healthy adults

Authors: *Y. GAZES[1], M. D. WALKER[2], S. LEE[3], H. RYU[4], R. P. SLOAN[5], Y. STERN[4]; [1]Columbia Univ., New York, NY; [2]Endocrinol., [3]Psychiatry and Biostatistics, [4]Neurol., [5]Psychiatry, Columbia Univ. Med. Ctr., New York, NY

Abstract: The benefits of aerobic exercise for cognition in older adults have been reported repeatedly. Growing evidence suggests female and male participants receive differential benefits from aerobic exercise, but understanding of the factors contributing to sex-differences is still lacking. We conducted a randomized control trial of stretching (34 female and 16 male participants) versus aerobic exercise (32 female and 12 male participants) in cognitively healthy adults aged 20-67 years. This 6-month study required 3 visits (baseline, month 3 and month 6). At baseline and month 6, cognitive assessments, cardiorespiratory exercise testing (VO_2 max), Magnetic Resonance Imaging scans, and blood draw were performed; at month 3, all of the above except MRI and blood draws were conducted. In this analysis, we examined the associations between changes in VO_2 max, body mass index (BMI), cognitive assessments (executive function, processing speed, and episodic and working memory) and plasma levels of estradiol, follicle stimulating hormone, beta-2 microglobulin (B2M), interleukin-6, soluble interleukin-6R (sIL6R), tumor necrosis factor alpha (TNFa), and C-reactive protein. With age and education controlled, we found that changes in VO_2 max, BMI, and cognition have differential associations with hormonal and neuroimmune proteins between male and female participants (interaction with sex). Specifically, male participants showed improved executive function in association with decreased levels of TNFa (beta= -.845, p=.024) and sIL6r (beta= -.0005, p=.0094) while females did not show such associations. Decreased B2M in male participants was similarly associated with improved processing speed but not in female participants (beta= -1.2, p=.0004). Further analyses showed that menopausal status was an important confounder, contributing to the lack of associations observed in female participants. Limiting the analysis to females and controlling for age, education, and menopausal status, changes in estradiol were associated with changes in episodic memory in the aerobic group and not in the stretching group (estradiol*group: beta= -.25, p=.032). These results together suggest that different factors contribute to the differential improvement in cognitive changes with aerobic exercise training, with levels of immune proteins driving the cognitive improvements in male participants and levels of estradiol contributing to cognitive improvements in female participants.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

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Title: Age related processes impacting processing speed: a diffusion model analysis

Authors: *C. SANCHES, C. YOUNG, H. ROMERO-KORNBLUM, Y. COBIGO, M. MANDELLI, W. CHIONG;
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Abstract: Longer reaction times are observed with increasing age across many cognitive tasks. However, the mechanisms underlying such slowing are still unclear and may reflect age impacts in different processes. Applying the drift diffusion model, increased overall reaction time could reflect a reduction on the speed of information uptake (*drift-rate*), a more cautious response criterion (*boundary-separation*), or reduced efficiency of sensory encoding and motor execution (*non-decision time*). The neurobiological underpinnings of age-related slowing are also still unclear. One hypothesis is that it relates to the loss of cerebral white matter integrity that has been observed with aging. We used a drift diffusion model approach to determine which processes underlie age-related slowing for varied cognitive tasks. Diffusion tensor imaging was used to compute measures of white matter integrity to enter a mediation analysis with age as a predictor variable and values for the drift diffusion model parameters as outcome variables. We recruited neurologically healthy older adults (age 45-95yo, mean = 77yo) from a cohort including in-person behavioral and neuroimaging measurements, and we selected participants that completed different online tasks, including a delay discounting (n = 140), an emotion recognition (n = 183), and a word memory task (n = 163). Of those, 95, 96 and 91 had imaging data for the delay discounting, emotion recognition and memory tasks, respectively. In our three tasks, overall reaction time significantly increases with age and is significantly associated with all three computed drift diffusion parameters. However, bivariate correlations between age and each of the parameters showed that for the delay discounting task only *non-decision time* is significantly associated with age (ρ=0.324, $p<0.001$) whereas both *drift-rate* and *non-decision time* are significantly associated with age for the emotion recognition (ρ=-0.175, p=0.018; ρ=0.413, $p<0.001$) and word memory tasks (ρ=-0.173, p=0.027; ρ=0.383, $p<0.001$), suggesting that associations between age and non-decision time may be the most consistent component of age-related slowing. Regarding white matter integrity, fractional anisotropy (FA) decreased and mean diffusivity (MD) increased with age across widespread regions of the brain in our cohort. For the emotion recognition task only, white matter integrity partially mediates the association between age and *drift-rate* (indirect pathway with FA as mediator: b = -0.0016, p = 0.013; MD

as mediator: b = -0.002, p = 0.065) and between age and *non-decision time (*indirect pathway with FA as mediator: b = 0.015, p = 0.023; MD as mediator: b = 0.018, p = 0.107).

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Title: Slow wave sleep is associated with nucleus accumbens volume in elderly cognitively-unimpaired adults

Authors: *Á. NÁRAI[1,2], N. BÁTHORI[3], É. KISS-BANKÓ[1], A. BIHARI[1], M. HAVADI-NAGY[1], A. MANGA[1], V. TOMACSEK[4], G. ERőSS[1], V. GÁL[1], B. WEISS[1], P. HERMANN[1], P. SIMOR[4], Z. VIDNYÁNSZKY[1];
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Abstract: Aging is characterized by a gradual deterioration of brain structure and sleep quality, and the degree of deterioration is implicated in many psychiatric and neurodegenerative diseases. However, the relationship between age-related changes in sleep and brain structure remains poorly understood. Slow wave sleep (SWS), the deepest non-rapid eye movement sleep stage, appears to be particularly vulnerable, as it declines linearly throughout adult life. Since previous research in animal models revealed that nucleus accumbens (NAc) might play an important role in controlling slow wave sleep, here we aimed to investigate the association between subcortical volumes with an emphasis on the NAc region and SWS in cognitively unimpaired older adults (N=67). Structural MRI measurements were performed on a Magnetom Prisma 3T MRI scanner. Subcortical volumes were derived from FreeSurfer and volume deviation scores were calculated using normative modeling. Sleep was recorded longitudinally in all participants at home up to 7 nights using a four-channel portable EEG device (Dreem 2) and sleep stages were annotated automatically. Regression modeling revealed a positive association between SWS duration and NAc volume as well as a negative association between SWS and caudate nucleus (CN) volume. Additionally, CN was also positively associated with rapid-eye movement (REM) sleep time. These results suggest that NAc is involved in SWS regulation in humans, which is consistent

with previous experimental research in rodents. Furthermore, our findings also revealed an association between CN and the duration of SWS and REM, suggesting that both the ventral and dorsal striatum may play an important role in regulation of sleep and its impairment during aging.

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Poster

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Guangdong Project (2017GC010590 to BL)

Title: Mitochondrial calcium transporter (MCU) mediates aging-related abnormalities in metabolism-excitation coupling and cognitive impairment

Authors: *S. YANG, S. ZHANG, L. CHEN, S. ZHAO, Z. LUO, L. HUANG, B. LI;
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Abstract: Aging is associated with cognitive decline; however, the precise neural mechanisms driving this connection remain ambiguous. We found that neuronal calcium transients in the cortical neurons of aged mice were significantly lower, indicating reduced neuronal excitability. To explore the influence of metabolism on this age-associated decline in neuronal excitability and cognitive impairment, we performed metabolomics analysis on the prefrontal cortex of aged mice. We found that purine catabolism was significantly enhanced in aged mice. The increase in purine catabolism led to a significant elevation of uric acid levels, effectively suppressing the firing frequency of action potentials. To investigate the effects of elevated uric acid on neuronal excitability and cognitive function *in vivo*, we increased the uric acid levels in the prefrontal cortex of young mice with a diet rich in uric acid. We found that this significantly reduced the excitability of cortical neurons and resulted in apparent cognitive impairment. To further validate these findings, we employed febuxostat to inhibit uric acid production in aged mice. Intracerebroventricular injection of febuxostat effectively reduced uric acid concentrations, elevated neuronal excitability, and improved cognitive function in aged mice. These findings suggest that elevated uric acid levels are crucial in aging-related reductions in neuronal excitability and cognitive function. Furthermore, we examined the mechanism underlying the

aging-induced abnormality in purine metabolism. Aging coincided with an increase in MCU expression in the mitochondria of cortical neurons. MCU upregulation resulted in heightened mitochondrial Ca^{2+} signaling, aberrant mitochondrial morphology, disrupted metabolic networks, increased purine catabolism, elevated uric acid production, reduced neuronal excitability, and impaired cognitive function in mice. In contrast, the reduction of MCU expression reversed these processes. These results suggest that MCU upregulation is a critical factor in aging-related impairments.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Title: Microglia regulation by C1q and CD47 may contribute to myelin loss and associated cognitive impairment in the aging monkey brain

Authors: *S. A. DEVRIES, B. CONNER, C. DIMOVASILI, M. MEDALLA, D. L. ROSENE; Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Severe cognitive impairment affects many individuals during normal aging despite the absence of neurodegeneration from Alzheimer's disease (AD). Studies of normal human aging, however, are often confounded by unrecognized early-stage AD. The aging monkey provides considerable insight into normal human aging since they do not develop AD but exhibit cognitive impairment similar to humans. Research in both humans and monkeys has shown that neurons are not lost in normal aging. Instead, structural changes including synapse loss and myelin damage occur, including splitting of the myelin sheath, which, in the cingulum bundle, is associated with cognitive impairment. While synapse and myelin pathology may occur independently, another possibility is that synapse loss is secondary to white matter damage. Studies suggest the involvement of microglia, the resident immune cell of the central nervous system, which exhibit increased inflammatory and phagocytic profiles in white matter along with a decreased efficacy in degrading myelin during aging. Microglia-mediated phagocytosis is modulated by immune "eat me" and "don't eat me" signaling proteins. Previous research has focused on "eat me" signals, including complement components, while the "don't eat me" signals have largely been ignored. This study investigated the balance between the "eat me" signal, complement component C1q and the "don't eat me" signal CD47 relative to age-related myelin loss and microglia phagocytosis in the cingulum bundle. To do this, archived tissue

available from 32 cognitively tested male and female rhesus monkeys (7 to 30 years of age) was selected. Cognitive testing for learning and memory included the delayed-nonmatch to sample (DNMS) and delayed recognition span (DRST) tasks which are used to calculate a cognitive impairment index (CII) that confirmed age-related cognitive impairment. Multilabel immunofluorescence was used to investigate C1q and CD47 localization to myelin as well as to determine if microglia phagocytosis is associated with age-related changes in C1q and CD47. Additionally, RNAscope was performed to measure glial *C1q* and *CD47* RNA. Overall, results show significantly elevated C1q along with diminished CD47 with age as well as increased phagocytic microglia. This suggests that with age, phagocytic microglia increase in the cingulum bundle and receive increased "eat me" signals from C1q along with reduced "don't eat me" signaling from CD47, suggesting a possible mechanism for age-related myelin loss and associated cognitive impairment.

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Poster

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Hungarian National Academy of Scientist Education / UNKP

Title: Impact of aging on multifractal functional connectivity and its association with cognitive performance: an EEG study

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Abstract: Background: Intact brain network function is a prerequisite for higher-order mental processes, which decline with aging, especially regarding fluid cognitive abilities. Underlying changes in task-related functional connectivity also occur in a temporal scale-free (fractal) manner that has not been characterized yet. **Objective:** Here, we investigated the impact of aging on multifractal functional connectivity (MF-FC) derived from resting- and task-state electroencephalography (EEG) recordings and assessed its relationship with cognitive performance. **Methods:** We recruited young (<45 years old, *n*=24, 12 females) and elderly (>60 years old, *n*=18) participants free of neuropsychiatric diseases. The measurement protocol consisted of: *i*) EEG recordings during resting states (eyes open and closed) and during a pattern recognition paradigm administered at three difficulty levels and *ii*) a standardized neuropsychological assessment (Cambridge Neuropsychological Test Automated Battery; CANTAB). To characterize MF-FC, bivariate scale-free exponents (Hurst, *H*) and their distribution were estimated from simultaneously recorded EEG signals. Taking *H*(2) or *H*(-15)-*H*(15)=dH_{15} values as estimators of MF-FC, local and global graph theoretical parameters of each node and the corresponding brain network were determined, respectively. **Results:** We found the elderly group having significantly lower connection strengths of brain graphs, reflecting reduced global scale-free coupling both in resting and in task state for networks reconstructed either from *H* or dH_{15}. Age-related loss of scale-free coupled dynamics during either resting or task states significantly correlated with worse pattern recognition memory performance and longer latencies during this CANTAB test. **Conclusion:** These findings suggest that age-related changes of EEG-based MF-FC predict impaired pattern recognition performance revealed by CANTAB.

Disclosures: P. Mukli: None. **C. Bonin Pinto:** None. **A. Vass:** None. **O. Stylianou:** None. **F. Racz:** None. **Z. Kaposzta:** None. **A. Czoch:** None. **A. Yabluchanskiy:** None. **K. Farkas:** None. **G. Csukly:** None. **A. Eke:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

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Knight Initiative for Brain Resilience Innovation Grants

Title: Unraveling the role of dentate spikes in memory formation and age-related cognitive decline

Authors: *E. HWAUN, J. S. FARRELL, I. SOLTESZ;
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Abstract: The dentate gyrus of the hippocampus supports episodic memory formation through organized neural population dynamics. Among these dynamics, dentate spikes have emerged as a prevalent yet understudied phenomenon characterized by synchronous bursts of neural activity. Intriguingly, we recently demonstrated that these dentate spikes were coupled with brief periods of arousal, suggesting their potential involvement in memory encoding processes. Our recent experimental evidence supports this notion, as inhibiting dentate spikes in young mice has been shown to disrupt associative memory formation. These findings raise an intriguing question: could the impairment in associative memory often observed in older animals be attributed to disrupted dentate spikes? Age-related changes in the brain may contribute to alterations in dentate spikes and subsequent memory deficits. The perforant pathway, a major hippocampal input that triggers dentate spikes, tends to atrophy as animals become older. This age-related decline in the perforant pathway suggests the possibility of disrupted dentate spikes in older animals, potentially contributing to associative memory impairments. Building upon these observations, our further investigations have revealed that the rate of dentate spikes triggered by startling tones or air puff stimuli decreases in older mice. This decline in dentate spike activity coincides with the diminished performance of these older mice in an associative place preference task that relies on dentate spikes. The emerging picture suggests that a dysfunctional dentate network, characterized by a reduced abundance of dentate spikes, may be a critical factor in impairing memory encoding processes in aged animals. Understanding the underlying mechanisms of dentate spike disruption and its impact on memory formation is crucial for developing strategies to alleviate age-related cognitive decline. Further research is warranted to elucidate the precise mechanisms by which disrupted dentate spikes contribute to associative memory impairments in older animals, potentially paving the way for novel interventions aimed at preserving memory function in the elderly population.

Disclosures: **E. Hwaun:** None. **J.S. Farrell:** None. **I. Soltesz:** None.

Poster

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Title: Three-dimensional structure of dendritic spines and synapses in the hippocampal area CA1 of an aged, cognitively unimpaired marmoset

Authors: *M. KUWAJIMA[1], C. GLAVIS-BLOOM[2], C. R. VANDERLIP[2], S. W. NOVAK[3], U. MANOR[3], J. H. REYNOLDS[2], K. M. HARRIS[1];
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Abstract: The hippocampus is critical for learning and memory and shows some of the earliest morphological and functional changes with age. To elucidate ultrastructural correlates of age-related cognitive decline, we began analyzing synapses and neuropil in the hippocampus of an aged common marmoset (*Callithrix jacchus*) that was cognitively unimpaired, using 3D reconstruction from serial section electron microscopy (3DEM). One female marmoset (aged 9 yr 7 mo at the time of perfusion-fixation) was used for this study. This animal performed similar to young adults over the course of several thousand trials in the Delayed Recognition Span Task, which is a working memory task that critically depends on both the prefrontal cortex (PFC) and hippocampus (Glavis-Bloom et al., 2022; 2023). The animal was perfusion-fixed under deep anesthesia with mixed aldehydes. A coronal section of the brain containing the hippocampus was processed for EM imaging of 212 serial ultrathin sections from the middle of stratum radiatum in area CA1. In the 3DEM dataset, 3 segments of oblique dendrites (9.19-14.78 µm in length) were identified based on their comparable microtubule content (range 13-18). These dendrites had a spine density of 2.29 ± 0.45 per µm of dendritic length (mean ± SEM; range 1.83-3.27) and a synapse density of 2.59 ± 0.56 per µm (range 1.96-3.81). Of 86 synapses total, 5 were located on the dendritic shaft (2 asymmetric and 3 symmetric synapses). The area of the asymmetric synapses on spines ranged 0.012-0.50 µm^2 with median of 0.051 µm^2 and mean of 0.062 ± 0.011 µm^2. The mean synapse area in CA1 is comparable to our previous findings in the dorsolateral PFC (layer III) of the same animal (Glavis-Bloom et al., 2023). Of 81 spines found along the dendrites, 15 (19%) contained the spine apparatus (SA), 9 (11%) had a single tubule of smooth endoplasmic reticulum (SER), 34 (42%) had endosomal compartments, and 6 (0.7%) had polyribosomes (PR). SA co-occurred with PR in one spine (1.2%), while endosomes were colocalized with SA in 8 spines (9.9%), a SER tubule in 5 spines (6.2%), and PR in 4 spines (4.9%). Because the SA is important for synapse enlargement during long-term potentiation in the rodent CA1, we analyzed the relationship between the synapse size and organelle content in the spine. This revealed that spines with SA had significantly larger synapses (0.25 ± 0.038 µm^2) than those without an organelle (0.045 ± 0.0024 µm^2; $p < 0.0001$, Dunn's multiple comparisons test). The presence of other organelles in the spine did not affect synapse size. Thus, the SA may

serve to maintain large (presumably stable) synapses in normal young, as well as aged, cognitively intact animals.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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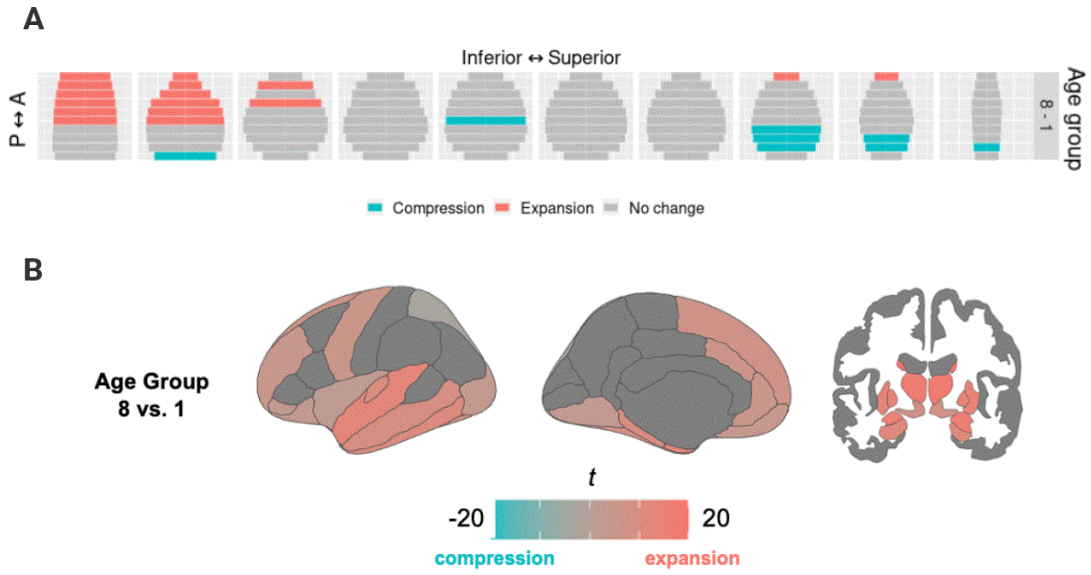
Program #/Poster #: PSTR570.23/VV47

Topic: H.12. Aging and Development

Title: Compression and expansion in the aging brain is associated with clinical and cognitive outcomes

Authors: *Y. Y. ESCALANTE[1], J. N. ADAMS[1], M. A. YASSA[1], N. JANSSEN[2,3]; [1]Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA; [2]Univ. de La Laguna, San Cristobal de La Laguna, Spain; [3]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Structural brain changes, such as decreases in brain size, gray matter loss, enlarged ventricles, and sulcal widening, occur during aging and cognitive impairment. These changes have been observed using methods such as regional volume, thickness, and voxel-based morphometry. However, these methods do not provide information on whole-brain patterns of compression/expansion nor insight into regional changes. Here we tested how the Euclidean distance between landmarks identified on the edge of the brain or in the center of individual regions are affected by age, clinical status, and cognitive function. We analyzed 2,039 structural MRIs from the Open Access Series of Imaging Studies (OASIS) dataset from older adults (42-97 years old; 56% F) in a cross-sectional manner. MRIs were preprocessed using Freesurfer version v6.0. Age effects were evaluated by comparing eight groups with equal numbers of MR sessions (n=~250), using the youngest age group as the control group (42.7-59.9 years old). We first examined how the outer edges of the brain changed across anterior-posterior and superior-inferior gradients. Increasing age was associated with more expansion in anterior and inferior brain regions and with compression in posterior and superior parts of the brain (Fig1A). In addition, we analyzed 39 of the same brain regions across hemispheres (homologues). Distances between homologues showed a progressive pattern of expansion in subcortical, medial and lateral temporal lobes and compression in posterior parietal areas (Fig 1B). These results suggest that patterns of compression and expansion occur in specific regions of the brain during aging. Finally, patterns of both whole-brain and regional homologue expansion and compression were exaggerated in older adults with clinical impairment (CDR>0) and associated with episodic memory and executive function, even when controlling for age. Our study suggests that the brain undergoes patterns of expansion and compression with normal aging, which may be more severely affected in clinical disorders and contribute to cognitive outcomes.



Disclosures: **Y.Y. Escalante:** None. **J.N. Adams:** None. **M.A. Yassa:** None. **N. Janssen:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

Location: WCC Halls A-C

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Program #/Poster #: PSTR570.24/VV48

Topic: H.12. Aging and Development

Title: The effect of aging on the mu and posterior alpha rhythms using the resting-state electroencephalography: Source localization

Authors: ***J. PARK**[1], W.-E. WANG[1], R. HO[1], Q. NGUYEN[1], S. COOMBES[1,2];
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Abstract: With increasing age, the peak alpha rhythm is slowed during resting-state with eyes closed. A slowed alpha rhythm is evident across the whole scalp, but is thought to be generated by two distinct sources which are evident in the mu rhythm over central areas, and the occipital alpha rhythm. The purpose of this study is to determine whether age impacts the power and frequency of the dominant alpha rhythm equally across source generators or whether the impact of age varies across sources. We recruited 28 healthy subjects into a young group (20.4 years)

and 26 healthy subjects into an older group (64.5 years). High-density EEG with 128 channels was recorded for 10 mins with eyes closed and was analyzed with the processing steps: down sampling, filtering, channel removal, ICA, IC-label, IC removal, epoch rejection, DIPFIT, and k-means clustering. Source level analysis revealed ten clusters of dipoles. Among them, four clusters were located in the occipital lobe consistent with the source of the posterior alpha rhythm, while two clusters were located in the primary somatosensory cortex (S1) consistent with the mu rhythm. The peak alpha rhythms in the bilateral occipital lobe were significantly slower in the older group compared to the younger group. For S1, a significant and slower peak alpha rhythm was evident in the left but not right cluster the older group compared to the younger group. The effect sizes (Hedges' g) of occipital clusters were greater compared to the S1 clusters. Together, our findings show that both mu and occipital alpha rhythms are slowed with aging, but the slowing is asymmetric in the somatosensory cortex, and is more pronounced in occipital regions.

Disclosures: **J. Park:** None. **W. Wang:** None. **R. Ho:** None. **Q. Nguyen:** None. **S. Coombes:** None.

Poster

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Topic: H.12. Aging and Development

Support: The Hong Kong Research Grants Council Collaborative Research Fund (Ref: C7069-19G)
The Hong Kong Research Grants Council General Research Fund (Ref: 17600522)

Title: The Association Between Brain Functional Network Properties and Resilience in Old Adults

Authors: *Y. GU, T. M. C. LEE;
The Univerisity of Hong Kong, Hong Kong, China

Abstract: Resilience, widely recognized as the ability to recover and thrive amidst stress, is a fundamental aspect of human functioning that empowers individuals to adapt and respond effectively to diverse challenges and adversities. Investigating the neural mechanisms that underlie resilience can provide valuable insights into adaptive processes and mental well-being. Therefore, this study investigated the relationship between brain network properties and resilience. This study involved 78 old healthy adults (age = 64.24 ± 5.55). Resilience was assessed using the Connor-Davidson Resilience Scale. Resting-state functional magnetic resonance imaging data collected from each participant were employed to calculate a 264 × 264 functional connectivity matrix with 0.15 sparsity. Twelve graph theory measures were then

applied to the individual functional connectivity matrix to capture the network properties of the brain. The relationship between these graph theory measures and resilience scores was determined using Pearson correlation. The present study observed a significant positive association between degree centrality and resilience in nine nodes (Rs > 0.248, Ps < 0.04) within the default mode network. In contrast, a negative correlation was identified between degree centrality and resilience in seven nodes (Rs < -0.237, Ps < 0.05) encompassing the sensorimotor, fronto-parietal, salience, and default mode networks. Regarding betweenness centrality, we observed a positive correlation with resilience in five nodes (Rs > 0.257, Ps < 0.033) within the default mode network and visual network. Conversely, we noted the negative correlation with resilience in nine nodes in the sensorimotor network, fronto-parietal network, salience network, subcortical network, and default mode network (Rs < -0.242, Ps < 0.04). For the nodal efficiency, we found that resilience was positively correlated with seven nodes (Rs > 0.241, Ps < 0.046) of the default mode network and negatively correlated with four nodes (Rs < -0.247, Ps < 0.041) within the sensorimotor, subcortical, and default mode networks. These findings reveal the functional connectivity basis of psychological resilience and highlight the default mode network as crucial in facilitating adaptive responses to stress and challenges.

Disclosures: **Y. Gu:** None. **T.M.C. Lee:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Topic: H.12. Aging and Development

Support: Ministry of Health, Singapore (MOH-OFYIRG19may-0012)

Title: Healthy older adults had more variable dynamic brain states during dual task switching

Authors: ***E. K. K. NG**[1], J. J. X. KOI[1], J. H. ZHOU[1,2];
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Abstract: Systemic theories propose that age-related alterations in functional synchrony within and between brain networks reflect a continuous neural adaptation to enable older adult to handle cognitive demands adequately. In the case of task switching, despite its high demands for executive functioning, older adults do not always suffer from larger switch cost than younger adults. What underlying network reorganization enables such competence remains unclear. We examined the properties of dynamic functional connectivity when young (N = 28, 15 F, 22-33 yrs) and older adults (N = 58, 32 F, 57-77 yrs) performed dual task switching during 3T multiband fMRI. We expected the default DMN and control FPN networks to be less stable and integrated in older adults, and their dynamics to relate with switch cost. In each task trial, participants followed an instruction cue to respond to a target letter based on its position or

identity via button press. MRI data were preprocessed using published pipeline and included nuisance regression and high-pass filtering (.1 Hz). Regional timeseries were obtained from a functional brain parcellation atlas and subject to Leading Eigenvector Dynamics Analysis (LEiDA) and kmeans clustering to characterize brain dynamics as the unfolding of several recurring phase-locked states. Each time point was assigned to a state, and the transition probabilities within and between states were subject to partial least square analyses. Older adults responded slower ($p<.001$) than young adults but did not show worse switch cost (no group-switch interaction). A 4-state LEiDA solution featured a global state, a DMN state, a motor state, and an FPN state. Older adults had lower probability of staying in the DMN and motor states, as well as more frequent transitions among the 3 non-global states (permuted $p < .001$). Higher probability of staying in the DMN state was further associated with lower switch cost ($p = .02$ uncorrected for multiple comparisons) across all participants. Despite the absence of disadvantage in switch cost, older adults completed the task with an apparently less efficient functional configuration. Our finding that older adults having lower same-state transitions (DMN-DMN) was consistent with previous report of shorter lasting LEiDA states in older adults with poorer cognitive ability. The switch cost association with the DMN state bolstered the importance of this network in externally-oriented cognitive tasks. Its consistent temporal engagement may be crucial for older adults to maintain competent cognitive performance, as their neural architecture might be rebalanced to be more reliant on the DMN.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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The Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B) (fiscal year 2022-2023) to CC and SI

Title: Mild cognitive impairment exhibits neural deficits in working memory capacity but intact selective filtering function

Authors: ***N. YUVASUTA**[1], S. PROM-ON[2], K. BENJASUPAWAN[3], P. WIWATPHONTHANA[5], K. LERTLADALUCK[6], C. CHUNHARAS[4], S. ITTHIPURIPAT[6];

[1]Engin., [2]King Mongkut Univeristy's of Technol. Thonburi, Bangkok, Thailand; [3]Cognitive Clin. & Computat. Neurosci. Lab, Fac. of Med., [4]Intrnl. Med., Chulalongkorn Univ., Bangkok, Thailand; [5]Neurosci. Ctr. for Res. and Innovation, Learning Inst., King Mongkut's Univ. of Technol. Thonburi, Bangkok, Thailand; [6]King Mongkut's Univ. of Technol. Thonburi, Bangkok, Thailand

Abstract: Mild cognitive impairment (MCI) serves as a transitional stage between healthy aging and various types of dementia, including Alzheimer's disease (AD). Previous clinical studies have demonstrated poorer selective attention and working memory performance in MCI patients compared to healthy individuals. However, the neural substrates underlying these deficits in MCI remain poorly understood. In this study, we aimed to investigate the neural deficits associated with storage capacity and selective filtering in visual working memory among Thai MCI patients and healthy individuals. We measured EEG activity while participants performed a variant of the delay match-to-sample task, which required remembering varying numbers of relevant color targets while ignoring varying numbers of irrelevant distractors or without distractors. Two event-related potentials (ERPs), the posterior contralateral delay activity (CDA) and frontal biasing activity were used to track working memory capacity and selective filtering functions, respectively. The MCI group exhibited significantly poorer working memory performance compared to the healthy control group, irrespective of the number of targets and distractors. The CDA amplitude was significantly lower in the MCI group compared to the control group, particularly when working memory capacity was full (approximately 3-4 targets with no distractors). That said, there was no significant difference in frontal biasing activity between the healthy aging and MCI groups. Overall, these findings suggest that MCI exhibits neural deficits in the posterior parietal cortex, resulting in reduced working memory capacity without impacting the selective filtering function of the frontal cortex.

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Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Program #/Poster #: PSTR570.28/VV52

Topic: H.12. Aging and Development

Support: NIA R21 AG047944, DoE SC 0001753

Title: Effects of Estradiol Level on Effective Connectivity in Postmenopausal Individuals

Authors: *A. A. TESTO[1], J. MAKAREWICZ[2], E. MCGEE[3], J. DUMAS[2];
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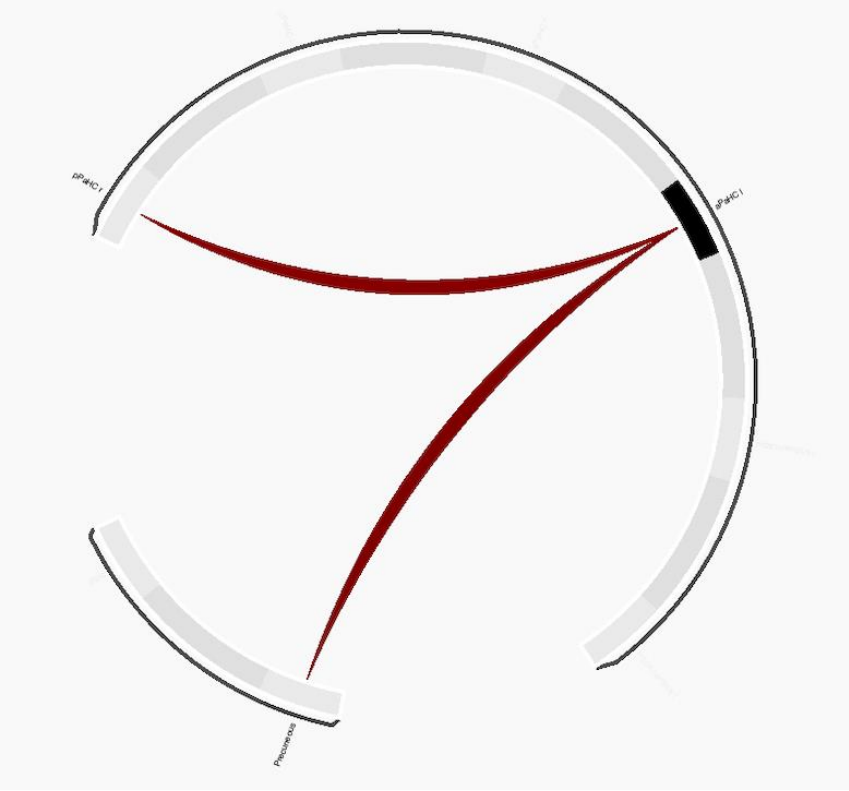
Abstract: Background: Previous studies have found that estrogens play a role in functional connectivity in the brain, however, little research has been done regarding how effective connectivity changes as a result of low estradiol levels that follow the menopausal transition. Effective connectivity characterizes the influence a brain region exerts on other anatomically distinct regions within the brain. The purpose of this study is to examine the effects of estradiol level on effective connectivity in individuals who have completed the menopausal transition.
Methods: Structural and BOLD resting state MRI scans of 88 cognitively healthy postmenopausal individuals (mean age=56.33(SD=2.53), mean number of years since menopause=5.91(SD=4.24)) were collected and used to generate connectivity values in CONN toolbox version 20.b, an SPM-based software. A regression analysis was run using estradiol level (*M*=8.91(SD=6.37)) collect via blood draw the same day as the MRI. Regions of interest included the hippocampus, parahippocampus, dorsolateral prefrontal cortex, and precuneus.
Results: A positive effect of estradiol levels was found during ROI-to-ROI regression analysis F(3,84)=5.88; p-FDR=0.009. Estradiol enhanced effective connectivity between the parahippocampal gyrus anterior division left and the precuneus (t(86)=3.10, p-uncorrected=0.003, p-FDR=0.011) as well as the parahippocampal gyrus anterior division left and parahippocapal gyrus posterior division right (t(86)=3.05 p-uncorrected=0.003 p-FDR 0.011).
Conclusions: These results illustrate the effect of estradiol level on effective connectivity in postmenopausal women. They have implications for understanding how the functioning of the brain changes for women after menopause that may eventually lead to changes in cognition and behavior in older ages.



Disclosures: **A.A. Testo:** None. **J. Makarewicz:** None. **E. McGee:** None. **J. Dumas:** None.

Poster

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Program #/Poster #: PSTR570.29/VV53

Topic: H.12. Aging and Development

Title: Age-related differences in functional connectivity patterns along the hippocampal long axis

Authors: *C. I. CHARLES, S. M. BIRR, C. R. BOWMAN;
Psychology, UW-Milwaukee, Milwaukee, WI

Abstract: Age-related differences in functional connectivity patterns along the hippocampal long axis
Cara I. Charles, University of Wisconsin-Milwaukee – Dept. of Psychology
Saisha M. Birr, University of Wisconsin-Milwaukee – Dept. of Psychology
Caitlin R. Bowman, University of Wisconsin-Milwaukee – Dept. of Psychology
The hippocampus is a critical structure for the formation of new episodic memories and is known to undergo age-related structural and functional decline. Recent work in young adults has proposed a functional gradient along the hippocampal longitudinal axis in which anterior portions support generalized, course memory representations, and the posterior tail supports detailed, fine-grained representations. In young adults, this distinction is apparent in differential patterns of functional connectivity between the hippocampus and cortical regions that are differentially involved in memory abstraction and memory specificity. Here, we investigated whether functional connectivity differences along the hippocampal longitudinal axis also differ across the adult lifespan. We used data from the Cambridge Center for Aging and Neuroscience that includes resting state fMRI data from 650+ individuals aged 18-88 years. We examined whole-brain functional connectivity patterns for the head, body, and tail of the hippocampus and their age-related differences in these patterns. Across all age groups, the hippocampal head showed uniquely strong connectivity with adjacent regions like the amygdala and temporal pole, and the tail showed uniquely strong connectivity with portions of visual cortex and the posterior cingulate. The hippocampal body showed a connectivity pattern that overlapped with both the hippocampal head and tail patterns. We formally compared the similarity of connectivity maps between pairs of hippocampal segments (e.g., similarity of the hippocampal head and hippocampal body whole brain connectivity maps) and tested for age-related differences in this similarity metric. Surprisingly, we found that patterns functional connectivity across hippocampal subregions tended to become more distinct in older age. That is, segments of the hippocampus tended to have more idiosyncratic functional connectivity patterns in older age. The effect was particularly strong for the right hippocampal head connectivity compared to both the right hippocampal body and tail. Coupled with known age-related decline in hippocampus-dependent memory function, these findings suggest that more differentiated patterns of hippocampal connectivity can be a sign of poorer cognition.

Disclosures: **C.I. Charles:** None. **S.M. Birr:** None. **C.R. Bowman:** None.

Poster

PSTR570. Neural Mechanisms of Natural Cognitive Aging: Anatomical and Functional

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Program #/Poster #: PSTR570.30/VV54

Topic: H.12. Aging and Development

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MOST Grant 110-2321-B-006-004
MOST Grant 107-2410-H-002-124
NIH Grant 061886-01

Title: Greater distance judgment distortion is associated with more subjective than objective representational similarity in extrastriate functional responses in older compared to younger adults

Authors: C.-S. WANG[1], Y.-H. LEE[1], J.-Y. CHUANG[1], T.-S. WANG[1], P.-K. WANG[1,2], W.-C. CHAO[1,3], C.-Y. CHEN[1], Y.-S. SU[1], *J. O. S. GOH[1];
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Abstract: There is much evidence that judgment of spatial distances evinces greater distortion in older compared to younger adults. It has been suggested that such age differences in distance processing stems from egocentric spatial navigation strategies in older adults that subjectively modulate neural coding of traversed distances. In this present study, we evaluated the neural correlates underlying subjective distortion in older adult mental representations of spatial distance. 24 younger and 24 older adults freely navigated and memorized a virtual environment consisting of paths and landmarks. Subsequently, they estimated distances from a given start point to goal landmarks during functional magnetic resonance imaging scanning. Both age groups under- and over-estimated closer and farther distances, respectively. However, older adults showed greater distance distortion than younger adults. Representational similarity (RS) analysis revealed both age groups showed strong RS in neural responses to subjectively judged than actual objective distances across several brain areas. Critically, subjective RS was significantly greater than objective RS in older than younger adults in the extrastriate cortex. Our findings suggest that older brains engage greater internal modulation of visual imagery implicated in extrastriate processing that distort perceptual processing during spatial navigational retrieval.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.01/VV55

Topic: H.12. Aging and Development

Support: R01 AG072714
FL DoH grant 21A11
McKnight Brain Research Foundation

Title: Effects of cannabis on cognition in young and aged rats

Authors: *S. ZEQUEIRA[1], E. A. GAZAROV[2], A. A. GUVENLI[1], A. S. SENETRA[3], T. R. HAWKINSON[4], T. MEDINA[4], S. B. KEOHANE[4], M. FEBO[2], T. HIRANITA[11], L. R. MCMAHON[5], R. C. SUN[4,6], A. SHARMA[3], C. R. MCCURDY[7,8,9], B. SETLOW[2,8,10,9], J. L. BIZON[9,1,10,8];
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Abstract: Cannabis use is growing rapidly among older adults. As the number of older adults in the US is expected to reach 90 million by 2050, it is imperative to understand the potential cognitive impacts of cannabis use in this population. This is especially true given that cannabis use in young adults can impair cognition, and that many aged individuals already exhibit such deficits, particularly in forms of cognition supported by prefrontal cortex (PFC) and hippocampus. We evaluated the effects of chronic oral administration of delta-9-tetrahydrocannabinol (THC) on a delayed response task that assessed PFC-dependent working memory and a water maze task that assessed hippocampal-dependent spatial memory in young adult (5 months) and aged (23 months) Fischer 344 x Brown Norway F1 hybrid rats of both sexes. Rats were initially trained on the delayed response task until reaching stable performance. In agreement with prior findings, aged rats were impaired compared to young adults, particularly at longer delays. Rats then consumed either plain gelatin or gelatin containing 1 mg/kg THC daily in their home cage. Drug was administered several hours after daily behavioral testing to dissociate chronic from acute effects. Working memory was assessed after three weeks of daily consumption. There were no effects of THC on working memory in young adult rats; however, aged rats consuming THC performed reliably better than aged rats consuming control gelatin. Rats were then trained on the water maze while continuing to consume gelatin following daily training. While aged rat performance in water maze was worse than young, no reliable effects of THC were observed at either age. In a second set of experiments, acute effect of cannabis smoke exposure on cognition was assessed. In a PFC-dependent delayed response task, acute cannabis smoke enhanced working memory accuracy in aged males but impaired accuracy in aged females, while having no effects in young adults of either sex. In contrast, acute cannabis smoke impaired performance on a hippocampus-dependent trial-unique non-matching to location task, irrespective of age or sex. These findings indicate that cannabis and THC can provide cognitive

benefits to older subjects under some conditions. Pharmacokinetics of THC and its metabolites were assessed using blood samples following both oral and smoked routes of administration, and revealed no significant differences between young and aged rats. Using Matrix-assisted laser desorption/ionization mass spectrometry (MALDI) imaging, brains will be analyzed to quantify changes in brain metabolism following chronic THC in young and aged rats

Disclosures: **S. Zequeira:** None. **E.A. Gazarov:** None. **A.A. Guvenli:** None. **A.S. Senetra:** None. **T.R. Hawkinson:** None. **T. Medina:** None. **S.B. Keohane:** None. **M. Febo:** None. **T. Hiranita:** None. **L.R. McMahon:** None. **R.C. Sun:** None. **A. Sharma:** None. **C.R. McCurdy:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.02/VV56

Topic: H.12. Aging and Development

Support: WaNPRC P51OD010425
Pfizer, Inc. (CAPECOD, E.A.B)

Title: Age-related pattern separation impairment in rhesus macaques

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Abstract: Pattern separation refers to the ability to distinguish between similar experiences and is a crucial feature of episodic memory. The Mnemonic Discrimination Task (MDT) is a behavioral task designed to measure pattern separation and critically depends on the dentate gyrus and CA3 subfield of the hippocampus. In humans, performance on the MDT declines with age and this impairment is exacerbated in Alzheimer's disease. To gain a deeper understanding of the neural mechanisms underlying age-related deficits in pattern separation, we adapted the MDT for use in rhesus macaques. The rhesus macaque presents exceptional similarities to humans in terms of neuroanatomy and physiology, making it an ideal animal model for human cognition. We examined pattern separation ability in a delayed match-to-sample paradigm with five macaques, including two young adult and three aged animals. In this task, each trial began with the presentation of a sample image, followed by a series of intervening nonmatching stimuli, "foils". After a variable number of foils (0-6), the sample image was presented again, and monkeys were rewarded for releasing the lever for this matching image. In a subset of trials, we introduced perceptually similar images, "lures", among the intervening nonmatching stimuli. The lures encompassed different levels of similarity, allowing us to assess the impact of varying levels of difficulty on pattern separation. We found an overall effect of age group (Linear Mixed Model: $\beta_1 = 0.136$, CIs = [.044, .228], $p = 0.005$) and difficulty level ($\beta_2 = -0.445$, CIs = [-.587, -

.303], $p < 0.001$) on performance. Aged macaques exhibited impaired pattern separation compared to young adults across all difficulty levels (Level 1: $p = 0.086$, Level 2: $p = 0.039$, Level 3: $p = 0.009$, Bonferroni corrected). Conversely, there was no age-related impairment on trials that only employed perceptually distinct foils ($p = 0.787$), demonstrating intact recognition memory in aged monkeys. Additional analyses suggested that the observed age-related impairments in the MDT cannot be attributed to changes in motor speed, attention, or impulsivity. Taken together, these findings provide compelling evidence for age-related impairments in pattern separation in rhesus macaques. This study paves the way for future neurophysiological studies aimed at elucidating the neurobiological mechanisms underlying this impairment.

Disclosures: C.R. Vanderlip: None. **M.L. Jutras:** None. **M.A. Yassa:** None. **E.A. Buffalo:** None.

Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.03/VV57

Topic: H.12. Aging and Development

Support: PREP Scholars Program- University of South Carolina Graduate School of Biological Sciences to AMO
South Carolina Honors College to KEC
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NIH Grant 2RF1AG050518 to JRF
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NIH Grant K01AG061263 to JAM

Title: Normal aging modifies sensitivity of orexinergic neurons to time-restricted feeding and ketogenic diet

Authors: *A. M. ORTH, K. E. COBB, K. B. PATEL, J. R. FADEL, J. A. MCQUAIL; Pharmacol. Physiol. Neurosci., Univ. of South Carolina, Columbia, SC

Abstract: Age-related deficits in brain glucose utilization are implicated in memory loss and risk for Alzheimer's disease. Recent studies suggest that a ketogenic diet, which is nutritionally complete, but supplies most calories from medium chain triglycerides (MCT), rather than as carbohydrates, can normalize glucose homeostasis and neural activity in advanced aging. However, the mechanism of this diet in the brain remains to be determined. Orexin (hypocretin)-producing neurons of the lateral hypothalamus sense changes in blood glucose, modulate food intake, and regulate activity of basal forebrain, hippocampal, and prefrontal cortex neurons. The present study tests the hypothesis that the ketogenic diet will increase the activity of orexin neurons, especially in the aged brain. Young adult (6 months) and aged (24 months) male and

female F344 x Brown Norway F1 hybrid rats were assigned a time-restricted (TRF) ketogenic, MCT-enriched diet (76% MCT oil; TRF-KMCT), a time/calorie-matched control diet (65% carbohydrates; TRF-CARB), or *ad libitum* (AL) access to standard chow (65% carbohydrates; AL-CHOW). Analysis of blood metabolites in AL-CHOW rats revealed sex-differences in regulation of blood glucose in aging, with marginally elevated blood glucose observed in aged males whereas age-matched females were susceptible to hypoglycemia following overnight fasting. Blood metabolite measurements in TRF rats revealed that KMCT reliably suppressed blood glucose and elevated blood beta-hydroxybutyrate (BHB), confirming efficacy to induce ketosis in aging rats of either sex. After 8 weeks, the diencephalon was harvested from all rats, fixed, sectioned in the horizontal plane, and dual-labelled for orexin-A, the major orexin peptide, and c-Fos, a neuronal activation marker. Single- (orexin) and double-labeled (c-Fos+orexin) neurons were counted exhaustively on sections from every animal. Diet reliably influenced the proportion of c-Fos+orexin-containing neurons and post hoc comparisons revealed these profiles were greater for either TRF-CARB or TRF-KMCT-fed aged rats relative to age-matched AL-CHOW. In young adults, c-Fos+orexin neurons were only increased between AL-CHOW and TRF-KETO. Collectively, our data demonstrate that orexin neurons of the aging brain are generally sensitive to restricted feeding and not necessarily diet composition, whereas the young adult brain is sensitive to ketogenic diet and not merely restricted feeding. These results indicate that time- or calorie-restricted feeding may be efficacious to normalize neural and cognitive deficits in normal aging and that benefits may not be specific to the ketogenic diet.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

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Topic: H.12. Aging and Development

Support: NIH Grant R01AI155887 to JLK
NIH Grant P20GM103641 to JAM, JLK, RTE, KTV, FH
VA Grant VISN7 RDA to FH
NIH Grant K01AG061263 to JAM

Title: Time-restricted feeding and ketogenic diet regulate gut microbiome independently of memory in aging rats

Authors: *T. J. COX[1], K. B. PATEL[1], K. E. COBB[1], T. E. PEACOCK[2], J. M. MOATS[1], K. T. VELAZQUEZ[2], R. T. ENOS[2], F. HOLLIS[1], J. L. KUBINAK[2], J. A. MCQUAIL[1];
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Abstract: The gut microbiome can influence brain health via the modulation of inflammatory responses, secretion of neuroactive substances, and interactions with the enteric nervous system. Aging is characterized by physiological and cognitive declines as well as gut dysbiosis, or an overall decrease in microbial diversity that is typified by reduced abundance of healthful bacteria (e.g., Akkermansia, Bifidobacterium) and exacerbated by outgrowth of harmful bacteria (e.g., Porphyromonadaceae). Prior work from our lab has identified rejuvenating effects of a ketogenic diet enriched with medium chain triglycerides (KMCT) on metabolism, body composition, physical function, and cognition in aging rats. Controlled changes to food intake or macronutrient profile of diets are credibly poised to regulate bacterial abundance and diversity in the gut, so we sought to determine if specific benefits of time-restricted feeding (TRF) and/or KMCT are associated with changes in the gut microbiome of normally aging rats. Fecal pellets were collected from young adult (8 months) and aged (26 months) male and female F344×Brown Norway F1 rats that were previously assigned to a TRF-KMCT diet or carbohydrate-enriched diets of ad libitum access to rat chow (AL-CHOW) or a time-restricted/calorie-matched control diet (TRF-CARB). 16S analysis revealed significant effects of diet and biological sex. Dissimilarity was highest in AL-CHOW and lowest in TRF-KMCT whereas diversity of observed species was lowest in AL-CHOW and highest in TRF-KMCT. TRF or diet composition contributed differing effects on discrete phyla. TRF-KMCT enhanced the abundance of Actinobacteria and Verrucomicrobia and reduced abundance of Firmicutes. TRF reduced the abundance of Bacteroidetes and Tenericutes. TRF-CARB reduced abundance of Proteobacteria, but this effect was reversed with TRF-KMCT. Further, diet interacted with age and sex to increase abundance of Actinobacteria in aging males on TRF-KMCT, but not in diet- and age-matched females. We also observed that age tended to blunt outgrowth of Verrucomicrobia by KMCT. Follow-up correlations with memory performance previously assessed in the Morris water maze revealed no reliable associations with abundance of Actinobacteria or Verrucomicrobia. In summary, our data affirm that KMCT is well-positioned to enhance gut microbiome diversity and combat dysbiosis in concert with time-restricted feeding. However, these effects may differ among aging males and females and further analysis, at deeper levels, is needed to establish associations with cognition or other metabolic and physical effects of diet.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.05/VV59

Topic: H.12. Aging and Development

Support: Vassar College Internal Support
NIH/NIDDK STEP-UP Program To FP

NIH Grant P20GM103641 to JAM
NIH Grant K01AG061263 to JAM

Title: Aging and ketogenic diet have sex dependent and brain region specific effects on astrocytes

Authors: *O. MCLERON[1], Z. DING[2], L. THOMPSON[2], G. CIACCIO[2], F. PINON[2], J. MCQUAIL[3], L. NEWMAN[2];
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Abstract: Insulin resistance increases with age, leading to increases in blood glucose, particularly after consumption of carbohydrates. This dysregulation of blood glucose can lead to increases in reactive oxygen species and cognitive impairments. The ketogenic diet, which is nutritionally complete and relies on medium chain triglycerides for caloric content, can reduce the need for insulin and has the potential to enhance cognitive functioning across the lifespan. Astrocytes in particular have been shown to play an important role in controlling blood flow through neurovascular coupling, processing of glucose, and the storage and breakdown of glycogen in learning and memory. It has been proposed that these aforementioned astrocytic processes can be disrupted, both by aging and neurodegenerative diseases. Our current study examines specific astrocyte proteins to determine if changes with age can be mitigated by ketogenic diets. Young adult (6 months) and aged (24 months) male and female F344 x Brown Norway F1 hybrid rats were assigned a time-restricted ketogenic MCT-enriched diet (76% MCT oil; TRF-KMCT), a time/calorie-matched control diet (65% carbohydrates; TRF-CARB), or *ad libitum* (AL) access to standard chow (65% carbohydrates; AL-CHOW) for 8 weeks. Glutamine synthetase, an astrocyte-specific enzyme involved in glutamate and GABA recycling, and glial fibrillary acidic protein (GFAP), a structural protein that increases with inflammation, were examined in the parahippocampal cortex (PHC) and the caudate putamen (CPu). Previous studies have indicated the PHC can show a sharp decline with age, whereas CPu shows relatively better maintenance of function and size. When examining the PHC:CPu ratio of glutamine synthetase expression, females fed the control TRF-CARb diet showed significant decline of expression with age, while males did not. This effect in females was mitigated in the TRF-KMCT groups. These results open the door for more exploration into changes in astrocyte function with age, and the potential role of ketogenic diet in reducing the glucose dysregulation that leads to cognitive impairment.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.06/VV60

Topic: H.12. Aging and Development

Support: University of South Carolina Office of Undergraduate Research to CEM
University of South Carolina Office of the Vice-President of Research to JAM
NIH Grant K01AG061263 to JAM

Title: A conditional delayed response test for early detection of memory loss in aging male and female rats

Authors: H. A. DUFALA, T. J. COX, C. E. MURPHY, *J. A. MCQUAIL;
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Abstract: Operant and spatial tests of rodent cognition aim to dissociate components of age-related memory loss, but brain regions and mnemonic processes naturally interact to support behavioral performance during cognitive tasks. Augmenting task complexity may enhance detection of early cognitive aging and neurobiological mechanisms. Beginning at 4 (young adult) or 12 months (middle-aged), we tested male and female F344 rats on two operant, delayed response tasks. On both tasks, one lever was randomly presented for the rat to sample and, following variable delays, memory was probed in a choice phase when the sampled lever was re-presented alongside a different lever. On the delayed matching task, rats were consistently reinforced for pressing the same lever presented in the sample phase before and after delays spanning 0-24 seconds. On the conditional delayed matching and non-matching task, response contingencies varied, but the correct choice condition was reliably signaled by non-illumination (match) or illumination (non-match) of cue lights before, during, and after delays that spanned 0-18 seconds. Choice accuracy of middle-aged rats on the delayed matching task was comparable to young adults across all delays. However, after switching to the conditional delayed matching and non-matching task, accuracy declined for both age groups and performance was significantly worse during non-matching trials. While increasing delay also dependably reduces accuracy across conditions, we even observed less accurate performance on trials with no delay. As performance stabilized, middle-aged rats demonstrated persistent impairments. Age tended to interact with response condition and delay to impair memory, which is likely attributed to worse performance on non-matching trials following longer delays. In summary, a conditional delayed matching and non-matching task is more sensitive to detect earlier declines in cognition relative to a simpler delayed matching task. Ongoing work will determine the degree to which performance on delayed response tasks affiliates with differences on reversal learning and set-shifting, tests dependent on the prefrontal cortex, or place-learning and delayed match-to-location tasks in the Morris water maze, to interrogate hippocampal function. In contrast to those classical behavioral assays, these delayed response tests produce stable patterns of daily performance. As such, delayed response tests are useful to implement longitudinal testing of cognition across the full rodent lifespan or to assess individualized responses to experimental interventions designed to slow or reverse memory loss associated with aging.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.07/VV61

Topic: H.12. Aging and Development

Support: University of South Carolina Office of Undergraduate Research to CHR, NJH, CEM
NIH Grant K99AG078400 to CMH
NIH Grant K01AG061263 to JAM

Title: Chronic stress and aging interact to influence working memory and expression of glutamate- and GABA-related genes in the prelimbic cortex

Authors: *C. H. RYAN[1], T. J. COX[1], H. M. GANDY[1], N. J. HAMMOND[1], C. E. MURPHY[1], H. A. DUFALA[1], C. M. HERNANDEZ[2], J. A. MCQUAIL[1];
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Abstract: Working memory depends on optimal coordination of glutamatergic and GABAergic signaling within the prefrontal cortex (PFC), a brain region that is highly sensitive to effects of stress and aging. Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and an imbalance to the brain's excitatory-inhibitory dynamic are common elements of stress-associated neuropsychiatric disorders and Alzheimer's disease (AD). The glucocorticoid hypothesis proposes that effects of aging may be attributed to cumulative effects of stress and stress-hormone exposure over the lifespan, but no studies have formally compared the effects of normal aging or chronic stress on working memory and expression of glutamate- and GABA-related genes in the PFC. We investigated the effects of 21 days of exposure to twice-daily, varied stressors on PFC-dependent working memory of young adult (4-6 months) and aged (22-24 months) F344 male and female rats. Chronic stress had no effect on working memory of aging females. In males, chronic stress interacted with age to impair working memory of young adults but, surprisingly, improved working memory of aged rats compared to age-matched, unstressed controls. PFC tissues were harvested from rats and mRNA was isolated from PFC subregions to examine expression of glutamate and GABA-related genes by low-density PCR array. Preliminary analysis focused on 55 genes associated with glutamatergic or GABAergic signaling and determined that expression of Slc6a11 (GAT3 GABA transporter) was reliably greater in the prelimbic cortex as a function of biological age. Expression of Grm4 (glutamate metabotropic receptor 4), Plcb1 (phospholipase C beta 1), and Grin2b (glutamate ionotropic receptor NMDA type subunit 2B) were enhanced in the prelimbic cortex after chronic stress. No genes examined were regulated by age or stress in the infralimbic cortex. Among subtle findings for specific genes, correlative analysis of Log2FC values for effect magnitude and direction of age and stress revealed a significant correlation across all 55 genes, but only in the prelimbic cortex. Considering these molecular similarities at the level of glutamate and GABA gene expression, ongoing RNA-sequencing studies will investigate the degree to which other molecular pathways are modulated by chronic stress and aging. These data will be foundational to understand the multidirectional effects of stress and neuroendocrine signaling on memory and neural function

over the full lifespan, and lead to tailored therapeutics that may rescue PFC signaling in aging and AD via restored HPA axis feedback.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

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Program #/Poster #: PSTR571.08/VV62

Topic: H.12. Aging and Development

Support: NIH Grant K01AG061263 to JAM
Magellan Scholars Award to CEM, NJH, CHR

Title: Advanced aging attenuates cognitive consequences of chronic stress in male and female rats

Authors: *C. MURPHY, T. J. COX, N. J. HAMMOND, C. H. RYAN, H. A. DUFALA, J. A. MCQUAIL;
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Abstract: Cognition is jointly susceptible to decline following stress or advanced aging. Indeed, the Glucocorticoid Hypothesis and Allostatic Load Hypothesis propose that brain aging is partly due to the cumulative effects of stress and stress hormone exposure over the lifespan. A corollary observation is that advanced aging erodes sensitivity to behavioral and neurobiological sequelae of stress. However, few studies have systematically examined effects of chronic stress on cognition over the full lifespan and, more specifically, in aging males and females. The lack of consideration for sex as a biological variable is critical given that aging women are at greater risk for Alzheimer's disease, men and women may experience stressors of differing frequency or nature, and stress responses may diverge between the sexes. As such, we investigated the influence of chronic stress on male and female F344 rats at 4 or 22 months (mo.) on spatial learning in the Morris water maze (MWM). Rats of each sex and age were randomized to unstressed (UNS) or repeated restraint stress (RRS; 6 hours/day). RRS was initiated 14 days prior to the first day of MWM and continued after daily testing for a total of 24 days. In Days 1-8 of MWM, rats were trained to escape onto a hidden platform that was fixed in the center of one quadrant of the maze. Memory for the platform location was assessed during probe trials when the platform was temporarily lowered to prevent immediate escape. On Days 9 and 10, the hidden platform was relocated to the opposite quadrant to evaluate reversal learning. On Day 10, a visible platform was used to assess cue learning and non-spatial performance. Analysis of probe trial performance revealed expected, impairing effects of age or stress. Planned contrasts revealed that RRS significantly impaired cognition of 4-mo. rats, but not 22-mo. Following

reversal training, 22-mo. UNS rats perseverated in searching the quadrant that previously held the training platform whereas age-matched RRS rats showed no spatial preference. RRS did not affect cue training in either age group, although velocity of RRS rats was faster compared to UNS, independent of age. In summary, our data confirm that age strongly moderates effects of stress on cognition. Ongoing work will increase sample sizes to compare interactions of stress and aging between males and females. Further, we will study 12-mo. rats to identify the time course over which aging influences behavioral responses to stress. These ongoing studies will determine cause-and-effect relationships between stress and cognition in aging males and females to better understand how stress contributes to age- and sex-related susceptibility to memory loss.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.09/VV63

Topic: H.12. Aging and Development

Title: Associative memory in late life: independent contributions of SES and hippocampal volume

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Abstract: Hypotheses: Socioeconomic status (**SES**) is related to memory ability, especially associative memory (eg, Peterson et al, 2017, *Cortex*). SES is also related to hippocampal volume (eg, Butterworth et al, 2012, *SCAN*). Given that the hippocampus plays an essential role in associative memory (eg, Lafontaine et al., 2020, *Hndk Clin Neurol*), this suggests that hippocampal volume may mediate the SES-AM relation. Here we test this hypothesis using UK Biobank data. **Method**: We analyzed data from the UK biobank (ages 44-82), excluding heavy drinkers, those who were morbidly obese, and those without MRI, SES info[1] or AM performance, yielding n=15,795. SES was measured with a principal component used previously (Kweon et al, 2022, *Sci Advances*). AM was measured by the Biobank's verbal Paired Associate Learning test (Field 2561). Statistical mediation, which would be consistent with a causal role for HV in the SES-AM relation, was tested with bootstrapped mediation.**Results:** Controlling for gender and age in all regressions, and reporting standardized betas, SES was related to both left and right hippocampal volume ($\beta = 0.081$, $p < 0.0001$; $b = 0.076$, $p < 0.0001$, respectively). SES was also related to memory performance ($\beta = 0.194$, $p < 0.0001$). Finally, we tested the relation between hippocampal volume and memory, again finding the expected relation ($\beta = 0.037$, $p < 0.0001$ and $\beta = 0.028$, $p = 0.0012$ for L and R, respectively). These three pairwise relations make it natural to explain the relation of SES to memory via the mediating influence of hippocampal

volume, but this was not the case. Adding hippocampal volume to the SES-memory model had virtually no effect on the SES-AM relation (with either L or R hippo in the model, the SES-AM relation was reduced by one half of a percentage point). Therefore, whatever aspect of hippocampal function varies with volume, it is different from that involved in the SES-memory relation. In conclusion, in a large late-life sample, SES, memory, and hippocampus volume are all inter-related pairwise, but hippocampal volume does not mediate the SES-AM relation. Late life memory is predicted separately by SES and hippocampal volume. Future studies will examine these same relations for the volume of hippocampal subfields.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.10/VV64

Topic: H.12. Aging and Development

Support: Goldwater Foundation to NJH
University of South Carolina Office of Undergraduate Research to NJH, CEM, and CHR
NIH Grant K01AG061263 to JAM

Title: The role of the locus coeruleus in working memory during stress and aging

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Abstract: The locus coeruleus (LC) is the main source of norepinephrine (NE) to the cerebral cortex, where this modulatory neurotransmitter calibrates neural activity and cognitive functions in response to arousing or stressful stimuli. While the LC is among the first regions to accumulate pathology in Alzheimer's disease, the effects of normal aging on LC cellular integrity and activity are sparse and conflicting. Due to these contradictions and the fact that the LC mediates arousal and coordinates neural and behavioral responses to perceived stressors, influences of the LC-NE system on cognition during normal aging may vary in response to stress. To address these questions, we examined the effects of chronic variable stress (CVS) on working memory and the LC in aging (4-6 or 22-24 months) male and female F344 rats. Working memory was assessed using a two-lever delayed match-to-sample operant task. In unstressed (UNS) rats, working memory accuracy of female rats was superior to males, who exhibited marked, age-dependent working memory deficits. Exposure to CVS, entailing twice-daily exposure to stressors (forced swims, physical restraint, predator urine exposure, and cage floods) for 21 days after daily working memory testing, led to significant improvement of

working memory in aged males relative to age-matched UNS controls, returning working memory to a level of accuracy indistinguishable from young rats. To determine whether the LC is involved in this sex- and age-specific behavioral effect of stress, we harvested brains from all rats immediately following the final working memory testing session, approximately 18 hours after the final stress exposure. The hindbrain, containing the LC, was dissected, fixed, and prepared for immunohistochemical staining. Ongoing studies will quantify the number of tyrosine hydroxylase (TH+) neurons in the LC and measure the proportion of persistently active LC neurons containing the long-lived Fos splice variant ΔFosB. We predict that chronic stress may reverse age-related changes in TH expression in the LC or increase the fraction of active LC neurons in males, and these increases will associate with working memory rescue. Once complete, our results will establish interactions among biological sex, aging, and LC-NE cellular responses to stress and how these changes may influence working memory. These data will improve our understanding of the role this brain nucleus plays in mental health and cognitive well-being over the lifespan and may direct the development of future therapeutics aimed at curtailing cognitive decline.

Disclosures: **N.J. Hammond:** None. **T.J. Cox:** None. **H.A. Dufala:** None. **C.H. Ryan:** None. **C.E. Murphy:** None. **J.A. McQuail:** None.

Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.11/WW1

Topic: H.12. Aging and Development

Support: University of South Carolina School of Medicine Office of the Associate Dean of Research and Graduate Education to DJH
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NIH K01AG061263 to JAM
NIH P20GM109091 to JAM

Title: Sex differences in NMDAR composition moderate efficacy of targeted therapeutics to reverse age-related memory loss

Authors: *D. J. HOROVITZ, M. A. TIEMAN, A. E. SIKORA, J. A. MCQUAIL;
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Abstract: N-methyl-d-aspartate receptors (NMDARs) are crucial for learning and memory. NMDAR dysfunction has been implicated in age-related memory loss and the pathophysiology of Alzheimer's disease. Divergent contributions to plasticity and neurodegeneration are likely attributed to discrete subpopulations of NMDARs that segregate between synaptic and extra-synaptic sites, contain different proportions of GluN2A and GluN2B subunits, and participate in dissociable intracellular signaling pathways. NAMENDA is a non-selective NMDAR channel

blocker, so it is chiefly presumed to restrict neurotoxic Ca2+ influx but may also limit physiological NMDAR activation. Serine is an essential co-activator of synaptic NMDARs, providing a therapeutically attractive route to optimize NMDAR signaling and reverse memory loss. We screened aged, 22-month-old, male and female F344 rats for spatial learning deficits in a multi-day, place-learning version of the Morris water maze relative to performance of 4-month-old young adults. Impaired, aged male and female rats were re-tested on a delayed match-to-place task to confirm persistent cognitive impairment before treatment with 0, 1, 3, and 10 mg/kg MPC, a d-amino acid oxidase inhibitor to prevent enzymatic degradation of serine. Treatment with MPC induced a dose-dependent improvement in memory performance of aged males. Surprisingly, age- and baseline memory-matched females showed no improvement with MPC. In a separate cohort of untreated rats, we immuno-precipitated GluN2B-containing NMDARs from hippocampal homogenates and observed a trend towards greater association with the synaptic scaffold PSD-95 in aged males, relative to young males, whereas aging females showed no differences. Qualitative studies that immuno-precipitated or immuno-depleted GluN2A suggest aging may increase abundance of GluN1/GluN2A/GluN2B tri-heteromeric NMDARs and attenuate levels of GluN1/GluN2B di-heteromeric NMDARs and levels of both are lower in females compared to males. In summary, our data demonstrate the potential to reverse memory loss in aging via modulation of brain serine, but such effects appear to be sex-specific due to differences in abundance and composition of brain NMDARs found in aging males vs. females. Ongoing work will focus on these sex differences and examine influences of MPC and NAMENDA on intracellular signaling cascades associated with synaptic plasticity and neuronal viability. Our experiments will uncover the neurobiological basis for sex disparities in age-related memory disorders and spur the development of NMDAR-directed therapeutics and adjuvants for aging men and women.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

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Program #/Poster #: PSTR571.12/WW2

Topic: H.12. Aging and Development

Support: NRF-2019R1A5A2026045
HI22C0724
HR22C1734
Korea Biobank Project 6637-303

Title: Ex vivo identification of transcriptional regulation landscape and potential therapeutic targets against ER stress using Alzheimer's disease patient-derived dermal fibroblast: findings from BICWALZS cohort

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Abstract: Background: Endoplasmic reticulum (ER) stress is considered as a central pathophysiology for neurodegeneration in Alzheimer's disease (AD). Many studies using in vitro cell line or in vivo mouse model have shown the possible association between ER stress and neurodegeneration. However, that connection was not elucidated well in AD patient-derived cell or brain organoids. In this study, we aimed to identify transcriptional regulation landscape and potential therapeutic targets against ER stress using AD dementia patient-derived dermal fibroblast. Method: For the purposes of ex vivo research, we performed skin biopsy and stabilized dermal fibroblasts in vitro. Total of 20 AD dementia patients and 22 cognitively normal older adults were assessed. All of AD dementia patients were amyloid positive on PET imaging, APOE e4 carrier, and cognition was impaired. On the contrary, all of cognitively normal older adults were amyloid negative on PET imaging, and APOE e4 noncarrier. We applied thabsigargin (10 nM) to dermal fibroblast for 24 hours to promote ER stress. After that, to identify transcriptional regulation landscape against ER stress, RNA sequencing was performed. Result: Total of 252 transcripts (up=162, down=90) were commonly differentially expressed in both of AD dementia and cognitively normal older adults derived dermal fibroblast by ER stress-thabsigargin. These transcripts were enriched in GO categories related to protein folding in ER, response to ER stress, and ERAD pathway. Interestingly, 80 transcripts (up=33, down=47) were only differentially expressed in AD dementia dermal fibroblast by ER stress-thabsigargin. These transcripts were enriched in GO categories related to cell division, miotic cell cycle, and microtubule-based movement. Total of 60 transcripts (up=41, down=19) were only differentially expressed in cognitively normal older adults dermal fibroblast by ER stress-thabsigargin. These transcripts were enriched in GO categories related to cholesterol biosynthesis process, DNA replication checkpoint, and cAMP-mediated signaling. Conclusion: We identified transcriptional regulation landscape by ER stress-thabsigargin in AD dementia and cognitively normal older adults dermal fibroblast. Further bioinformatic analysis with computational tools and validation experiments will be performed to find potential therapeutic targets against ER stress.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.13/WW3

Topic: H.12. Aging and Development

Support: German Research Foundation CRC 1436 (project ID 425899996)

Title: Physical fitness as a proxy of cognitive reserve in healthy older adults - preliminary results

Authors: *S. SCHWARCK[1,2], N. VOCKERT[2], P. MÜLLER[2], E. N. MOLLOY[2,3], L. FISCHER[2], A. HOCHKEPPLER[2,1], B. SCHUMANN-WERNER[2,1], N. BEHRENBRUCH[2], M. KREIßL[3], M. R. KREUTZ[4,5], E. DUZEL[6,1,2], A. MAASS[2];
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Abstract: Cognitive reserve plays an important role in successful aging, by maintaining memory performance despite brain changes. Higher cognitive reserve, often approximated by education years or IQ, seems to mitigate the age-related decrease in hippocampal volume. Although physical activity is well-known to counteract cognitive decline, physical fitness as potential cognitive reserve proxy has been rather neglected or only been assessed via questionnaires. As such, we hypothesized that the association between age-related hippocampal atrophy and worse cognitive performance would be attenuated by high aerobic and muscular capacity. 70 cognitively unimpaired older adults (age: 72.84 ± 7.98yrs; 24 female) underwent a comprehensive neuropsychological test battery assessing, i.a., global cognitive performance using CERAD-Plus (Consortium to Establish a Registry for Alzheimer's Disease). Their physical fitness was measured in the form of both aerobic (VO_{2max}) and muscular capacity (z-normalized composite: grip strength, appendicular skeletal muscle mass and walking performance). Whole hippocampal volumes (wHCV) were extracted from their T1w MRI images (3T MEMPRAGE, 0.8x0.8x0.8mm resolution) using the T1-ASHS segmentation algorithm (manual correction of 51 hemispheres). Linear regression models were performed to test the hypothesis that aerobic and muscular capacity each moderate the relationship between wHCV and cognitive performance including sex and age as covariates. Linear regression yielded a positive association between wHCV and global cognitive performance as assessed with the CERAD ($p = .024$). Furthermore, muscular capacity moderated this association ($p = .039$), but aerobic capacity did not ($p = .197$). Our results support evidence for muscular capacity, but not aerobic capacity, as cognitive reserve proxy. As such, the global cognitive performance of participants with lower muscular capacity seems to be dependent on the extent of structural integrity of the hippocampus. In contrast, the relationship is attenuated in participants with high muscular capacity, suggesting a link between higher physical fitness and cognitive reserve processes. However, our findings are preliminary, given the future addition of more participants.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.14/WW4

Topic: H.12. Aging and Development

Support: Else Kröner-Promotionskolleg „Jena School for Ageing Medicine (JSAM)

Title: Optimizing MCI data preparation for progression rediction using a user friendly lightweight data management system

Authors: *A. SCHWEINAR[1], F. WAGNER[2], S. FESTAG[3], C. SPRECKELSEN[3], S. BRODOEHL[2];
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Abstract: Alzheimer's is the most common neurodegenerative disease, affecting 55 million people worldwide. Early diagnosis is crucial for slowing its progression. Mild Cognitive Impairment (MCI) is a precursor to dementia and provides an ideal stage for early detection. Artificial intelligence (AI), specifically Random Forest algorithms, can analyze complex data from MCI patients to identify risk factors for progression. Data collected routinely from MCI patients over several years are particularly suitable for predicting progression using AI. However, the management of research data in neurology faces challenges, including discoverability, completeness, standardization, and privacy. Researchers and clinicians believe that user-friendly, flexible data management systems that facilitate data standardization, management, and processing are a good solution. Existing data management systems like REDCap or Loris are complex and lack customization.Our study aimed to develop a lightweight, user-friendly, and customizable data management system to prepare MCI data for determining the relative importance of parameters on progression using Random Forest. We collected MCI data from 56 patients who had visited the Neurological Memory Center at the University Hospital of Jena more than twice between 2014 and 2022, including various parameters like laboratory results, neuropsychological tests, imaging, etc. Of the 56 patients, 30 developed dementia; 25 were female and 31 were male, ranging in age from 55 to 85 at the start of the study. Random Forest uses multiple decision trees trained on random subsets of data. Predictions are made by majority voting or averaging the tree predictions, and feature importance can be evaluated. Random Forest analysis was performed with dementia progression as the classifier, eliminating parameters with excessive missing data. Our data management system successfully prepared the data for analysis, as validated by a usability test with 10 employees. Random Forest identified important parameters for progression such as neuropsychological scores, medication use, age, folic acid, and creatinine. Gender had less significance. The system proved effective in storing, standardizing, and preparing data. This study opens opportunities for future research on the influence of individual parameters on MCI progression using Random Forest.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.15/WW5

Topic: H.12. Aging and Development

Support: 1K99AG078402-01
P30 AG050886

Title: Exploring metabolism as an intermediary in the gut-brain-axis using the TgF334 rat model of Alzheimer's disease

Authors: *A. BANERJEE[1], E. PARKER[2], T. W. BUFORD[1], A. R. HERNANDEZ[1];
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Abstract: While nearly 6 million Americans are currently living with the debilitating effects of Alzheimer's disease (AD), the lack of treatment options and impoverished understanding of the underlying causes of cognitive decline, metabolic impairment, impaired gut function and other symptoms leads to severe impairments in quality of life. In addition to cognitive impairment, AD is associated with neuropathology, impaired metabolic function and gut microbiome dysbiosis. However, the relationships between gut health (including the gut microbiome), metabolism and cognitive decline remains largely unknown, despite strong evidence that the gut-brain-axis is an important intermediary in neurodegenerative disease. Moreover, normal aging also influences both gut microbiome composition and peripheral metabolic health, demonstrating the importance of including geroscience as a factor. Gut dysbiosis can result in impaired insulin resistance as well as obesity, both of which increase the risk of developing AD. Therefore, this work aims to elucidate how altered gut microbiome composition can influence cognitive outcomes in an aged rat model of AD to identify potential targets for therapeutic intervention. In particular, this work investigates whether the gut is able to exert its influence over cognition through metabolic intermediates, as the gut microbiome directly influences metabolite production and energy homeostasis. Our data indicate that aged female TgF344-AD rats have impaired cognitive performance, glucose metabolism, short chain fatty acid excretion and lifespan. Moreover, significant differences in gut microbiome composition across genotypes are regionally dependent along the length of the intestinal tract and fecal samples. Several differential analysis methods revealed distinct amplicon sequence variants (ASVs) within each intestinal region at multiple taxonomic levels. PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was utilized to predict functional abundances based these sequences, which revealed several Enzyme Classifications that significantly differed based on genotype, helping to discern the functional relevance of changes in gut microbiome populations. Collectively, these data further strengthen the gut-brain-axis's role in AD and can be utilized to generate potential therapeutics targeting the gut for the amelioration of age and AD related cognitive and neurobiological impairments.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.16/WW6

Topic: H.12. Aging and Development

Support: NIH/NIA Grant R01AG038465
NIH/NIA Grant R01AG026158-11

Title: Sociability and cognition across the adult lifespan

Authors: *R. BABUKUTTY, J. B. HOPKINS, C. H. LEIGHTON, O. T. T. MYERS, A. COORS, Y. GAZES, S. SANZ SIMON, Y. STERN;
The Taub Inst. for the Res. on Alzheimer's Dis. and the Aging Brain, Columbia Univ. Med. Ctr., New York, NY

Abstract: Background: Maintaining healthy social relationships is crucial to preserving cognitive performance as we age. Despite previous literature showing the protective effects of sociability on cognition, it is unclear which cognitive abilities are most impacted by this relationship. Here, we examine how sociability, quantified by the size and quality of our social networks, influences cognitive performance as measured by four established reference abilities: fluid reasoning, memory, vocabulary, and processing speed. **Methods:** Our sample consisted of 207 cognitively healthy, right-handed adults (mean age=58.55±15.5 years, range=26-84 years, 120 females) from the five-year follow-up of the Cognitive Reserve and the Reference Ability Neural Network Studies. Participants completed self-reported questionnaires on Perceived Social Support (PSS) and Social Engagement Network Size (SNS), where higher PSS and SNS is indicative of higher sociability. Participants also underwent neuropsychological testing to assess the four reference abilities and screen for dementia and mild cognitive impairments. **Results:** Exploratory factor analysis was performed on PSS and SNS to extract a factor score for sociability (SOC). A bivariate Pearson correlation coefficient was used to assess the relationships between SOC, age, and the four reference abilities. As seen in previous studies, age is negatively correlated with fluid reasoning, memory, and processing speed. Additionally, we found a negative correlation between age and SOC, $r(205) = -.247$, $p < .001$. Most significantly, we found a positive correlation between SOC and processing speed, $r(205), = .242$, $p < .001$. There were no significant relationships between SOC and the remaining reference abilities. **Conclusions:** Of the four reference abilities, processing speed is the most positively correlated with SOC, suggesting that participants with higher sociability may have faster processing speed. As age has a substantial influence on cognitive performance, sociability may be an important mediator between age and cognition. This mediatory relationship may impact the observed correlations between sociability and the four reference abilities. However, further research is required to model the directionality of this relationship.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

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Program #/Poster #: PSTR571.17/WW7

Topic: H.12. Aging and Development

Support: NIA R01AG066430

Title: Heart rate variability and basal forebrain activity during proactive interference across the lifespan

Authors: *E. RILEY[1], C. CAMMARATA[2], A. K. ANDERSON[1], E. D. DE ROSA[1]; [1]Psychology, Cornell Univ., Ithaca, NY; [2]Duke Univ., Duke Univ., Durham, NC

Abstract: Introduction: Cognitive aging, neurodegeneration, and Alzheimer's disease are strongly related to age-related declines in basal forebrain (BF) function. The BF is also a critical node in the central autonomic network and is hypothesized to provide beat-by-beat inhibitory control of heart rate. Here we examined age-related differences in BF-centered networks as a common central origin for cognitive and autonomic (vagally-mediated HRV) regulation in young, middle aged and older adults. We used a proactive interference (PI) task known to recruit the BF. **Methods:** Our sample contained 72 individuals (32 younger, 17 middle aged, 23 older). Participants completed a block-design PI task with multi-echo fMRI to measure BOLD and photoplethysmography to measure pulse. In the first of two runs of the task, participants discriminated on which side of the screen contained a target color (A+B-). In the second run, the participants responded to new targets, while the targets from the first run became distractors (C+A-), to provoke PI as participants learned the new targets. **Results: Behavior:** Young adults performed best on the task ($p < 0.001$), with 92% accuracy at baseline, falling to 88% accuracy when PI was provoked. Middle aged adults were 74% accurate, falling to 68% and older adults were 77% accurate, falling to 67%. Accuracy recovered in all groups. **HRV:** Young adults had higher heart rate variability (RMSSD) than middle aged or older adults ($p < 0.001$). Overall, heart rate variability decreased in young adults during task blocks (ON) compared with rest blocks (OFF) ($p < 0.001$; RMSSD actually increased in older adults during task blocks ($p = 0.003$.)) RMSSD trended downward in all age groups during PI ($p = 0.08$). **BOLD:** In the brain, we found significant clusters of voxels at the BF and BF-related cortical structures that were significantly more active in young adults than in middle aged or older adults during the PI portion of the task ($p = 0.003$). BOLD activity was selectively related to PI ($p < 0.001$) in young adults. **Conclusions:** Expected autonomic responses to the PI task, i.e. decreased HRV, were selectively observed in younger adults; older adults actually showed the reverse (increased HRV) despite much more difficulty with the task. The BF was more active in younger adults than other groups. Overall, hypothesized cholinergic modulation of both the BF and the heart linked with cognitive activity in young adults and may change significantly throughout the lifespan and therefore may be a useful index of cognitive status.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Topic: H.12. Aging and Development

Support: Schweppe Sholar Award
Sloan Research Fellowship

Title: Optogenetic Stimulation of PPC Restores Early Exploratory Behaviors and Learning-dependent Decision Making

Authors: *S. KORDE[1], Y. HAN[1,2,3], B. LIAN[1], J. SAMBANG[1], A. OWUSU-OFORI[1,2], M. DIASAMIDZE[1], L. M. WONG[1], N. PICKERING[1], S. BEGIN[1], E. J. HWANG[1];
[1]Cell Biol. and Anatomy, Stanson Toshok Ctr. for Brain Function and Repair, Rosalind Franklin Univ. of Med. and Science, Chicago Med. Sch., North Chicago, IL; [2]Dept. of Neurosci., [3]Dept. of Computer Sci., Lake Forest Col., Lake Forest, IL

Abstract: The rapidly evolving world requires us to persistently learn new information and rules to make adaptable decisions, a task that becomes more challenging with age. This decline might be attributed to the observed decrease in exploration among older adults, which would limit the discovery of solutions suitable for novel and non-stationary problems. Accumulating evidence suggests that exploratory decisions are dependent on the posterior parietal cortex (PPC). The PPC is more intensely engaged when people opt for exploratory decisions rather than exploitative ones during reinforcement learning, and inhibitory PPC stimulation curbs visual exploration. Thus, modifying PPC activity could potentially augment exploratory behavior, subsequently improving the capacity for learning-dependent decision-making. Our study aimed to validate this hypothesis using a mouse model. We initially characterized decision-making and learning in mice ranging in age from 3 to 22 months (both female and male) using the International Brain Laboratory (IBL) decision-making task. In this task, mice are shown a Gabor patch stimulus either on the right or left side of the screen. They are required to use their forepaws to turn the wheel, moving the stimulus to the center of the screen to receive a water reward (a leftward turn is needed for the right side stimulus and vice versa). It has been established that young adult mice can learn this stimulus-choice-outcome rule through multiple trial-and-error sessions. We trained each mouse for up to 40 sessions (one session per day). In line with the prior report, a majority of mice (20 out of 21 mice; 95%) in the youngest group (N=21; age 2.7 ± 0.18 (mean ± s.d.) months) reached a correct choice rate of over 80%. In contrast, only 2 out of 16 mice in the oldest group (N=16; age 20.6 ± 0.60 months) managed to achieve a similar correct choice rate. Furthermore, we observed that early exploratory choices (e.g., win-switch trials) significantly decrease with age, mirroring human behavior. To investigate whether manipulation of the PPC could reverse this age-related reduction in exploration, we virally expressed an excitatory opsin, ChRmine, in the PPC of aged mice (N=7; age 19.9 ± 0.05 months). We then optogenetically stimulated the PPC during the initial 16

training sessions. Remarkably, by stimulating the PPC, we were able to restore early exploratory behavior in aged mice, thereby enhancing their learning in a novel decision-making task to a level similar to young adult mice. Collectively, our results support our hypothesis and imply that strengthening exploration could be a promising strategy to improve learning-dependent decision-making in older adults.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.19/WW9

Topic: H.12. Aging and Development

Support: NIH Grant R56AG068149

Title: Distinct fMRI Subsequent Memory Effects for Scene Targets and Lures in the Mnemonic Similarity Task

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Abstract: The mnemonic similarity test (MST), assessing the ability to discriminate between previously studied items and perceptually similar lures, has been proposed as a behavioral correlate of hippocampal pattern separation. Successful performance on the MST is assumed to rely on a "recall-to-reject" strategy where participants recollect study items to identify non-studied lures. This assumption implies that study items associated with a later-presented, successfully identified similar lure will engage encoding operations similar to those engaged by study items that go on to be identified as such. Thus, it would be expected that the neural correlates of successful encoding of correctly identified study items and items whose lures are correctly identified should be highly similar. To test this prediction, 23 younger (18-30 years) and 24 older (65-75 years) humans of both sexes underwent a scanned encoding phase followed by an out-of-scanner memory task. During the encoding phase, participants viewed a series of images of scenes and objects. At test, the items comprised of exact repetitions of previously studied items, items that were perceptually similar to a studied item, and entirely new images. The task was to classify each image as either 'Old', 'Similar', or 'New'. The fMRI data acquired at study were employed to estimate subsequent memory effects (SMEs) for scene images that were correctly identified as 'old' on the later memory test, and for images whose corresponding lures were correctly identified as 'similar'. Due to an insufficient number of item misses, we did not analyze object trials. Relative to incorrectly identified images, study images that were correctly

identified elicited enhanced BOLD responses ('positive' SMEs) in the parahippocampal place area (PPA), occipital place area (OPA), and the retrosplenial complex (RSC). In striking contrast, the analogous contrast for studied images associated with correct lure identification did not give rise to detectable SMEs. Moreover, in a direct contrast, correctly identified study items elicited greater SMEs in the PPA, OPA, and RSC than did the items whose lures were correctly identified on the memory test. All the effects were age-invariant. The robust SMEs for targets, but not for lures, suggests that while later memory for studied items likely relies on a recollection-like signal, the successful identification of lures depends on a different kind of signal, possibly related to an acontextual sense of familiarity. The findings challenge the assumption that successful identification of similar lures in the MST is dependent on encoding a high precision representation of the originally experienced item.

Disclosures: **A.N.Z. Aktas:** None. **S. Srokova:** None. **J. Koen:** None. **M.D. Rugg:** None.

Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.20/WW10

Topic: H.12. Aging and Development

Support: NIH Grant R01GM128183
National Institute of General Medical Sciences

Title: The $GABA_A$ receptor α5-selective positive allosteric modulator, MP-III-022, rescues postoperative cognitive impairments in aged mice

Authors: *J. LYU[1,2], R. NAGARAJAN[2], M. KAMBALI[2], M. WANG[1,2], D. SHARMIN[3], J. M. COOK[3,4], U. RUDOLPH[2];
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Abstract: Perioperative neurocognitive disorder (PND), i.e., cognitive deficits persisting more than four weeks after surgery, is a relatively common complication in elderly human patients. Studies have shown that GABAergic neurotransmission may be reduced in aged brains, potentially contributing to cognitive dysfunction. The mechanism underlying PND is not fully understood, but the presence of microglia is essential. PND is apparently dependent on inflammation induced by surgery. Our hypothesis is that a reduced functioning of the α5-$GABA_A$ receptor system in the aged brain is a main contributing factor to postoperative cognitive deficits. Notably, α5-selective negative allosteric modulators have been shown to reverse anesthesia-induced cognitive deficits in young adult animals, indicating that an increase in α5-$GABA_A$ receptor function may impair learning and memory at a young age. We tested whether an α5-selective positive allosteric modulator (α5-PAM), MP-III-022, can reduce postoperative

cognitive deficits in aged mice. In this study, MP-III-022 (1mg/kg/day in the drinking water) was administered to the mice 3 days prior to laparotomy and throughout the study. Mice with ablation of dentate gyrus (hilar) somatostatin interneurons (4-5 months old), a model of hippocampal aging, and chronologically aged mice (21-24 month old) were used. In both types of mice, we found that laparotomy reduced correct alternations in the Y maze, novel object recognition, learning and reversal learning in the water maze, and contextual fear conditioning, while these changes were absent with chronic MP-III-022 treatment, indicating prevention or reduction of postoperative cognitive impairments. Laparotomy caused a reduction of dendritic spine density which was reversed by MP-III-022. The α5-PAM MP-III-022 essentially completely abolished laparotomy-induced cognitive deficits and structural alterations. This study confirms that α5-mediated modulation of learning and memory functions in aged mice is different compared to what has been reported in young adult mice. Our data also validates our somatostatin interneuron ablation model of hippocampal aging. Overall, positive allosteric modulation of α5-$GABA_A$ receptors is a promising strategy to prevent or reverse surgery-induced cognitive impairments and thus potentially to attenuate PND in the elderly.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.21/WW11

Topic: H.12. Aging and Development

Support: 3R01AG061200-05S1
R01AG061200
R01MH101130

Title: Timing of “blackout period” for transient amnesia caused by PDE11A deletion in mice can differ by genetic background and age

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Abstract: Phosphodiesterase 11A4 (PDE11A4), a dual-specific cAMP/cGMP hydrolase, is preferentially enriched in the CA1 and subiculum subregions of the ventral hippocampus. Deletion of PDE11A on a C57BL/6J genetic background induces a “blackout period” for recent long-term social memories that ultimately leads to an enhancement of remote long-term memories (LTM) in females and males. Although this has been discerned in mice of a C57BL/6J background, it is not yet determined if the transient “blackout period” of social memories produced by PDE11A deletion is a strain-specific phenomena. Therefore, we determined if the

same phenotype would manifest when PDE11A was deleted from BALB/cJ mice, a genetic background in which PDE11A4 protein expression and function significantly differ due to the encoding of a threonine at amino acid 499, as opposed to an alanine in C57BL/6J mice. Female and male *Pde11a* wild-type (WT) and knockout (KO) mice on a 98.8% BALB/cJ genetic background were tested for recent and remote LTM on Social Transmission of Food Preference (STFP), Social Odor Recognition (SOR), and Non-Social Odor Recognition (NSOR). Indeed, deletion of PDE11A from BALB/cJ mice also resulted in a transient amnesia for STFP and SOR, but not NSOR in both young adult (2-6 months) and aged (18+ month) mice. Interestingly, however, the timing of the "blackout period" triggered by deletion of PDE11A was delayed in BALB/cJ mice relative to that observed in C57BL/6 mice. PDE11A deletion on the C57BL/6J background produces transient amnesia for STFP and SOR with normal short-term memory 1 hour after training, impaired recent LTM 24 hours after training, and enhanced remote memory 7 days after training in both young adult and aged mice. In contrast, PDE11A deletion on the BALB/cJ background resulted in normal recent LTM 24 hours after training, impaired recent LTM 48 hours after training, and enhanced remote memory 14 days after training in young adult mice. In aged mice, the blackout period was further delayed, with normal recent LTM 48 hours after training, impaired remote long-term memory 7 days after training, and spontaneously recovered memory 21 days after training. Studies are ongoing to investigate the biochemical underpinnings of the shifted "blackout period" observed in BALB/cJ mice, with a focus on stress-related signaling pathways. These findings not only provide insight into the molecular mechanisms regulating the transition from recent to remote LTM across the lifespan, they may also suggest that recent and remote LTMs are processed in parallel as opposed to sequentially.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

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Program #/Poster #: PSTR571.22/WW12

Topic: H.12. Aging and Development

Support: NIA R00AG058748
NIH R01AG074330

Title: D2/3 receptor occupancy measured with [^{11}C]-raclopride and functional brain network reconfiguration in healthy older adults

Authors: *T. MORIN[1,4], C. CIAMPA[2], J. PARENT[3], J. L. COWAN[2], A. ADORNATO[2], K. O'MALLEY[2], J. HOOKER[4], A. BERRY[1,3];
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Abstract: Aging is associated with declines in both the dopamine system and in memory ability. Methylphenidate increases the availability of dopamine at the synapse by inhibiting dopamine reuptake. We investigated effects of 20mg oral methylphenidate on memory and fMRI brain activity in cognitively normal older adults. Because the effects of dopaminergic drugs are highly dependent on baseline dopamine function, we used [^{11}C]raclopride PET imaging to measure both baseline D2/3 receptor non-displaceable binding potential (B_{ND}), and dopamine release following methylphenidate (% change in B_{ND}). In this study we recruited 22 older adults from the ongoing longitudinal Brandeis Aging Brain Study (mean age = 70.0 years, standard deviation = 4.47, range = 63-82; 11 males, 11 females) who were carefully screened for contraindications for methylphenidate and PET/MRI imaging. Participants underwent two simultaneous [^{11}C]raclopride PET/MRI scans on the same day first after receiving a placebo, and then after receiving 20mg methylphenidate. During each PET/MRI scan, we measured fMRI BOLD activity during rest and during a memory encoding task with high and low monetary incentives ($5 vs $0.01 per item). Memory was tested 24 hours later. Preliminary results suggest methylphenidate enhanced memory for items in the high monetary incentive condition ($T(20) = -2.91$, p &lt 0.01). Individual differences analyses demonstrate that the effect of high monetary incentive on memory was strongest for older adults with low baseline D2/3 receptor occupancy (Placebo: r = -0.71, p &lt 0.05). Analyses of fMRI data have focused on pre-to-post-task resting state network reconfiguration (quantified using Variation of Information), and find lower pre-to-post-task resting state network reconfiguration was associated with better overall memory performance in a drug-dependent manner (Placebo: r = -0.047, p = 0.86 ; Drug: r = -0.45, p &lt 0.05). Overall, these results suggest that dopamine availability is linked to memory for rewarding events and functional network dynamics in older adults.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.23/WW13

Topic: H.12. Aging and Development

Support: NIH/NIA R24 AG065172
James S. McDonnell Foundation

Title: Patterns of large-scale functional brain network decline are conserved across aging mice and humans

Authors: *E. WINTER-NELSON[1], E. BERGMANN[2,3], M. CHAN[1], L. HAN[1], S. KLAUSNER[1], Z. ZHANG[1], A. KAVUSHANSKY[3], J. ASLEH[2,3], J. ALGOO[2], I. KAHN[2,3], G. WIG[1,4];
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Abstract: In human individuals, healthy and pathological aging are accompanied by alterations in resting-state functional correlation (RSFC) large-scale brain networks. In healthy adults, increasing age is associated with decreasing RSFC brain system segregation, which is a measure quantifying the extent to which brain systems are functionally differentiated. Reduced system segregation is linked to lower cognitive ability and alterations in brain function. Independent of Alzheimer's Disease-related pathologies, declining system segregation is prognostic of dementia severity in older adults. Changes in RSFC system segregation have also been shown to vary with respect to socio-environmental factors during adulthood. Motivated by these observations from human fMRI research, establishing a cross-species model of brain network organization and decline across the lifespan could bypass methodological limitations in human lifespan research and allow for identification of the mechanisms and factors underlying age-associated brain network alterations. In a densely-sampled fMRI dataset of 3-month-old(mo) male C57BL/6 mice (n=7) imaged during resting wakefulness across multiple days, we applied established methods from human brain network research to construct and analyze large-scale RSFC networks. We first adapted the Allen Institute Mouse Brain Atlas (CCFv3) into a set of unbiased brain network nodes and then used these nodes to form mouse RSFC networks, to which we applied graph theoretic analysis. Consistent with human RSFC networks, mouse networks exhibit a modular organization such that areas belonging to the same system are more highly correlated with one another than they are with areas in different systems. Several mouse RSFC systems exhibit considerable overlap with large-scale systems derived from axonal connectivity and are consistently identified across individuals. In a cross-sectional comparison, system segregation is greater in 3mo mice than in 12mo mice (n=8; $t(13)=5.78$, $p<.001$). Given the known limitations of cross-sectional designs, we also computed segregation longitudinally for a set of 6 mice scanned at 6mo, 9mo, and 12mo; a mixed-effects linear model revealed that segregation declined within individuals across this timespan ($F(1,16)=5.20$, $p=.037$), paralleling results from human longitudinal research. These results fortify examination of RSFC networks as a viable framework through which to investigate similarities and incongruencies between rodent and human models of large-scale brain network changes across the lifespan, as well as environmental and genetic contributions to age-related cognitive decline.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.24/WW14

Topic: H.12. Aging and Development

Support: NIA R01AG066430 to E De Rosa and A Anderson

Title: Heart rate dynamics associated with aging and learning in Long-Evans rats

Authors: *M. K. MACMILLAN[1], E. RILEY[1], E. D. DE ROSA[1], A. K. ANDERSON[2];
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Abstract: A bi-directional relationship between cardiac health and brain health has been proposed to be mediated by the vagus nerve, which connects the central autonomic network with the heart. Here we examine heart rate dynamics in young and older rats during sleep and waking baseline conditions to describe non-cognitive changes with aging, along with task dependent changes in young rats during olfactory learning. In these pilot data, three older (>24 mos.) and four younger (6 mos.) male Long-Evans rats were implanted with a wireless telemeter (ADInstruments, TR50BB) to measure ECG and allowed to recover. Biopotential data were recorded at 2 kHz. ECG was collected nightly continuously for 20 minutes during sleep. Each day, 10 minutes of waking baseline data were recorded and 10 minutes of operant tasks of different levels of complexity: shaping without odors (no odor [NO], odor/no odor discrimination [ONO], or odor-pair discrimination [PD]). LabChart's heart rate variability (HRV) algorithm was used to detect normal beats and to reject ectopic beats and data was further visually inspected for noise or signal dropout. Heart rate dynamic metrics calculated included temporal measures of RR intervals, RMSSD (root mean square of successive deviations in RR interval), and a spectral LF/HF HRV ratio.Aging: For young and old rats, RR intervals were greater in sleep than waking ($p<.001$), and old rats had marginally longer RR intervals than young rats ($p=.058$) at waking. A task by group interaction indicated that decreases in RR intervals between sleeping and waking was greater for young rats compared to old rats ($p = 0.049$). LF/HF was greater in young rats than old rats in sleeping ($p=.013$) and waking ($p=.004$).Learning: In young rats, RR intervals were significantly different between PD and NO ($p =.0014$), ONO and NO ($p<.001$). Additionally, RR intervals were greater in waking compared to all task conditions, NO, ONO, and PD (all $p<.001$). Finally, RMSSD was marginally greater in ONO compared to waking ($p=.053$), and when collapsed across tasks (NO, ONO, PD), task was different from waking ($p=.0454$).In sum, we find evidence of task and age related changes in the neural regulation of the heart. Convergent data will be presented that examine the role of age related altered cholinergic central nervous contributions to heart rate dynamics.

Disclosures: M.K. MacMillan: None. **E. Riley:** None. **E.D. De Rosa:** None. **A.K. Anderson:** None.

Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.25/WW15

Topic: H.12. Aging and Development

Support: ReALity Innovation Fund

Title: Translating Molecular Signatures into Cognitive Performance: A Systemic Analysis of Healthy Aging in the HBBA Project

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Abstract: The Healthy Body and Brain Aging (HBBA) project uses a systemic approach from the brain to the body to delve, among others, into the complex interplay of genetic, epigenetic, and neurocognitive factors in healthy aging. Our cohort, displaying remarkable cognitive performance across several domains, despite high age provides an exceptional context for studying the mechanisms of cognitive resilience in aging. We have uncovered significant associations between PhenoAge, a measure of epigenetic age, and brain structures implicated in cognitive decline. Lower PhenoAge, after adjusting for chronological age, correlates with larger volumes of critical brain areas such as the hippocampus and basal forebrain, as well as enhanced structural interhemispheric connectivity. This points to a strong link between systemic aging, brain health, and cognitive function. Intriguing genetic findings also surfaced, with certain single nucleotide polymorphisms (SNPs) in *TACR3* and *BDNF* associated with aging and cognitive resilience respectively. Interestingly, the *TACR3* SNP rs2765 appears to impact the discrepancy between phenotypic and chronological age, with especially high underestimation of chronological age in individuals carrying the wild-type allele. The BDNF polymorphism Val66Met, on the other hand, seems to modulate cognitive resilience through increased hippocampal volumes. These insights underline the value of our systemic approach, weaving together genetic, epigenetic, and cognitive aspects of healthy aging. Future work will include expanding our analysis to proteomics, immunoaging, and telomere biology. Through the HBBA project, we aim to further illuminate the neuromolecular networks that underpin healthy aging and cognitive resilience, and to foster strategies for promoting healthy brain aging.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.26/WW16

Topic: H.12. Aging and Development

Support: John G. Kulhavi Professorship in Neuroscience
E. Malcom Field and Gary Leo Dunbar Chair of Neuroscience

Office of Research and Sponsored Programs at Central Michigan University

Title: Gender differences in the age-related cognitive ability in progesterone-treated mice

Authors: *K. ADAMS[1], D. DOYLE[1], O. SMITH[1], L. GARMO[1], U. ZEKONYTE[2], M. CHOUDHURY[1], A. UPRETY[1], N. DAY[1], B. SRINAGESHWAR[1], J. ROSSIGNOL[1], G. DUNBAR[1];
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Abstract: Progesterone is a neurosteroid and sex hormone that has been shown to reduce behavioral and neuropathological deficits following traumatic brain injury. However, less is known about its efficacy in reducing age-related cognitive deficits. The goal of the present study was to assess the potential impact of progesterone on cognitive ability as a function of sex and age. To this end, daily subcutaneous injections of progesterone (5 mg/kg) or vehicle (30% 2-hydroxy beta-cyclodextrin) were given to young (4-month-old) and old (20-23-month-old) male and female mice. The cognitive abilities of the mice were assessed using the water-T-maze and passive avoidance behavioral tasks. A total of 17 mice were initially analyzed, while additional animals are currently under study. To date, our data from this study demonstrated that progesterone may produce cognitive benefits in younger, male mice, but these benefits were not conserved within older male mice. Furthermore, results indicated potential detrimental effects of progesterone on the ability of both young and old female mice to perform cognitive behavioral tasks. These results are similar to previous unpublished findings from our lab. Further work is underway to help determine whether the addition of this dose of exogenous progesterone may have exceeded an optimal level of progesterone conferred by greater stores of endogenous progesterone in female mice. Although progesterone treatments tended to facilitate cognitive functioning in aged male mice, which was observed in our previous unpublished finding, only the young male mice benefitted significantly from the progesterone treatment in the present study. Our results indicate that progesterone treatments have varied effects on males and females at different ages, and that finding an optimal dose for specific ages and sex may be a critical variable for optimizing its therapeutic efficacy.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.27/WW17

Topic: H.12. Aging and Development

Support: NIA Grant AGO06265

Title: “Practiced” Processing is Responsive to Learning Across the Lifespan - Structural and Demographic Influences on Cognitive Aging

Authors: *E. T. SMITH, J. P. HENNESSEE, J. BACCI, D. C. PARK;
Ctr. for Vital Longevity, Univ. of Texas at Dallas, Dallas, TX

Abstract: The finding that cognitive processes tend to diminish with age is generally accepted, but the myriad factors which influence individual trajectories of cognitive aging are less well understood. The present study examined longitudinal change in five such capacities - episodic and working memory, speed of processing, reasoning, and verbal fluency - in an adult lifespan sample, and identified qualitatively distinct patterns of decline and separable predictors of rate of decline between these measures. Episodic memory, working memory, and processing speed demonstrated the expected longitudinal pattern of decline, demonstrated the expected accelerating pattern of decline over the lifespan with large heterogeneity between the trajectories of individual participants, closely resembling past research of cognitive decline. Reasoning and verbal fluency also demonstrated a decline over the lifespan when examined cross-sectionally, but universally showed a strong practice or learning effect across assessments within-participants of all examined ages - suggesting a preserved learning capacity which selectively impacts some aspects of cognition. Based on this finding, we pursued two cognitive constructs for further analysis - a “Practiced Processing” construct which included those measures that demonstrated significant practice/learning effects (reasoning and fluency), and a “Stable Processing” construct which included those measures that did not (memory and speed). Regression analysis identified separable predictors of individual rates of change on these two cognitive constructs. Annual change in Stable Processing was positively predicted by mean cortical thickness, which replicates recent findings by our group and others that cognitive decline in healthy aging is reflective of whole-brain gray matter decline. Conversely, annual change in Practice Processing was positively related to years of education and negatively related to total volume of white matter hypoentensities, and uniquely was found to be age-invariant. This latter finding specifically suggests that the reasoning and fluency measures which sensitive to learning and practice throughout the lifespan, but are also uniquely susceptible to white matter neuropathology compared to the Stable Processing construct.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Topic: H.12. Aging and Development

Support: University of Maine 5T32GM132006
NIA R01AG054180
NIA RF1AG063755
NIA 1F31AG077860-01A1

Title: Investigating Dendritic Spine Morphology as a Mediator of Cognitive Outcomes in Aged Diversity Outbred Mice

Authors: *A. R. OUELLETTE[1], N. HADAD[1], K. GREATHOUSE[2], A. WEBER[2], J. HERSKOWITZ[2], C. KACZOROWSKI[1];
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Abstract: The intersection of genetic diversity, memory, and synaptic function is a critical component in better understanding age-related cognitive decline. Diversity Outbred mice offer the opportunity to investigate cognitive aging across a genetically diverse population in a controlled lab environment. DO mice exhibit an appreciable amount of variance in Contextual Fear Memory and Acquisition (CFM, CFA), which can be linked to individual differences in genetic background (Ouellette A. et al, 2022, *Cell Reports)*. Here, we investigate the role of dendritic spines as a mediator of individual age-related changes in memory. While there was not a decline in CFM or CFA between 8 and 18mo, we observed expectedly wide range in individual memory outcomes. Thin spine density significantly decreased between 8mo and 18mo, and stubby spine density increased. Spine volume across all spine types increased with age. Apical thin spine density explained 61% of the variance in CFM outcomes, while thin spine volume explained 79% and 43% of variance CFA in apical and basal dendrites respectively. Thin spine density, however, did not associate with CFA outcomes. We have linked dendritic spine density and morphology as a potential mediator of individual outcomes across a genetically diverse population. Our results suggest that there may be an age-related conversion of thin to stubby spine types, and that mice with fewer thin spines are more likely to have better CFM outcomes, coinciding with reports of thin spines as dynamic "learning spines" rather than "memory storage" spines (Hayashi, Y. 2005, *Neuron*). We also show that the size of these "learning" thin spines may be more important memory acquisition than their total density.

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Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

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Program #/Poster #: PSTR571.29/WW19

Topic: H.12. Aging and Development

Support: Nanyang Technological University Internal Grant ARISE2017

Title: Using a multimodal imaging approach of magnetoencephalography and functional magnetic resonance imaging to determine the spatial-temporal dynamics of neural compensation in high performance cognitive aging.

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Abstract: Background: One of the hallmarks of successful cognitive aging, neural compensation, involves the recruitment of additional brain regions in response to variations in cognitive demands, which leads to enhancement of cognitive performance. Previous evidence for compensation consists largely of metabolic-type imaging, which revealed its spatial properties but lacks finer spatial-temporal dynamics of compensation. This study hypothesizes that temporal properties of compensation, such as the speed of recruiting the compensatory brain region, is positively related to cognitive performance. Method: The study adopts a multimodal approach of using functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG) where participants undergo the two sessions with the same experimental protocol, each with a respective imaging modality. Eleven young (mean age = 25, SD = 5.4 years, 4 females) and eleven old (mean age = 63.3, SD = 7.3 years, 3 females) participants were recruited and tasked to go through an experimental protocol that includes six sessions of single letter n-back memory task for both imaging modalities. The order of one, two and three-back memory tasks were counterbalanced using a balanced latin square design across both the MEG and fMRI sessions. The old participants were considered high-performing if the scores are within one standard deviation or higher than the average young adult. Representational similarity analysis (RSA) was employed to integrate MEG-fMRI data to reveal the spatio-temporal brain activity during the n-back trials of 250ms and the progression of neural compensation in the old adults. Thereafter, region specific evoked responses were derived to measure the latencies to the recruited brain regions. Results: The young adults have an accuracy score of 96.0%, 93.5% and 73.3% of one-, two- and three-back task scores respectively. Four old adults are categorized as high performers. The other seven old “low performers” scored 95.6%, 93.3%, 64.5% for the n-back tasks. The fMRI general linear modelling revealed that the high-performing old adults recruit additional brain regions when engaged in more cognitively demanding tasks. The MEG-fMRI representational convergence through RSA displayed additional recruitment in frontal-temporal cortical region during the 100-250ms window for the high-performing old adults. The evoked responses of these additional recruitments suggest a relationship where shorter latency is associated with higher cognitive performance. Conclusion: Neural compensation on successful cognitive ageing is influenced by a dynamic combination of both spatial and temporal properties.

Disclosures: W. Sim: None. **K. Lee:** None. **B. Gulyás:** None.

Poster

PSTR571. Learning and Memory in Aging: Neuromodulation, Pharmacology, Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR571.30/WW20

Topic: H.12. Aging and Development

Support: Dassault Systemes Foundation G-PROP000230

Title: Complementary reorganization of whole-brain neural connectivity at both extremes of age:The unitary interplay in fiber dynamics and collateral neural processing in brain across human life-span.

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Abstract: INTRODUCTION:.The whole-brain connectivity of the normal adult human brain has been well investigated. However, there is yetlimited comprehension of whole-brain connectivity and its compensatory adaptation at the vulnerable period of life,namely at the extremes of age: younger and older-age. Maladaptive networking at these two age segments areassociated respectively two critical brain dysfunctionalities: Neurodevelopmental disorders and Neurodegenerativedisorders. Here we investigate whole brain connectivity and remodelling in these two vulnerable segments, as timeelapses, and thereby identify common unitary processes, so that translational insights from one age segment could bepertinent to the other..METHODS:.We investigate the life spanning process, using 3 tesla MRI-DTI scans of 382 individuals (IXI scan library), across 15-90 years of age. Using our methodology of whole brain tractography methodology [1], we devise a novel networkmotif-based connectomics approach, offsetting the age-induced alteration of neuronal fiber density. For explanatoryphysiological analysis, we develop a quantitative biophysics-based cellular formulation in terms of neuron andextracellular bodies...RESULTS:.We find that as life-span ageing occurs, the brain's information flux, as estimated by the average basal fiber span andthe number of peripheral nodes shows a positive U-shaped quadratic behaviour with the minimization point at the 6thdecade. In the young-age segment (upto 30 years) there is gradual decrease of both parameters, while in the old-agesegment (after 60 years), both parameters monotonically increase..CONCLUSION:.We can account for both findings by the dynamics of collateral neural processing during the life-span..(1) Connectivity:.In young age, there is formative activation of peripheral arcuate fibers between gyri for actuating association area map(scaffolding model), the activation decreases in adulthood due to automatization. On the other hand, in the elderly,there is peripheral collateral neural activation increases using neural reserve, to compensate cognitive decline. We finfthat the alteration of the parameters (fibre span, peripheral nodes) of the two age transitions are complimentary to eachother..(2) Fiber span:In both age extremes, there is increased extracellular objects, as postsynaptic dense bodies (child), or neuritedeposition (elderly). These objects enhances tissue tortuosity, increasing fiber span..Thus complementary physiological processes in younger and older age elucidates higher peripheral nodal activationand basal fiber span.[1] W Otte et al, *Neuroimage, 109:* 171-189, 2015.

Disclosures: P.K. Roy: None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.01/WW21

Topic: H.13. Schizophrenia

Support: K01DA043615

Title: Frontal theta abnormalities in Individuals at clinical high risk (CHR) for psychosis

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Abstract: Neurophysiological studies in individuals with schizophrenia suggest abnormalities in resting-state electroencephalographic (EEG) activity, specifically in the bands. However, the specific patterns in interactions between these frequency bands, which can potentially add to a more objective and robust identification of individuals at risk for psychosis, have not been studied. To this end, we compared resting-state theta relative power between CHR and healthy controls (HC), during the eyes open (EO) and closed (EC) states. Resting EEG data (60-channel, 5 minutes of EO, and 9 minutes of EC) was obtained on 42 CHR (23 males) and 29 HC (14 males). Symptom severity was assessed using the Structured Interview for Psychosis-Risk Syndromes (SIPS). Signal processing was conducted using MATLAB (v.R2023a, MathWorks) with EEGLAB [v.14_1_2b], Brainstorm [v.May 1023], and custom software, following good-practice guidelines. EEG theta (4 - 7 Hz) band features were analyzed using a 2 (condition: EO, EC) x 2 (group: CHR, HC) ANOVA. Results show that relative theta power was more significant in the EO compared to the EC condition ($p<.001$), and the difference between EO and EC theta power was greater in CHR compared to HC ($p=.031$). It is important to note that a number of cognitive functions, including memory and attention, have been linked to increased theta power. In fact, increased theta wave activity has been associated with more severe negative symptoms in schizophrenia, even in first-episode psychosis and those at CHR. This link implies that these patterns in EEG slow wave activity exist in CHR individuals and that they precede the onset of frank psychosis.

Disclosures: R. Shaik: None. **T. Bel-Bahar:** None. **K. Ferster:** None. **S. Herrera:** None. **A. Srivastava:** None. **M. Cotter:** None. **S. Haas:** None. **G.A. Cecchi:** None. **C. Corcoran:** None. **M.A. Parvaz:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.02/WW22

Topic: H.13. Schizophrenia

Title: Effects of the atypical antipsychotic and d3/d2 partial agonist cariprazine on effort-based choice: implications for understanding the neuropharmacology of avolition

Authors: *A. ECEVITOGLU[1], G. EDELSTEIN[2], K. BEARD[1], A. MARTÍNEZ VERDÚ[3], R. OLIVARES-GARCÍA[4], A. KOVACH[1], R. CONRAD[1], M. CORREA[4], J. D. SALAMONE[2]; [2]Univ. of Connecticut, [1]Univ. of Connecticut, Storrs, CT; [3]Univ. Jaume I, Univ. Jaume I, Castelllón de la Plana, Spain; [4]Univ. Jaume I, Univ. Jaume I, Castellón de la Plana, Spain

Abstract: Schizophrenia is characterized by positive symptoms, cognitive dysfunctions, and negative symptoms such as avolition, which is a motivational impairment defined as a decrease in goal-directed behavior. The “typical” or “first generation” antipsychotic drugs are dopamine (DA) D2 receptor antagonists, however, they are relatively ineffective at treating negative symptoms. The need for better therapeutics led to the development of “atypical” antipsychotics that have multiple targets and exhibit fewer side effects. Third generation antipsychotic drugs include cariprazine (trade name Vraylar) which is a D3 preferring D3/D2 receptor partial agonist/antagonist. Both clinical and preclinical data suggest that cariprazine is effective in treating both positive and negative symptoms. However, there are no studies focusing specifically on avolition. To test the influence of cariprazine on avolition, the current study investigated the effects of cariprazine on effort-related choice behavior in male Sprague-Dawley rats. Rats were trained on the fixed ratio (FR) 5/chow feeding choice task, in which they were given the option of lever pressing for a preferred reward (high carbohydrate pellets) vs. approaching and consuming a less preferred reward (lab chow). When administered alone cariprazine shifted choice behavior, inducing a low-effort bias marked by reduced lever pressing for pellets, but increased intake of the concurrently available chow. Previous work has shown that D2 antagonists such as haloperidol and eticlopride produced a similar low-effort bias in rodents. This shift in choice behavior was partially reversed by the adenosine A2A antagonist istradefylline, consistent with previous studies showing that A2A antagonists reversed the effects of D2 antagonists. Cariprazine did not alter the preference or intake of these foods in parallel free-feeding tests. Furthermore, cariprazine failed to reverse the effort-related effects of the VMAT-2 inhibitor and DA-depleting agent tetrabenazine. Given these results, it is reasonable to suggest that in this model of avolition, cariprazine is acting functionally as a D2 receptor antagonist due to its weak partial agonism of DA receptors. Furthermore, avolition as measured by the exertion of effort in goal-directed activity could be dissociable from the other negative symptoms. Taken together, these findings underline the importance of developing novel therapeutics, especially for the treatment of negative symptoms of schizophrenia, and support the idea that individual negative symptoms may have distinct neural mechanisms.

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Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.03/WW23

Topic: H.13. Schizophrenia

Title: Cell type-specific transcriptional changes following chronic treatment of clozapine in mouse prefrontal cortex

Authors: K. PARK[1], N. KANG[2], S.-H. HUH[2], S.-J. SEO[1], **Y. KIM**[4], S. WON[1], *S. KIM[3]; [1]Dept. of Publ. Hlth. Sci., Seoul Natl. Univ. Grad. Sch. of Publ. Hlth., Seoul, Korea, Republic of; [2]Dept. of Neuropsychiatry, [3]Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [4]Dept. of psychiatry, Nowon Eulji Med. Center, Eulji Univ., Seoul, Korea, Republic of

Abstract: Schizophrenia is a major psychotic disorder with complex genetic underpinnings. Clozapine is one of the representative antipsychotics for its unique and superior efficacy. Understanding the mechanisms of action of clozapine is still challenging, which could provide insights into the therapeutic as well as pathogenetic mechanism of schizophrenia. In this study, clozapine was systemically injected into male C58BL/6 mice for 21 days, and single-cell RNA sequencing was applied to their prefrontal cortical tissue samples. Differentially expressed genes (DEGs) for each cell type were identified via single-cell RNA sequencing, including microglia, astrocytes, neurons, endothelial cells, and vascular smooth muscle cells, and analyzed their intercellular interactions. Clozapine-induced changes in gene expression were most prominent in microglia, and the cell population clusters within microglia were altered by clozapine exposure. Pathway analyses of gene expression in microglia highlighted the cytokine secretion, B cell, and T cell-related pathways as particularly engaged by clozapine. Cell-cell interaction analysis demonstrated the clozapine-induced interactive changes among microglia, endothelial cells, and vascular smooth muscle cells. Comparative analysis with human data from the comparative toxicogenomics database (CTD), genetic loci implicated by genetic studies of schizophrenia, and genes in interaction with clozapine also revealed enriched clozapine-induced DEGs in microglia. As a result, eleven genes, including *Nfkb1*, *Nfe2l2*, *Egr1*, *Fos*, and *Atp6v1b2*, were demonstrated to have convergent evidence. In conclusion, single-cell RNA sequencing could provide important insights into the mechanisms of action of clozapine, a representative antipsychotic with superior efficacy, which has links to the genetic background of pathogenetic and therapeutic mechanisms in schizophrenia.

Disclosures: K. Park: None. **N. Kang:** None. **S. Huh:** None. **S. Seo:** None. **Y. Kim:** None. **S. Won:** None. **S. Kim:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.04/WW24

Topic: H.13. Schizophrenia

Title: Preclinical pharmacokinetic-pharmacodynamic (PK/PD) characterisation of behavioural and physiological responses to the novel GPR52 agonist HTL0041178

Authors: *C. P. MACSWEENEY[1], S. J. BRADLEY[1], S. POULTER[1], L. A. STOTT[1], A. J. H. BROWN[1], M. BARNES[1], B. GRAYSON[2], N. IDRIS[2], J. C. NEILL[2], S. P. WATSON[1]; [1]Sosei Heptares, Cambridge, United Kingdom; [2]Div. of Pharm., Univ. of Manchester, Manchester, United Kingdom

Abstract: GPR52, an orphan G protein-coupled receptor, has been proposed as a target for the treatment of positive, negative and cognitive symptoms of schizophrenia. High expression of the receptor on D_2 receptor-expressing medium spiny neurons and D_1 cortical neurons suggests that a GPR52 agonist will selectively modulate dopaminergic and glutamatergic signalling without causing the adverse effects associated with antipsychotics. The pharmacokinetic properties of HTL0041178, a selective GPR52 agonist, were measured in preclinical species and pharmacodynamic effects at behavioural, cognitive and physiological endpoints were explored in rat and mouse. Hyperlocomotor responses to d-amphetamine (rat - 0.5 mg/kg, SC), caffeine (rat - 15 mg/kg, SC; mouse - 15 mg/kg, IP) and istradefylline (rat - 10 mg/kgc, IP) were explored following 60 minutes pre-treatment with HTL0041178 (doses ranging from 1 to 30 mg/kg, PO). Male Sprague-Dawley rats and C57BL/6J mice were used in the locomotor studies. Reversal learning was tested following HTL0041178 treatment (1-30 mg/kg, PO) in female Lister Hooded rats previously treated with PCP (2 mg/kg, IP) twice daily for 7 days. The effects of HTL0041178 (1-30 mg/kg, PO) on basal plasma prolactin levels were measured in male Sprague-Dawley rats. Terminal plasma and brain samples were taken for measurement of HTL0041178 concentrations. Further studies were performed to understand the pharmacokinetics and bioavailability in preclinical species. HTL0041178 dose-dependently inhibited the hyperlocomotor responses to amphetamine, caffeine (both rat and mouse) and istradefylline, and reversed the learning deficit in subchronic PCP-treated rats. Furthermore, plasma prolactin levels decreased following treatment with HTL0041178. There was a consistent pharmacokinetic-pharmacodynamic relationship overall across these behavioural and physiological measures. HTL0041178 also demonstrated excellent pharmacokinetic properties across preclinical species, with predicted low clearance and good bioavailability in human. Overall, these studies support the progression of HTL0041178 and provide the basis of an initial estimated therapeutic dose-range for exploration in early clinical studies.

Disclosures: **C.P. Macsweeney:** A. Employment/Salary (full or part-time):; Sosei Heptares. **S.J. Bradley:** A. Employment/Salary (full or part-time):; Sosei Heptares. **S. Poulter:** A. Employment/Salary (full or part-time):; Sosei Heptares. **L.A. Stott:** A. Employment/Salary (full or part-time):; Sosei Heptares. **A.J.H. Brown:** A. Employment/Salary (full or part-time):; Sosei Heptares. **M. Barnes:** A. Employment/Salary (full or part-time):; Sosei Heptares. **B. Grayson:** None. **N. Idris:** None. **J.C. Neill:** None. **S.P. Watson:** A. Employment/Salary (full or part-time):; Sosei Heptares.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.05/WW25

Topic:

Title: 40 hz click train related narrow band gamma oscillations: induced or evoked?

Authors: *D. GAUTAM, B. ALLEN, E. KREPPS, A. SHIELDS, J. PHAM, D. SIVARAO; East Tennessee State Univ., Johnson City, TN

Abstract: Click trains at discrete frequencies elicit time-locked evoked response in EEG when averaged across multiple trials, called the auditory steady state response (ASSR). There is evidence in the literature that ASSR represents simple back-to-back super-positioning of transient evoked responses. Band-pass filtering the ASSR around a driving frequency (±2 Hz) result in apparent narrow band oscillations. It is however unclear if these represent endogenous induced oscillations resonating to the click train frequency or simply discrete evoked responses that look like oscillations due to filtering. In this study we characterized narrow band oscillations recorded from M2, a part of the rodent prefrontal cortex, in awake female SD rats in response to click trains at different frequencies (10, 20, 40 and 80 Hz) and contrasted them with unfiltered ASSR in terms of their onset, offset and evolution of phase synchrony. We found that all click trains evoked a robust ASSR, with a prominent sensory registration response of P1-N1-P2 followed by smaller steady state evoked responses. However, narrow band-passing of these data differed substantially from ASSR within and across frequencies. Strong and transient narrow band oscillations that corresponded to the P1-N1-P2 response were noted at 10 and 20 Hz but not at 40 and 80 Hz ASSR. At steady state, there was little phase synchrony at 10 Hz while phase synchrony was intermittent at 20 Hz. At 40 Hz, phase synchrony developed slowly, stabilizing ~ 250 ms after train onset. Once established, it continued through the duration of stimulation and lasted for another 150 ms post-stimulus. Oscillations at 80 Hz too showed robust but slowly developing phase-locking that outlasted the stim train by ~ 200 ms. These oscillations were specific to driving frequency as there was little synchrony at other gamma frequencies (e.g., ~ 50±2 Hz). In summary, there was a significant divergence in how narrow band oscillations evolved vs ASSR depending on the driving frequency. Strong but transient alpha (~ 10 Hz) and beta (~ 20 Hz) band oscillations were noted at train onset, temporally coincident with the N1 response. However, there was little narrow band synchrony at steady state. On the other hand, gamma oscillations were not associated with P1-N1-P2 response but developed slowly and outlasted the stim train. Moreover, at steady state, gamma oscillations were robustly phase locked to stimulus. Thus, narrow band gamma oscillations display both induced (not time-locked to stim onset and offset) and evoked (phase-locked to stim train at steady state) characteristics.

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Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.06/WW26

Topic: H.13. Schizophrenia

Support: Silvio O. Conte Center P50 MH103222

Title: Kynurenine Aminotransferase II Inhibition by Glycyrrhetinic Acid: An In Vitro and In Vivo Study

Authors: *K. V. SATHYASAIKUMAR[1], S. BEGGIATO[2], R. SCHWARCZ[1];
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Abstract: Kynurenic acid (KYNA), a metabolite of the kynurenine pathway of tryptophan degradation, is thought to play an important role in the mechanism(s) underlying normal and abnormal cognitive processes, acting as an antagonist of α7 nicotinic and NMDA receptor function. Specifically, increased brain KYNA levels may have detrimental effects in schizophrenia (SZ) and other psychiatric diseases (Plitman et al., 2017; Sellgren et al., 2019). KYNA is synthesized from its immediate bioprecursor kynurenine - either by non-enzymatic oxidation or through irreversible enzymatic transamination by kynurenine aminotransferases. In the mammalian brain, kynurenine aminotransferase II (KAT II) is the principal enzyme responsible for the neosynthesis of rapidly mobilizable KYNA and therefore constitutes an attractive target for pro-cognitive interventions (Schwarcz et al., 2012). Glycyrrhetinic acid (GA) is a bioactive constituent of the traditional Japanese medicine Yokukansan, which has been shown to alleviate clinical symptoms in people with SZ (Miyaoka et al., 2013) and to improve cognitive function in people with senile dementia (Mizukami et al., 2009). Based on the discovery that GA selectively inhibits the activity of recombinant human and mouse KAT II (Yoshida et al., 2019), we now examined the effect of GA on KAT II activity in crude brain and liver tissue homogenates of mice, rats and humans *in vitro* (Sathyasaikumar et al., 2013), showing IC_{50} values in the micromolar range in all cases. We then evaluated the effect of intraperitoneally (i.p.) administered GA (200 mg/kg) on KYNA neosynthesis by *in vivo* microdialysis in the medial prefrontal cortex (mPFC) of adult mice (C57Bl/6J, 2-4 months), both alone or 20 min prior to a challenge with systemically administered kynurenine (50 mg/kg, i.p.). Applied alone, GA reduced extracellular KYNA levels by ~30% (**P<0.01, main peak effect, 2-way ANOVA, N=4). GA also inhibited the *de novo* production of KYNA from its immediate precursor by ~25% (*P<0.05, main peak effect, 2-way ANOVA, N=6-7/group), at the same time increasing extracellular glutamate levels by ~20% (*P<0.05, main peak effect, 2-way ANOVA, N=6-7/group). These results indicate that GA may be a useful novel tool for testing the relevance of fluctuating brain KYNA levels in physiology and pathology.

Disclosures: K.V. Sathyasaikumar: None. **S. Beggiato:** None. **R. Schwarcz:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

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Title: Synergistic impact of microglia-mediated adverse effect of adolescent THC exposure and 16p11.2 duplication on prefrontal cortex maturation and social memory

Authors: *Y. HASEGAWA[1], J. KIM[1,2], G. URSINI[1,3], Y. JOUROUKHIN[4], X. ZHU[1], Y. MIYAHARA[1], F. XIONG[1], S. MADIREDDY[1], M. OBAYASHI[1], B. LUTZ[5,6], A. SAWA[1,7], S. P. BROWN[1,8], M. V. PLETNIKOV[4], A. KAMIYA[1];
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Abstract: Considering that recreational cannabis use is gradually legalized in several countries in the world, the deleterious effects of cannabis use during adolescence, a critical period for prefrontal cortex (PFC) maturation, has gained further attention as an environmental risk factor for psychiatric disorders. Cannabinoid receptor type 1 (Cnr1) is expressed not only in neurons and astrocytes, but also in microglia, which shape synaptic connections during adolescence. Nonetheless, the role of microglia in mediating the adverse cognitive effects of cannabis has been unexplored. In this study, we investigate the impact of adolescent delta-9-tetrahydrocannabinol (THC) exposure on microglial function of which disturbance may be exacerbated by genetic insults conferring the risk of psychiatric disorders, leading to aberrant PFC maturation and producing adult pathophysiology. In particular, we focus on the interplay between THC exposure and 16p11.2 duplication (16p11dup), a major copy number variation risk factor for psychiatric disorders. 16p11dup and control mice were chronically exposed to THC during adolescence or adulthood, followed by molecular, histochemical, biochemical, electrophysiological, and behavioral assays. THC treatment induced medial PFC (mPFC)-specific microglial apoptosis via Cnr1-mediated mechanisms. These microglial phenotypes were exacerbated by 16p11dup predisposition. We also found that these synergistic effects on microglia mediated by microglial Cnr1 resulted in neuronal subtype-specific deficits in intrinsic excitability of mPFC pyramidal neurons, leading to deficits in social memory. By performing

microglia-specific RNA-seq experiments, we identified key molecules which may mediate these phenotypes induced by the convergent action of adolescent THC treatment and 16p11dup. Our findings highlight the unexplored impact of adolescent THC exposure on microglial function. In particular, we identified the role of Cnr1 expressed in microglia for mediating its gene-environment effect on adolescent mPFC maturation and adult social memory in 16p11dup mice. We are currently investigating molecular mechanisms of how adolescent THC treatment impair microglia-mediated mPFC maturation in a neuronal subtype-specific manner.

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Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.08/WW28

Topic: H.13. Schizophrenia

Support: K99MH129613-02
R01MH120118-04

Title: Amelioration of selective cognitive symptoms by targeted restoration of Prefrontal excitatory/inhibitory balance in a 22Q11 deletion syndrome model

Authors: *A. MUKHERJEE[1], J. SCOTT[1], N. BAJWA[1], S. J. MOSS[2], M. M. HALASSA[1]; [1]Dept. of Neurosci., [2]Sch. of Med., Tufts Univ., Boston, MA

Abstract: Cognitive dysfunctions in Schizophrenia and other psychiatric illnesses are causally linked to atypical prefrontal cortex (PFC) function. Mounting evidence from patients and animal models suggests that altered PFC excitatory/inhibitory (E/I) balance underlies impairments in different cognitive domains such as attentional control, working memory and task switching. However, the mechanisms described for E/I imbalance and their precise relationship to cognition is poorly understood. To address this knowledge gap, we are dissecting the role of the main subclasses of PFC inhibitory neurons in balancing PFC activity patterns. In an effort to enhance cognitive function in Schizophrenia we have further devised preclinical translational strategies for the restoration of PFC E/I balance, via molecular or network stimulation approaches.
Here we show that abnormal PFC E/I balance in a mouse model of 22Q11 deletion syndrome manifests as reduced mediodorsal (MD) thalamus driven inhibition of PFC population activity and a separate reduction of evoked inhibition following afferent stimulation. These physiological phenotypes are associated with deficits in separate measures of cognitive control as evidenced by inflexibility in task switching and reduced capacity for working memory maintenance, respectively. Critically, these cognitive measures appear to be rescued by two distinct strategies

that likely converge on prefrontal E/I balance (1) A pharmacological approach to restore postsynaptic GABAergic transmission at a single neuron level, and (2) Optogenetic activation of the MD, which through its preferential connectivity to PFC Parvalbumin positive inhibitory neurons likely augments inhibitory control of activity at a network level. Such therapeutic enhancement of cognitive impairments associated with Schizophrenia has the potential to fulfill an unmet public health need and restore 1% of the global population to the workforce.

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Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.09/WW29

Topic: H.13. Schizophrenia

Support: BBRF Young Investigator Grant 31216

Title: Altered thalamocortical connectivity mediates belief-updating deficits in a moue model with a high-risk genetic mutation in schizophrenia

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Abstract: Schizophrenia is a severe neuropsychiatric disorder that affects 1% of people worldwide. Cognitive hypofunctions are prevalent in schizophrenia patients and lack effective treatments. One primary cognitive function of the brain is to update our beliefs of the world when the environment changes to make optimal decisions. Disruption of this belief updating process can lead to the loss of contact with reality, known as psychosis, which is a defining symptom of schizophrenia1. Belief updating was shown to be impaired in schizophrenia patients. To study the impaired belief updating in schizophrenia, we took two synergistic approaches: First, we used CRISPR/Cas9 gene editing to generate mouse models bearing point mutation (Grin2aY700X) recently identified in human schizophrenic patients by large-scale exome sequencing studies (Singh et al., 2022); Second, we developed a computationally trackable lever-pressing foraging task for mice, in which we can monitor how mice form and update their beliefs in a dynamic environment. With the mice model and the lever-pressing task, we found that mutant mice have a delayed belief-updating rate and unstable behavioral states. Our in vivo single unit electrophysiological experiments identified the distinct roles of the medial prefrontal cortex (mPFC) and mediodorsal thalamus (MD) in the lever-pressing task: while both mPFC and MD encode dynamic values, mPFC encodes the information in an earlier time window; MD neural activities encode behavioral states. Our optogenetic data showed that both inhibition of mPFC and mediodorsal thalamus MD slowed down the belief update rate, while inhibition of MD also destabilized the behavioral states of WT mice. Our ex vivo electrophysiology

experiment identified an increased excitatory/inhibitory (E/I) synaptic transmission on MD-PFC synapses in mutant mice. Further, we found that SSFO activation of MD can rescue the performance of mutant mice. Thus, we have identified that the altered MD-PFC circuit mediates the belief updating deficits in Grin2aY700X+/- mice. These results provide a potential therapeutic target for schizophrenia patients

Disclosures: **T. Zhou:** None. **Y. Ho:** None. **N. Hartley:** None. **K. He:** None. **M. Halassa:** None. **G. Feng:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.10/WW30

Topic: H.13. Schizophrenia

Support: MCHRI Uytengsu-Hamilton 22q11 Neuropsychiatry Research Award
UH22QEXTFY22-04
ERC —DISCONN, GA802371
NIH (1R21MH116473-01A1)

Title: Synaptic-dependent developmental dysconnectivity in 22q11.2 deletion syndrome

Authors: *F. ALVINO[1,2], S. GINI[2], D. SASTRE YAGÜE[2], A. MINETTI[3], A. GALBUSERA[2], C. MONTANI[2], M. PAGANI[2], C. SCHLEIFER[4], F. PAPALEO[1], M. V. LOMBARDO[2], M. PASQUALETTI[5], C. E. BEARDEN[4], A. GOZZI[2];
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Abstract: 22q11.2 Deletion Syndrome (22qDS) is a genetic syndrome associated with increased risk of developmental disorders such as autism and schizophrenia. Brain imaging studies have shown that people with 22qDS exhibit altered large-scale functional connectivity. However, the developmental course and neural underpinnings of these alterations remain undetermined. Here, we investigated the developmental trajectory of functional connectopathy in 22qDS in both a mouse model and in human 22qDS carriers. To this aim, we longitudinally mapped resting-state fMRI (rsfMRI) connectivity in juvenile (p33-p37) and adult (p105-p120) LgDel mice, an established mouse model of 22qDS (LgDel n=22; WT n=22, mixed sexes). We found that developmental connectopathy in LgDel mice undergoes a dramatic reconfiguration during the pubertal period, with widespread prepubertal fMRI hyper-connectivity reverting to focal hippocampal hypo-connectivity in adult LgDel mutant mice. We also found that fMRI hyperconnectivity in juvenile Lgdel mice is paralleled by a surplus of cortical dendritic spines, and that both of these phenotypes are normalized by pretreatment with the Gsk3B- inhibitor SB216763. To probe the generalizability of these observations to human 22qDS, we examined

fMRI connectivity in both pre-pubertal (N=52, 22qDS carriers n=21) and peri/post-pubertal (N=204, 22q11DS carriers n=118) 22qDS carriers. We found that functional connectopathy in human 22qDS similarly undergoes a reconfiguration from dominant hyperconnectivity in prepubertal carriers, to hippocampal and cortical hypoconnectivity in adulthood. Prompted by our mouse investigation, we next tested the hypothesis that this reconfiguration could be driven by Gsk3B-related synaptic mechanisms. In keeping with this notion, we found that cortical and subcortical regions exhibiting functional connectivity reversal are spatially enriched for gene transcripts encoding for synaptic proteins that interact with Gsk3B (hypergeometric test: OR = 2.91, p= 0.00005). Taken together, these findings provide evidence of synaptic-dependent, developmental dysconnectivity in 22qDS.

Disclosures: F. Alvino: None. **S. Gini:** None. **D. Sastre Yagüe:** None. **A. Minetti:** None. **A. Galbusera:** None. **C. Montani:** None. **M. Pagani:** None. **C. Schleifer:** None. **F. Papaleo:** None. **M.V. Lombardo:** None. **M. Pasqualetti:** None. **C.E. Bearden:** None. **A. Gozzi:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.11/WW31

Topic: H.13. Schizophrenia

Title: Acute inflammation induced by ultra-low dose lipopolysaccharide and restraint stress improve positive symptom-like behavior in a mouse model of schizophrenia by different mechanisms

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Abstract: Aim and objectives: Recently, there has been a focus on the brain inflammation hypothesis of schizophrenia. However, inflammation may have different effects on schizophrenia depending on the intensity, type, and duration of inflammation, and the relationship between them has not been fully elucidated. In this study, we examined the effects of mild acute brain inflammation induced by different causes on positive symptoms in an animal model of schizophrenia. **Method:** We created a model animal by repeatedly administering 1 mg/kg of methamphetamine (METH) to C57BL/6J male mice. Following a 1-week withdrawal period, METH was readministered to induce behavioral sensitization, a surrogate behavior of positive symptoms. Changes in behavior and striatal dopamine (DA) concentrations were measured under inflammatory conditions induced by intraperitoneal administration of 1 μg/kg of LPS or 2-hour restraint stress (RS) exposure. The LPS dose was determined to be comparable to the blood concentration of TNF-α in patients with schizophrenia. Then, TLR4, TNF-α, and COX-2

inhibitors were administered before inflammation was induced. Changes in striatal DA levels were measured using microdialysis. **Results:** The expression of behavioral sensitization was suppressed in both the LPS and RS groups, and this response was inhibited by pretreatment with a TLR4 inhibitor. The suppression of behavioral sensitization by LPS administration was inhibited by pretreatment with a COX-2 inhibitor, and that by RS exposure was inhibited by pretreatment with an anti-TNF-α antibody. During behavioral sensitization, METH re-administration caused an increase in striatal DA in both the control and the LPS groups. However, in the RS group, the increase in striatal DA was significantly attenuated compared to that in the control group, and this response was inhibited by pretreatment with an anti-TNF-α antibody. **Conclusion:** Our findings revealed that acute inflammation ameliorates positive symptoms in an animal model of schizophrenia. Furthermore, the mechanisms of action may differ depending on the cause of inflammation, which provides a basis for developing novel therapies targeting inflammation and predicting patients with a higher chance of successful anti-inflammatory treatment.

Disclosures: R. Shinohara: None. **S. Ishikawa:** None. **K. Ito:** None. **M. Tonosaki:** None. **N. Hashimoto:** None. **I. Kusumi:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.12/WW32

Topic: G.08. Other Psychiatric Disorders

Support: 1R15DA04926-01

Title: Positive allosteric modulation of the metabotropic glutamate receptor type 5 (mGlu5) reduces the enhanced rewarding and neural plasticity response to nicotine in a rodent model of psychosis

Authors: *A. M. CUOZZO, L. D. PEETERS, L. J. WILLS, K. L. IVANICH, S. E. TURNEY, L. P. BULLOCK, S. R. MASSEY, J. T. GASS, R. W. BROWN;
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Abstract: Nicotine has been indicated as a prevalent drug for substance abuse comorbidities in mental illness. Tobacco use is elevated in those suffering from psychiatric disorders, most notably in schizophrenia (SZ), where there is a three-to-fivefold increase in usage compared to the general population is observed. Our laboratory has established a rodent model of psychosis. In this model, male and female rats are neonatally treated with quinpirole (NQ), a dopamine (DA) D_2-like agonist from postnatal days (P)1-21, resulting in lifelong supersensitization of the DAD_2 receptor. Increases in dopamine D2 receptor sensitivity is a hallmark of psychosis. Interestingly, the DAD_2 receptor forms a triple mutually inhibitor heteromer in the dorsal striatum with the adenosine A(2A) and metabotropic glutamate receptor type 5 (mGlu5), such

that stimulation of the A(2A) or mGlu5 receptor results in decreased dopamine D_2 signaling. The present study was designed to analyze the role of the mGlu5 receptor in the associative aspects of nicotine s in adolescence using conditioned place preference (CPP). CPP is a behavioral task in which animals are conditioned with a reinforcing drug to prefer a particular environmental context. On P41-42, neonatal saline (NS) or NQ-treated animals were given pre-conditioning preference test, and then conditioned to nicotine from P43-50. On P51, animals were given a drug free preference test. Results revealed that NQ animals conditioned to nicotine demonstrated enhanced CPP, replicating our past work. Groups receiving 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (CDPPB), a positive allosteric modulator to mGlu5 before nicotine conditioning showed reduced rewarding effects of nicotine in CPP. Brain tissue was analyzed for brain-derived neurotrophic factor (BDNF), a neurotrophin involved in cell growth, as well as cell adhesion molecule cadherin-13 in the ventral tegmental area. Results revealed significant elevations of BDNF in NQ-treated rats given nicotine, and a sex difference in the increase in cadherin-13, with female NQ rats given nicotine demonstrating increases compared to all other groups. These effects were blocked by CDPPB. In addition, we analyzed mammalian target of rapamycin (mTOR)-dependent phosphorylation of p70S6 kinase in the nucleus accumbens (NAcc). The NQ group given nicotine demonstrated significant increases in NAcc P70S6 kinase compared to all other groups, suggesting increased synaptic growth, which was also blocked by CDPPB. Taken together, these results elucidate mGlu5 as a drug target for reducing the rewarding effects of nicotine via CDPPB administration in a model of substance abuse in psychosis.

Disclosures: A.M. Cuozzo: None. **L.D. Peeters:** None. **L.J. Wills:** None. **K.L. Ivanich:** None. **S.E. Turney:** None. **L.P. Bullock:** None. **S.R. Massey:** None. **J.T. Gass:** None. **R.W. Brown:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.13/WW33

Topic: H.13. Schizophrenia

Support: Fogarty/NIMH R21 MH120692 USA
VA CSRD 1I01CX002371 USA
Hawkes Bay Hospital, Hastings, NZ
Higher Education Commission, Islamabad, Pakistan

Title: Genome sequencing of consanguineous family implicates ubiquitin specific protease (USP53) variant in psychosis/schizophrenia: wild type localization to murine hippocampal CA 1-3/granular dentate regions and interactions with the AMPA synapse

Authors: A. KANWAL[1], S. A. SHEIKH[2], F. ASLAM[1], S. YASIN[1], Z. BEETHEM[3], N. PANKRATZ[3], C. R. CLABOTS[4], S. NAZ[1], ***J. V. PARDO**[5];

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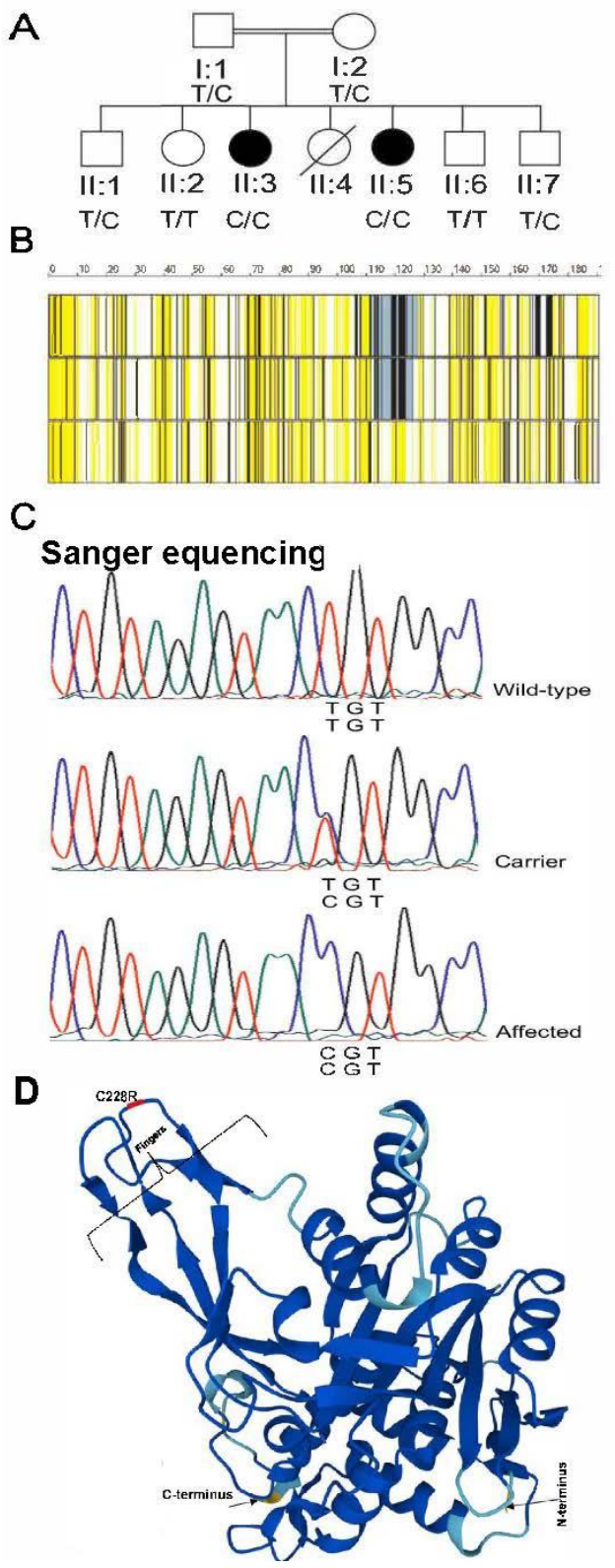
Abstract: A Pakistani family consisted of five offspring and first cousin parents, all healthy, and two daughters with a severe psychotic disorder without comorbidities. The pedigree showed apparent autosomal recessive transmission enabling searches for potentially causal Mendelian genes associated with common mental disorders (e.g., schizophrenia) or phenotypes (e.g., psychosis). Whole genome sequencing found one mutation (USP53 p.C228R) in a noncatalytic deubiquitinase satisfying unbiased a priori criteria for a variant of interest (VOI). No copy number variants surfaced. USP53 did not have any previously known CNS expression or association with mental disorders. Immunofluorescence studies indicated USP53 WT localized in mice to the hippocampus (CA 1-3) and granular dentate in a pattern like that seen with GRIA2 or GRIP2 staining. Pull-down co-immunoprecipitation studies showed USP53 interacted with GRIA2 and GRIP2. If confirmed, this USP53 variant appears a rare [MAF ~ $(10)^{-5}$] genetic form of schizophrenia. The variant likely phenocopies more common clinical disorders and points to the genetic heterogeneity of mental disease, an important limitation of the common disease/common gene hypothesis. Together, the genetic findings are consistent with and support a dominant hypothesis about one of schizophrenia's pathophysiologies: dysfunctional glutamate neurotransmission extending to the AMPA synapse. *Figure: A Pedigree; B Homozygoisty Mapping; C. Sanger sequencing; D. USP53 showing mutation (red) in Fingers region.*



Disclosures: **A. Kanwal:** None. **S.A. Sheikh:** None. **F. Aslam:** None. **S. Yasin:** None. **Z. Beethem:** None. **N. Pankratz:** None. **C.R. Clabots:** None. **S. Naz:** None. **J.V. Pardo:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.14/WW34

Topic: H.13. Schizophrenia

Support: R01 MH129343

Title: Cellular and neurodevelopmental consequences of a rare SETD1A missense variant associated with cognitive deficits and risk for psychosis

Authors: *S. ALI[1], R. LEASE[1,2], R. T. OSHONE[1], Y. AHMED[1], S. ARJONA[1], M. E. CORTES-GUTIERREZ[1], S. A. AMENT[1,3];
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Abstract: Loss-of-function variants in SET Domain containing 1A (SETD1A), a histone lysine methyltransferase, confer >10-fold increased risk for schizophrenia and related psychiatric disorders. Studies in mice suggest effects of SETD1A haploinsufficiency on memory and cognition, as well as impaired axonal branching and neuronal connectivity. However, there have been no studies of a naturally occurring SETD1A variant in human neural cells, and the mechanism through which SETD1A confers psychiatric risk remains poorly understood. Here, we characterize the cellular and neurodevelopmental consequences of a rare SETD1A missense variant, SETD1A P596L, that is enriched in the Old Order Amish founder population and associated with cognitive deficits and risk for psychosis. Induced pluripotent stem cells (iPSCs) from homozygous carriers of SETD1A P596L displayed decreased SETD1A protein abundance, decreased cellular proliferation, and increased susceptibility to DNA double-strand break repair, all of which mimic phenotypes of SETD1A protein-truncating variants, suggesting that SETD1A P596L is hypofunctional. SETD1A P596L iPSCs could be differentiated into neurons. However, we found differences in the morphology of neural stem cell and progenitor populations, including deficits in the numbers of neural rosettes and neurites. Immunohistochemical analyses suggest that these phenotypes correspond to accelerated cellular senescence. To test whether these changes relate to the effects of SETD1A P596L on histone 3 lysine 4 (H3K4) methylation, we restored histone methylation levels through the inhibition of KDM5, an H3K4-specific demethylase. KDM5 inhibition rescued all phenotypes in pluripotent cells, as well as the deficit in neurite extension in neural progenitors, but only when the inhibitor was present throughout the entirety of neural induction. Overall, SETD1A P596L is a hypofunctional variant that results in deficits in neural differentiation processes, primarily mediated by the variant's effects on H3K4 methylation.

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Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.15/WW35

Topic: H.13. Schizophrenia

Support: U01 MH108148
R01 MH129343

Title: Neuropsychiatric, neurocognitive, and neuroimaging phenotypes of a population-enriched SETD1A missense variant

Authors: *Y. AHMED[1], J. CHOE[1], E. M. HUMPHRIES[1], F. MCMAHON[2], A. R. SHULDINER[3], B. D. MITCHELL[3], P. KOCHUNOV[4], E. HONG[4], S. A. AMENT[1];
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Abstract: Rare, loss-of-function variants in SET Domain Containing 1A (SETD1A), a chromatin remodeling gene, confer >10-fold increased risk for schizophrenia, one of only a handful of genes known to have a large effect on risk for adult-onset psychiatric disorders. However, the spectrum of clinical phenotypes associated with SETD1A variants is poorly characterized, primarily because these variants are very rare in the general population. In founder populations, certain otherwise-rare variants are enriched due to population bottlenecks, making it feasible to recruit larger numbers of carriers for deep phenotyping. We discovered a SETD1A missense variant, SETD1A P596L, that is enriched >50-fold to a frequency of ~5% in the Old Order Amish founder population. Cellular assays suggested that SETD1A P596L causes partial SETD1A loss-of-function. We enrolled 55 carriers of SETD1A P596L into the Amish Connectome Project, which conducts neuropsychiatric, neurocognitive, and structural and functional brain imaging studies of Old Order Amish families with mental illnesses. Carriers had a nominally significant increased risk for bipolar disorder, with most affected carriers experiencing psychotic illness. We found quantitative deficits in cognitive domains, independent of diagnoses. Structural and functional brain imaging indicated no gross differences in brain structure. However, we detected trend effects on cortical thickness and white matter integrity, mirroring well-established patterns in schizophrenia patients. These results expand the phenotypic spectrum for SETD1A variants and demonstrate the value of founder populations for deep phenotyping in psychiatric genetics.

Disclosures: Y. Ahmed: None. **J. Choe:** None. **E.M. Humphries:** None. **F. McMahon:** None. **A.R. Shuldiner:** None. **B.D. Mitchell:** None. **P. Kochunov:** None. **E. Hong:** None. **S.A. Ament:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.16/WW36

Topic: H.13. Schizophrenia

Title: Mechanistic Profiling of M4 Ligands using Multiple Binding Probes

Authors: *J. HATCH, D. SMITH, L. TE, K. GUPTA, A. WONSEY-QUINN, E. KLEIN, G. CATIPAY, S. S. PIN, P. IREDALE, H. NGUYEN;
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Abstract: The M4 muscarinic acetylcholine receptor (mAChR) is one of 5 mAChR subtypes (M1-M5) in the G-protein coupled receptor (GPCR) superfamily. It is a 7-transmembrane $G_{\alpha i}$-coupled receptor expressed both pre- and post-synaptically in neurons of brain regions associated with psychotic and cognitive functions including the striatum, cortex, and hippocampus. The aim of this study was to elucidate the mechanisms of our investigational M4 compounds and literature tool molecules by using distinct probes in multiple biochemical and cell-based assays. This multifaceted approach allowed us to gain a deep understanding of M4-selective molecules and their impact on the biology. First, we integrated modern receptor pharmacology methods to analyze M4-activating molecules. This approach achieved functional profiling at the second messenger level and the proximal level, revealing that molecules appearing as full agonists with equal functional potency in the cAMP assay had different levels of intrinsic activities when evaluated at the G-protein activation level using GTPgS assay. We also performed M4 radioligand binding using steady state and kinetics experiments with multiple tritiated probes with distinct mechanistic profiles including antagonists, non-selective partial/full agonists, and selective M4 PAMs and full agonists. Evaluation using multiple tools allowed us to observe that compounds have a broad range of binding affinities, functional potencies, and kinetic properties across multiple binding sites. By combining these mechanistic data in various tissue types along with our functional assays, we can better distinguish our molecules and advance them toward downstream in vivo profiling. These full kinetic profiles give us a more complete depiction of the pharmacology of diverse molecules targeting the M4 receptor.

Disclosures: **J. Hatch:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **D. Smith:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **L. Te:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **K. Gupta:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **A. Wonsey-Quinn:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **E. Klein:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **G. Catipay:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S.S. Pin:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **P. Iredale:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **H. Nguyen:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.17/WW37

Topic: H.13. Schizophrenia

Title: Effects of novel M4 muscarinic acetylcholine receptor agonists on spontaneous and amphetamine-stimulated locomotor activity in mice

Authors: *J. LOCANTORE, P. STOLYAR, A. RUGGLES, S. LEISER, S. CARRIER, G. SUIDAN, S. CHAKILAM, H. NGUYEN, P. IREDALE;
Cerevel Therapeut., Cambridge, MA

Abstract: While currently available antipsychotics work primarily through blocking D2 dopamine or serotonin receptor signaling, stimulation of M4 muscarinic acetylcholine receptors (mAChR) is also known to produce antipsychotic-like effects. Recent clinical data with our selective M4 positive allosteric modulator, emraclidine, and the M1/M4-preferring agonist, xanomeline, have further validated this in individuals with schizophrenia. M4 receptors are highly expressed in the striatum, where emerging evidence suggests their activation can reduce striatal dopaminergic transmission and play a potential role in mediating the antipsychotic activity of xanomeline. Furthermore, both emraclidine and xanomeline show efficacy in preclinical psychosis assays. Here, we aimed to assess the behavioral and antipsychotic properties of selective M4 agonism. Achieving M4 selectivity may reduce unwanted off-target adverse symptoms and prevent the need to coadminister with peripherally-restricted mAChR antagonists. In naïve C57BL/6J mice, we profiled several novel M4 agonists first in spontaneous locomotor activity (sLMA), followed with testing in amphetamine-stimulated locomotor activity (aLMA), a preclinical psychosis assay. We find that selective M4 agonists cause dose-dependent decreases in both mouse sLMA and aLMA. Several compounds were found to be efficacious in aLMA at doses that did not impact sLMA, showing a desirable separation between efficacy and reduced spontaneous activity. These data indicate that selective M4 activation alone can indirectly reverse dopamine-driven increases in animal activity without significantly impacting baseline locomotion. These novel and selective M4 agonists demonstrated similar behavioral phenotypes to emraclidine and xanomeline, suggesting their potential as antipsychotic therapies that circumvent directly targeting dopamine receptors or activating off-target mAChR subtypes.

Disclosures: **J. Locantore:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **P. Stolyar:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **A. Ruggles:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S. Leiser:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S. Carrier:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **G. Suidan:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S. Chakilam:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **H. Nguyen:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **P. Iredale:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.18/WW38

Topic: H.13. Schizophrenia

Support: NSTC 110-2320-B-002 -030 -MY3

Title: Impact of chronic sleep insufficiency on heterozygous Disc1 mutant mice

Authors: *Y.-Z. LU[1], T.-Y. HE[1], C.-H. NG[1], L.-J. LEE[2];
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Abstract: Sleep insufficiency is a pervasive phenomenon among adolescents, and insufficient sleep is highly associated with the pathogenesis and progression of a wild range of psychiatric symptoms that usually emerge during late adolescence and early adulthood. In this study, we examined the impact of chronic sleep restriction (CSR) on adolescent normal wildtype (WT) and heterozygous *Disc1* mutant (*Disc1* Het) mice, a model of schizophrenia. Adolescent (P28) mice of the CSR group were kept on multiple platforms in a water tank 18 hours a day, 5 days a week, for three weeks. Mice in the normal sleep (NS) group were kept in the home cages. Since CSR is a risk factor for psychiatric disorders and stressors for hippocampal neurogenesis, we first evaluated the proliferation and maturation of newborn neurons in the subgranular zone. The number of Ki67-positive proliferative cells was comparable between genotypes in the NS group, whereas it was largely reduced by CSR in WT but not in *Disc1* Het mice. The number of DCX-positive maturing cells was similar between genotypes in the NS group; notably, in the CSR group, it was significantly increased in *Disc1* Het but not in WT mice. Given that hippocampal neurogenesis could be regulated by microglia, we next checked the features of Iba-1-positive microglia in the neurogenic niche. In the subgranular zone, the microglial density was comparable between genotypes in the NS group; interestingly, in the CSR group, it was decreased in WT yet increased in *Disc1* Het mice. Together, our results suggested an interplay between chronic sleep insufficiency and the mutation of the *DISC1* gene, especially in adolescent subjects.

Disclosures: Y. Lu: None. **T. He:** None. **C. Ng:** None. **L. Lee:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.19/WW39

Topic: H.13. Schizophrenia

Title: Distinct connectivity patterns of the ventral anterior cingulate cortex in schizophrenic patients compared to controls.

Authors: *A. NAVARRO-CEBRIAN, M. CHANG, H. TUJJAR, P. TALLON, E. NEWMAN; Univ. of Maryland, Col. Park, College Park, MD

Abstract: Previous research has indicated that the anterior cingulate cortex (ACC) plays a key role in the pathophysiology of schizophrenia. However, most studies have not differentiated between the ventral and dorsal subdivisions of the ACC. Understanding the different connectivity patterns of these subdivisions is of paramount importance, as neuroimaging evidence suggests an inverse functional relationship between them. Specifically, the ventral ACC shows functional connectivity with brain areas involved in internally-directed attention, such as the default mode network, while the dorsal ACC is connected to regions associated with externally-directed attention. Given the hyperactivity of the default mode network observed in individuals with schizophrenia, we hypothesized that the ventral ACC would exhibit stronger correlations with the default mode network in this population compared to controls. To test this hypothesis, we used publicly available neuroimaging data from Schizconnect, and analyzed resting-state fMRI data from 45 schizophrenia subjects and 51 matched controls. Our findings confirm that schizophrenic patients have an increased connectivity between the ventral ACC and regions within the default mode network, specifically medial temporal lobes (bilateral parahippocampal gyri; $p<0.0001$). Additionally, our data shows a decreased connectivity between dorsal ACC and areas involved in externally-oriented attention in schizophrenic patients versus controls ($p<0.0005$). In conclusion, these results suggest greater connectivity in schizophrenic patients in areas related to internally-directed attention or self-referential thought, and less connectivity between areas of a network important for externally-directed attention. We postulate that these different anticorrelations of the ACC in individuals with schizophrenia may explain the difficulties experienced by these patients in differentiating external from internal objects.

Disclosures: A. Navarro-Cebrian: None. **M. Chang:** None. **H. Tujjar:** None. **P. Tallon:** None. **E. Newman:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.20/WW40

Topic: H.13. Schizophrenia

Support: NSTC 110-2320-B-002 -030 -MY3

Title: Chronic sleep insufficiency affects dendritic features in mice

Authors: *T.-Y. HE, Y.-Z. LU, C.-H. NG, L.-J. LEE;
Grad. Inst. of Anat. and Cell Biol., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Chronic sleep insufficiency could be an environmental stress factor that might interact with genetic risk factors and contribute to the pathogenesis of psychiatric disorders. In the present society, adolescents are facing the challenge of sleep insufficiency, and the impact of insufficient sleep on young people should be a concern. In this study, we examined the effect of chronic sleep restriction (CSR) on adolescent (P28) normal wildtype (WT) and heterozygous Disc1 mutant (Disc1 Het) mice, a genetic model of schizophrenia. A multiple-platform paradigm (18 hours a day, 5 days a week, for three weeks) was used to restrict sleep in mice. Since sleep insufficiency affects the structure and function of the hippocampus, we examined the dendritic features in Golgi-stained and reconstructed granule cells in the dentate gyrus. The dendritic complexity was increased by CSR in WT mice but not as significant in Disc1 Het mice. In the Sholl analysis, increased intersections were noticed in the proximal regions of WT neurons. However, the length of dendritic segments was not influenced by CSR in both genotypes. Our results showed the differential changes following CSR between WT and Disc1 Het mice. Together, these results suggested an interplay between chronic sleep insufficiency and the mutation of the DISC1 gene, especially in adolescent subjects.

Disclosures: T. He: None. **Y. Lu:** None. **C. Ng:** None. **L. Lee:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.21/WW41

Topic: H.13. Schizophrenia

Support: The Pritzker Neuropsychiatric Disorder Research Consortium

Title: Cholecystokinin and vasoactive intestinal peptide mRNA differences in the frontal pole cortex of schizophrenia and bipolar disorder subjects

Authors: *D. M. KROLEWSKI[1], V. KUMAR[1], M. WASELUS[1], A. MEDINA[1], M. FOLTZ[1], R. M. MYERS[2], F. LEE[3], J. D. BARCHAS[3], B. G. BUNNEY[4], W. E. BUNNEY[4], H. AKIL[1], S. J. WATSON[1];
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Abstract: Background: Gene expression of cholecystokinin peptide (CCK) and vasoactive intestinal peptide (VIP) profile specific subpopulations of inhibitory GABA-ergic interneurons. CCK basket cells primarily synapse around excitatory pyramidal cell bodies whereas VIP types are well known to form disinhibitory circuits through connections with other GABA interneurons. The physiological dysfunction of these interneurons has been previously

represented by mRNA reductions in the dorsolateral prefrontal and orbital frontal cortices of schizophrenia (SZ) and bipolar disorder (BPD) postmortem patients (Fung et al., 2010, 2014). Our laboratory has used qPCR to investigate gene expression in the less well studied frontopolar cortex; a region linked to multiple functions including those related to emotion, nociception, and pain. The results uncovered a number of altered GABA-related genes in psychiatric disorders (Medina et al., 2023). To expand on these findings, we conducted a preliminary quantitative analysis of CCK and VIP mRNA in the frontopolar tissue of healthy controls (CTR), SZ, and BPD subjects by cortical layer. **Methods:** Frontopolar cortex from all diagnoses were obtained from the Brain Donor Program, University of California, Irvine. All samples had zero agonal factors and met stringent criteria for pH and PMI. Hybridization chain-reaction (HCR) fluorescent in situ hybridization (FISH) was performed on 30µm-thick fresh-frozen cryosections. Images were captured on an Olympus Fluoview 3000 with CCK+ and VIP+ cells quantified as stitched z-plane stacks with Amira and ImageJ software. **Results:** Our preliminary study, CTR (n=5), SZ (n=4), BPD (n=5), showed several notable findings. 1) The mean number of CCK+ cells in CTRs was similar in layers II-V, but considerably lower in layer VI. 2) Across all individual layers, BPD subjects displayed the lowest mean number of CCK+ cells vs. CTRs. 3) VIP+ cells were more numerous in layers II-IV of CTRs, but appreciably less in layers V/VI. 4) Across all layers, the mean number of VIP+ cells was lower in SZ vs. CTR, but even more so in BPD. 5) Although CCK and VIP interneurons are thought to be mostly separate populations, we did find about 12% overlap. The latter was most apparent in layers II-IV. **Conclusion:** Given the promising results of our preliminary analysis of CCK+ and VIP+ interneurons in the frontopolar cortex, we are currently processing HCR-FISH for CTR, SZ, and BPD subjects to complement these findings (totaling n=16/group). These data will allow for more complete understanding of cortical layer-based interneuron differences and thus the pathology of such networks in psychiatric disorders.

Disclosures: **D.M. Krolewski:** None. **V. Kumar:** None. **M. Waselus:** None. **A. Medina:** None. **M. Foltz:** None. **R.M. Myers:** None. **F. Lee:** None. **J.D. Barchas:** None. **B.G. Bunney:** None. **W.E. Bunney:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.22/WW42

Topic: G.08. Other Psychiatric Disorders

Support: 1R15DA04926-01

Title: Reinstatement of nicotine conditioned place preference in a heritable model of drug abuse vulnerability in psychosis: Characterizing the BDNF response in brain regions mediating drug reward

Authors: *L. D. PEETERS[1], L. J. WILLS[2], A. M. CUOZZO[1], K. L. IVANICH[1], R. W. BROWN[3];
[1]Biomed. Sci., East Tennessee State Univ. Quillen Col. of Med., Johnson City, TN; [2]East Tennessee State Univ. Biomed. Sci. Dept., Quillen Col. of Med., Johnson City, TN; [3]East Tennessee State Univ., Dept. of Biomed. Sci. East Tennessee State Univ., Johnson City, TN

Abstract: Schizophrenia is a severe mental illness often accompanied by high rates of cigarette smoking, reduced quit success, and high relapse rates, negatively impacting patient outcomes. Impaired synaptic plasticity is suggested to confer substance abuse and relapse vulnerability in schizophrenia. Brain-derived neurotrophic factor (BDNF) plays a complex role in learning and memory and has been reported to have brain region-dependent effects on addiction behaviors that are also phase dependent. However, the mechanisms underlying altered relapse-like behaviors in individuals diagnosed with psychosis are poorly understood. The present study analyzed changes in extinction and reinstatement of nicotine conditioned place preference (CPP) and resulting changes in BDNF in a novel heritable rodent model of psychosis, demonstrating increased dopamine D2 receptor sensitivity, to explore mechanisms contributing to changes in relapse-like behaviors. Characterizing the role of aberrant synaptic plasticity on relapse-like behaviors in a novel heritable rodent model of psychosis may elucidate treatment targets to facilitate successful smoking cessation. Male and female offspring of two neonatal quinpirole-treated (QQ) and two neonatal saline-treated (SS) Sprague-Dawley rats were tested on an extended CPP paradigm to analyze extinction and nicotine-primed reinstatement. Brain tissue was analyzed 60 min after the final nicotine injection for BDNF response in the ventral tegmental area (VTA) as well as two cortical projections, the infralimbic (IfL) and prelimbic (PrL) cortices via enzyme-linked immunosorbent assay (ELISA). Regression analysis was also conducted to determine the impact of factors accounting for the variance in BDNF in each region. Founder treatment, adolescent drug treatment, and reinstatement test performance were used as predictors. QQ animals demonstrated delayed extinction and more robust nicotine-primed reinstatement, as well as an enhanced BDNF response to nicotine in the VTA, IfL and PrL cortices compared to SS control animals. Founder treatment and adolescent treatment were found to be the best predictors of BDNF across brain regions. This study is the first to demonstrate altered relapse-like behavior in a heritable model of drug abuse vulnerability in psychosis. This altered pattern of behavior is hypothesized to be the related to aberrantly elevated activity-dependent BDNF protein in brain regions associated with drug reward during conditioning that persists through the extinction phase, rendering aberrantly salient drug associations resistant to extinction and enhancing relapse vulnerability.

Disclosures: L.D. Peeters: None. **L.J. Wills:** None. **A.M. Cuozzo:** None. **K.L. Ivanich:** None. **R.W. Brown:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.23/WW43

Topic: H.13. Schizophrenia

Support: NSTC 110-2320-B-002-030-MY3

Title: Test the synaptic over-elimination hypothesis of schizophrenia using heterozygous Disc1 mutant mice, an animal model of schizophrenia

Authors: *E.-C. CHANG[1], P.-Y. WANG[2], L.-J. LEE[3];
[1]Grad. Inst. of Brain and Mind Sci., Natl. Taiwan Univ., Taipei, Taiwan; [2]Grad. Inst. of Brain and Mind Sci., Natl. Taiwan Univ., Taipei City, Taiwan; [3]Grad. Inst. of Anat. and Cell Biol., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan

Abstract: The psychotic symptoms of schizophrenia usually emerge during late adolescence and early adulthood, a period of prominent synaptic elimination. An over-elimination hypothesis of schizophrenia had been proposed. However, due to the rarity of human brain samples, solid evidence supporting this hypothesis is still not available. The animal models of schizophrenia might fulfill the need. The interrupted *DISC1* gene had been identified in a Scottish family in which many members were diagnosed with psychiatric disorders such as schizophrenia, bipolar disorder, or major depression. We generated *Disc1* mutant mice and proposed the heterozygous (Het) *Disc1* mutants as a mouse model of schizophrenia. To test the over-elimination hypothesis of schizophrenia, we collected brain samples from wild-type and *Disc1* Het mice at different time points, including postnatal day (P) 14, 28, 42, 56, and 90, corresponding to early childhood, early adolescence, late adolescence, early adulthood, and adulthood, respectively. Since the excitatory synapses are largely located on the dendritic spines, Golgi-stained dendrites and dendritic spines in layer 2/3 pyramidal neurons in the mPFC were examined. We analyzed the density and shape of spines collected from segments of different orders in the basilar dendrites. In wild-type mice, the spine density increased from P14 to P28 and then dropped. However, in *Disc1* Het mice, the spine density was lower than in wild-type mice and the drop was not evident. Our results obtained from the basilar dendrites of layer 2/3 mPFC pyramidal neurons did not support the over-elimination hypothesis. Based on our findings, an underdevelopment hypothesis is suggested.

Disclosures: E. Chang: None. **P. Wang:** None. **L. Lee:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.24/WW44

Topic: H.13. Schizophrenia

Support: MH115188-01 NIH (NIMH)
NS125016 NIH (NINDS)
MH109450-01A1 NIH (NIMH)

Title: Synaptic organization of the dCA1-LS-VTA pathway and its role in novelty-induced hyperlocomotor behavior

Authors: *M. NAVARRETE MATHEWS, Y. WU, Y. ZHOU;
Biomed. Sci., Florida State Univ. Program In Neurosci., Tallahassee, FL

Abstract: An estimated 3% of the population will experience psychosis in their lifetime, yet we know little about its underlying neural circuitry. To address this knowledge gap, our lab created a mouse model in which the 14-3-3 proteins are functionally inhibited in the forebrain (14-3-3 functional knockout (FKO)). These mice display several phenotypes that are thought to correlate to symptoms of psychosis, including novelty-induced locomotor hyperactivity. We recently determined that this phenotype is mediated by hippocampal hyperactivity and overactive dopamine signaling, leading to the discovery of a dorsal hippocampus CA1 (dCA1) - lateral septum (LS) - ventral tegmental area (VTA) pathway. Based on previously characterized cellular organization of the dCA1-LS-VTA pathway, we hypothesize that it is composed of a dCA1 (glutamate neuron) - LS (GABA neuron) - VTA (GABA neuron) - VTA (dopamine neuron) neural circuit. To test this hypothesis, we are using the state-of-the-art viral-genetic tracing methods known as Tracing the Relationship between Inputs and Outputs (TRIO) and the cell type specific cTRIO to visualize the cell type specific synaptic connections within the dCA1-LS-VTA neural circuit. In addition, we utilize chemogenetic approaches to manipulate neuronal excitability in both a cell-type- and pathway-specific fashion within the dCA1-LS-VTA circuit in both wildtype and 14-3-3 FKO mice. By measuring the effects of neuronal activity manipulations on psychomotor behavior and dopaminergic signaling, we gain insight into how the three brain regions within the dCA1-LS-VTA pathway functionally influence each other and affect psychosis-related behavior. Importantly, both female and male mice are used in each experimental and control group so that any sex differences in the dCA1-LS-VTA pathway can be addressed. These studies allow for the deeper understanding of neural circuitry involved in the pathophysiology of psychosis, contributing valuable knowledge to the field that may be used in the development of more targeted treatments and earlier diagnosis.

Disclosures: M. Navarrete Mathews: None. **Y. Wu:** None. **Y. Zhou:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.25/WW45

Topic: H.13. Schizophrenia

Support: UCL Institute of Mental Health

Title: Genetic risk for schizophrenia and experience of hearing impairment both influence auditory brain function in a mouse model of 22q11.2 deletion syndrome

Authors: *C. LU[1], J. F. LINDEN[1,2];
[1]Ear Inst., [2]Dept. of Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Hearing impairment has been identified as a longitudinal risk factor for schizophrenia in the general population (Linszen et al. Neurosci Biobehav Rev 2016). The 22q11.2 chromosomal microdeletion is one of the strongest known genetic risk factors for schizophrenia; ~30% of carriers develop schizophrenia in adulthood (Schneider et al. Am J Psychiatry 2014). Up to 60% of 22q11.2 deletion carriers have mild to moderate hearing impairment, primarily from chronic middle-ear problems that typically emerge in childhood and persist in adulthood (Verheij et al. Clin Otolaryngol 2017). Here we used the *Df1/+* mouse model of 22q11.2 Deletion Syndrome (22q11.2DS) to investigate how genetic risk for schizophrenia and experience of hearing impairment might interact to affect brain function. The *Df1/+* mouse replicates the large inter-individual variation in hearing ability observed among 22q11.2DS patients and also exhibits auditory brain abnormalities consistent with disrupted cortical excitation/inhibition balance (Zinnamon et al. 2022 Biol Psychiatry Global Open Sci). We measured peripheral hearing sensitivity and cortical auditory evoked potentials (AEPs) in 29 *Df1/+* mice and 22 WT littermates, exploiting the large inter-individual variation in hearing ability among *Df1/+* mice to distinguish cortical effects of genetic background from those of experience with hearing impairment. To quantify alterations in cortical gain and adaptation, we analysed the growth of tone-evoked AEPs as loudness or inter-tone interval duration increased. These AEP growth measures were abnormal in *Df1/+* mice with normal hearing, but were also affected by hearing impairment. Our results suggest that auditory cortical abnormalities in 22q11.2DS may depend not only on the genetic deletion but also on experience of hearing impairment. In ongoing work, we are investigating the abnormalities in auditory cortical function at the single-neuron and neuronal population levels as well as in cortical evoked potentials.

Disclosures: C. Lu: None. **J.F. Linden:** None.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.26/WW46

Topic: H.13. Schizophrenia

Title: Functional Mechanistic Profiling of M4-Selective Agonists and Modulators

Authors: *A. WONSEY-QUINN, L. TE, E. KLEIN, G. CATIPAY, D. L. SMITH, K. GUPTA, J. HATCH, H. N. NGUYEN, P. IREDALE, S. PIN;
Cerevel Therapeut., Cambridge, MA

Abstract: The family of muscarinic acetylcholine receptors (mAChR), M1, M2, M3, M4, and M5, are G-protein coupled receptors, which play important roles in the CNS system.

Furthermore, they serve as attractive drug-discovery targets for multiple CNS conditions, including schizophrenia, where novel non-dopaminergic therapies would provide new potential treatment options. Selective M4 activation is hypothesized to alleviate positive symptoms associated with schizophrenia, without the common side effects of currently available non-selective muscarinic agonists. With this in mind, we have performed *in vitro* profiling on a wide range of small molecule modulators and agonists using a variety of pharmacological tools to explore the binding and activation proprieties of the muscarinic receptors. The present study described for tool compounds represents our M4-selective lead molecule strategy to evaluate the functional behavior of partial agonists, full agonists, and positive allosteric modulators (PAMs). By going through in-depth characterization of our lead molecules using the whole panel of muscarinic acetylcholine receptors, and exploring the different levels of agonism, modulation, binding properties, and the degree of receptor selectivity, we were able to select the most appropriate molecules to move further downstream toward *in vivo* studies. Our approach provides a significant advantage over using a single-assay compound ranking strategy. Results from our complex, multi-faceted *in vitro* approach, with a strong emphasis on mechanistic pharmacology, provide unique and valuable information to guide SAR and the design of *in vivo* studies, which we expect will increase our chance for success in improving the lives of patients suffering from schizophrenia and other CNS conditions.

Disclosures: **A. Wonsey-Quinn:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **L. Te:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **E. Klein:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **G. Catipay:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **D.L. Smith:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **K. Gupta:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **J. Hatch:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **H.N. Nguyen:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **P. Iredale:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S. Pin:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics.

Poster

PSTR572. Schizophrenia Therapeutics: Animal and Human Studies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR572.27/WW47

Topic: H.13. Schizophrenia

Support: R01 MH129343

Title: Single-cell multi-omic profiling of patient-derived induced pluripotent stem cells reveals the effects of a hypofunctional SETD1A variant on human neurodevelopment

Authors: ***R. LEASE**[1], M. E. CORTES-GUTIERREZ[2], R. T. OSHONE[2], C. COLANTUONI[3], *R. LEASE[2], S. AMENT[2];

[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Departments of Neurol. and Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Cell fate decisions during neural development are governed by the activity of gene regulatory networks in neural stem and progenitor cells. Chromatin remodeling genes play important roles in these developmental processes and have been strongly linked to neurodevelopmental disorders such as schizophrenia and autism. However, the specific effects of disease-associated mutations on gene regulation in developing neurons are poorly understood. Exome sequencing has revealed >10-fold increased risk for schizophrenia in carriers of rare loss-of-function variants in Set Domain containing 1A (SETD1A), a chromatin remodeling gene responsible for transcriptional activation through histone 3 lysine 4 (H3K4) methylation. Here, we investigate the effect of a naturally occurring hypofunctional variant, SETD1A P596L, on chromatin states and transcriptional activity at four unique stages of neural induction. We performed single cell multi-omic profiling of patient-derived homozygous SETD1A P596L iPSCs and matched non-carrier controls to determine cell fate decisions and reconstruct gene regulatory network activity during the induction of stem cells to neural progenitors. We find the largest effects of SETD1A P596L early in neural induction, where a subset of SETD1A P596L iPSCs rapidly lose pluripotency markers, prematurely exit the cell cycle, and precipitately activate neuronal gene networks. We verify these results through bulk RNA-seq in additional iPSC lines. Overall, we use single cell multi-omic RNA + ATAC-seq to reconstruct gene regulatory networks underlying the induction of iPSCs to neural progenitor cells, and identify deficits caused by SETD1A P596L.

Disclosures: R. Lease: None. **M.E. Cortes-Gutierrez:** None. **R.T. Oshone:** None. **C. Colantuoni:** None. **R. Lease:** None. **S. Ament:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.01/WW48

Topic: I.03. Anatomical Methods

Title: Automatic Skull Opening System

Authors: *K. OZMEN, Jr., M. YOUSEFI;
Neurosci., Koc Univ. Grad. Sch. of Hlth. Sci., Ankara, Turkey

Abstract: Abstract: This study aimed to evaluate the feasibility and advantages of automated cranial window opening in animal experiments. The cerebral cortex, comprising specialized neurons, can be modulated by external physical and chemical stimuli, allowing observation of neuronal activation using diverse techniques. Precise access to specific cortical regions is crucial in disease-related research. Cranial window opening serves as a valuable method for studying and manipulating these regions. In this study we aimed to evaluate the feasibility and advantages

of automated cranial window opening in animal experiments. Cranial window opening serves as a valuable method for studying and manipulating these regions. This investigation focused on the development and utilization of the craniobot device, designed to automate cranial window opening, in conjunction with existing tools, using wild-type mice. Methods: Wild-type mice were utilized for the study, and open skull cranial windows were created employing an automated cranial window opening device. The windows were opened to desired specifications in terms of size and location. Subsequently, the opened windows and underlying cortical tissues were observed to assess inflammation and tissue viability. Live microscopic observations were conducted to monitor the opened cranial window. Metabolic changes in targeted areas were quantified using laser speckle contrast imaging (LSCI) and the intrinsic optical imaging system (IOSI), providing measurements of metabolic activity and cerebrovascular reactivity (CVR), respectively. The collected data were systematically organized and compared to control groups. Results: Successful automated cranial window opening and subsequent controls were achieved precisely without compromising the well-being or cortical functions of the mice. Assessment of the opened tissue revealed no damage, with clean borders observed.
Conclusion: This study demonstrates the feasibility of automated cranial window opening and provides valuable insights into its utilization. The preliminary investigation conducted on cortical opening using the craniobot device will contribute to the development of future research endeavors focused on studying specific cortical regions with improved precision and efficacy.

Disclosures: **K. Ozmen:** None. **M. Yousefi:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.02/WW49

Topic: I.03. Anatomical Methods

Support: MnDRIVE RSAM
1R01NS11128
RF1NS126044.

Title: Integrating Deep Learning and Advanced Imaging For automated cranial microsurgeries

Authors: *Z. S. NAVABI[1], R. T. PETERS[3], J. A. O'BRIEN[1], B. R. GULNER[1], S. B. KODANDARAMAIAH[2,4,5];
[1]Mechanical engineering, [2]Mechanical Engin., Univ. of Minnesota- Twin Cities- Mechanical E, Minneapolis, MN; [3]Mechanical engineering, Univ. of Minnesota- Twin Cities- Electrical and Computer Engin., Minneapolis, MN; [4]Univ. of Minnesota-Twin Cities-Biomedical Engin., Minneapolis, MN; [5]Univ. of Minnesota-Twin Cities-Neuroscience, Minneapolis, MN

Abstract: Neural activity recording and modulation often require access to the mouse brain through acute ports or transparent cranial windows. Delicate craniotomy procedures are

performed on sub-millimeter-thick skull tissue to protect the underlying dura and brain. Current robotic craniotomy methods utilize impedance (Pak et al. 2014) or contact force sensing (Ghanbari* Rynes* et al. 2019) for skull profiling, but these approaches have limitations. Impedance sensing is prone to false positives and negatives, while contact profiling is time-consuming and provides limited information about skull thickness. Consequently, an accurate estimation of skull thickness is lacking, necessitating iterative bone excision.In this study, we employed Optical Coherence Tomography (OCT) as a non-invasive profiling technique for the dorsal skull of mice. We utilized a spectral domain OCT scanner with 1300 μm center wavelength, providing 10 μm axial resolution (in bone) and 20 μm lateral resolution. Deep-learning models, specifically U-net, were employed to extract dorsal and ventral skull surfaces from the captured 3D OCT images. The model was trained on a diverse dataset of 22 images from live and fresh cadavers of mice representing different ages, sexes, and strains. The model's accuracy in discriminating bone and background pixels reached 92%. We also introduced methods to measure and correct the refractive index of the live mouse skull in OCT scans. The accuracy of our measurements was cross-validated with other measurement techniques.Using our skull thickness measurement method and a 3-axis cartesian robot, we successfully automated various types of craniotomy on mice. Burr hole and 3 mm diameter craniotomies, as well as skull thinning preparations, were performed on different areas of live mouse skulls. Comparative analysis with manual surgery demonstrated improved success rates and reduced surgery times with our robotic approach. The results highlight the potential of our automated system in enhancing craniotomy outcomes and efficiency compared to experienced surgeons.

Disclosures: **Z.S. Navabi:** None. **R.T. Peters:** None. **J.A. O'Brien:** None. **B.R. Gulner:** None. **S.B. Kodandaramaiah:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.03/WW50

Topic: I.03. Anatomical Methods

Support: Japan Society for the Promotion of Science Grants-in-Aid: 21K06412

Title: Neuron specific autophagy-visualizing model in transgenic mice

Authors: *M. TANAKA[1], K. TAGUCHI[1], T. OKAZAKI[1], Y. WATANABE[2];
[1]Anat. and Neurobio., [2]Basic Geriatrics, Kyoto Prefectural Univ. Med., Kyoto, Japan

Abstract: Autophagy is an evolutionarily conserved intracellular clearance pathway in which cytoplasmic components are trafficked to the lysosome for degradation. When autophagy (macroautophagy) is induced, the isolation membrane sequesters a part of cytoplasm, including proteins and organelles to form the autophagosome. Then autophagosome is fused with a lysosome (autolysosome). Enclosed materials are degraded with autophagosome. Autophagy

occurs at low levels under basal conditions but can be induced by various stimuli including cellular stress. LC3, mammalian homologue of autophagy-related 8 family protein (ATG 8) is often used as a marker of autophagosome. Previously it was reported that GFP-LC3-RFP can be used as an autophagic flux probe by calculating the GFP/RFP signal ratio. In this study using this probe, we attempted to develop a transgenic (TG) mice to monitor the activity of autophagy in neurons by GFP-LC3-RFP gene is expressed under the Syn1 promoter. First, using primary cultures of hippocampal neurons, we confirmed intracellular aggregations of GFP-LC3 by the treatment of bafilomycin A, inhibitor of autophagy. Those GFP-LC3 aggregations were colocalized with p62, autophagy receptor. Then we examined the expressions of GFP-LC3 and RFP in the brain tissues in TG mice. GFP-LC3 was colocalized with mature neuronal marker, NeuN but not with GFAP, a marker of astrocytes. Basal expression of GFP-LC3 is different between cell body and neuronal processes in such as olfactory bulb, hippocampus and cerebral cortex. In mitral and pyramidal neurons, GFP-LC3 is mainly expressed in axons and dendrites, while RFP is observed in cell bodies. Moreover GFP-LC3 expressions are different among brain regions. Then we administered a potential autophagic inducer Li_2CO_3 to TG mice to enhance autophagy for a month. The GFP/RFP expression ratio in the hippocampus has a tendency of decreasing in both dendrites and cell bodies suggesting the increase of autophagy in these neurons. These results suggest the difference in basal autophagy activity of cellular components and brain regions and expression of GFP-LC3 in neurons of TG mice may reflect the activity of autophagy in vivo.

Disclosures: **M. Tanaka:** None. **K. Taguchi:** None. **T. Okazaki:** None. **Y. Watanabe:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.04/WW51

Topic: I.03. Anatomical Methods

Support: SBIR/NIH Grant R44MH119989

Title: Software and Reagents for Brain-Wide Snapshots of Neuronal Activity with Cellular Resolution Measurement of Npas4 and cFos

Authors: *D. WHEELER[1,2], N. GUANZON[1], J. ZEITOUN[1], A. REKSOATMODJO[1], Y. GALLEGOS[1], C. REDD[1], E. MAY[1], E. BLAES[1], M. PETERS[3], R. AZEVEDO[3,1], S. GANDHI[3,1];
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Abstract: Despite great advances in tissue labeling and imaging technology, until very recently high-resolution imaging more than a few hundred microns into a tissue has required slicing and mounting on slides. Providing access to the intricate anatomy of the whole intact brain, tissue

clearing offers neuroscientists unbiased and complete views of brain anatomy and function. Translucence Biosystems is developing an ecosystem of products to help enable a dimensional shift from 2D to 3D histology. Using iDISCO-based tissue clearing methods, imaging on the ZEISS Lightsheet Z.1 microscope with our Mesoscale Imaging System, machine learning-enabled whole-brain object segmentation with our BrainQuant3D/3TK software and new statistical methods for anatomics, the Translucence pipeline produces regionalized read-outs of cellular patterns across 100's of brain areas. Here, we describe our Stitchy software and our new Neuronal Activity Tissue Clearing Kits. Stitchy is a user-friendly standalone software tool developed to provide a simple solution for tiling and stitching terabyte-scale light sheet images. Stitchy reads raw files from common light sheet microscope systems, allowing for both automated and manual alignment and then stitching images to commonly used output formats like ims, ome.tif, and ngff. By reading native files and writing directly to the desired output, Stitchy avoids proprietary intermediates and provides significant speed increases. Our new tissue clearing kits provide a simple solution for brain-wide, cellular resolution measurement of neuronal activity taking advantage of the unique activity-dependent properties of the immediate-early gene (IEG) products Npas4 and cFos. Expression of cFos is driven by Ca^{2+}-signaling downstream of neuronal activity and is commonly used to mark active neurons. However, cFos expression is also driven by cAMP elevations and signaling pathways engaged by neurotrophins or other paracrine factors. In contrast, Npas4 expression is neuron-specific and tightly tuned to Ca^{2+}-dependent signaling pathways. Using our Activity Signaling Npas4 recombinant rabbit monoclonal antibody and a compatible cFos antibody, our kits reveal distinct, but overlapping, populations of neurons throughout the brain. Our reagent kits, Stitchy software and machine learning-enabled quantitative methods can help neuroscientists reveal brain-wide patterns of activity in response to behavioral, genetic and pharmacological manipulations.

Disclosures: **D. Wheeler:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **N. Guanzon:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **J. Zeitoun:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **A. Reksoatmodjo:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **Y. Gallegos:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **C. Redd:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **E. May:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc. **E. Blaes:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. **M. Peters:** None. **R. Azevedo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **S. Gandhi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc..

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.05/WW52

Topic: I.03. Anatomical Methods

Title: Recombinant Parkin Antibodies as Tools for Studying Neurodegeneration

Authors: N. V. SHARMA[1], T. KARUPPUCHAMY[1], F. A. MOINUDDIN[1], G. K. VISWANATHAN[1], R. R. DALMEIDA[1], *S. BALASUBRAMANIAN[2], A. A. KETKAR[1], S. R[1], K. KALIYAMOORTHY[1], J. KS[1], S. SAJJA[1], S. SUNDARRAJ[1], H. SRIDHARAN[1]; [1]R&D, Thermofisher Scientific, Bangalore, India; [2]Thermo Fisher Scientific, Bangalore, India

Abstract: Parkin, an E3 Ubiquitin Ligase, has been a subject of extensive research in the field of Neuroscience, especially since its role in juvenile Parkinsonism (AR-JP) was identified in the early 2000s. Given the significance of the protein in modulating dopaminergic selective nigral neurodegeneration ensuing early onset familial Parkinsonism, there is a significant ongoing effort to understand the role of this protein in PD pathogenesis, including but not limited to its expression, localization, and interaction with other cellular components, to provide insights into the disease and potentially guide the development of therapeutic interventions. However, studies on parkin have been impeded because of limited antibodies that are specific for this protein. Generating high quality and specific antibodies against Parkin is challenging due to it's relative small protein size, low levels of expression in most tissues or cell types, and post-translational modifications that can affect the protein's structure and function, potentially leading to changes in epitopes. Thus, highly specific and sensitive Parkin antibodies are the need of the hour for elucidating the molecular mechanisms underlying the pathogenesis of this condition. Here, we report the generation and characterization of a recombinant rabbit Parkin antibody whose specificity has been demonstrated using a Parkin knock out model, as well as relevant tissue, cell panels and well characterized cerebral organoids. The antibody readily picks up human, mouse, and rat Parkin, making it a valuable tool to study rodent models of neurodegeneration. We further discuss expanded formats offered on this clone, including a primary HRP conjugate, as well as an engineered format with enhanced sensitivity. We propose that the use of this set of recombinant Parkin-specific antibodies can address many of the existing issues around Parkin detection and quantification in difficult cell lines and tissue models, and further aid in our understanding of the debilitating neurogenerative Parkinson's disease. For Research Use Only. Not for use in diagnostic procedures.

Disclosures: **N.V. Sharma:** None. **T. Karuppuchamy:** None. **F.A. Moinuddin:** None. **G.K. Viswanathan:** None. **R.R. Dalmeida:** None. **S. Balasubramanian:** None. **A.A. Ketkar:** None. **S. R:** None. **K. Kaliyamoorthy:** None. **J. Ks:** None. **S. Sajja:** None. **S. SundarRaj:** None. **H. Sridharan:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

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Program #/Poster #: PSTR573.06/WW53

Topic: I.03. Anatomical Methods

Support: Canadian Institutes of Health Research Awards FDN-143206
CIHR PJT-183943
This work was supported by resources made available through the Dynamic Brain Circuits cluster and the NeuroImaging and NeuroComputation Centre at the UBC Djavad Mowafaghian Centre for Brain Health (RRID:SCR_019086)

Title: Adaptation of magnified analysis of the proteome for excitatory synaptic proteins in varied samples and visualization of cell-type specific distributions

Authors: *M. C. DELHAYE[1], J. LEDUE[1], K. ROBINSON[1], Q. ZHANG[2], S. OKU[1,3], P. ZHANG[2,1], A. CRAIG[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Case Western Univ., Cleaveland, OH; [3]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Visualizing the distribution of synaptic proteins in tissue has long been a technical challenge. Expansion microscopy approaches have the potential to overcome this challenge using standard confocal microscopes. However, few labs outside those developing these techniques have been applying them to answer biological questions related to synapses. Here we present a detailed protocol to study synaptic proteins using an adapted version of the Magnified Analysis of the Proteome (MAP; Ku et al., 2016. Nat. Biotechnol. 34:873-81), adapted and applied independently. MAP anchors the proteins contained in a tissue to a matrix of acrylamide and removes most of the other cellular components, allowing expansion of the resulting hybrid gel-tissue in water. We applied the adapted synaptic MAP procedure to mouse brain sections, focusing on the hippocampus. We first showed that MAP enables protein visualization at the individual synapse level for over 40 synaptic proteins using standard confocal microscopes, which is not the case for traditional immunohistostaining procedures. This gain is primarily due to improved antigen recognition associated with the denaturation and tissue clearing, and secondarily to the physical 4-fold expansion. Synaptic MAP worked well with fresh-fixed or PFA perfused tissue, and with brains stored in formalin, thus could be combined with slice electrophysiology or traditional histochemistry and applied to clinical specimens. Our Formalin-Fixed Paraffin-Embedded FFPE-MAP procedure is optimized for visualizing synaptic proteins in pathology specimens. Synaptic MAP combined with super-resolution microscopes enables nanodomain visualization within synapses. Synaptic MAP of AMPA (panAMPA) and NMDA (GluN1) receptors along with a postsynaptic density marker (panMAGUK) was performed in hippocampal CA1 *stratum radiatum* of Dlx5/6-Cre Ai32 mice which express YFP-ChR2 in interneurons. Quantitative analysis on 3 mice, from both sexes, revealed a significantly lower content, greater variability, and greater correlation with postsynaptic density volume for AMPA receptors on pyramidal cells than on interneurons. Conversely, NMDA receptors were more abundant at pyramidal cell than interneuron synapses. We further reveal cell type differences in the excitatory synaptic complement of Shank family proteins, differences which may relate to their roles in neurodevelopmental disorders. To summarize, we present a MAP clearing and expansion protocol optimized for synaptic proteins under various fixation conditions and use this to reveal cell-type specific distributions of excitatory synaptic proteins in mouse hippocampus.

Disclosures: **M.C. Delhaye:** None. **J. Ledue:** None. **K. Robinson:** None. **Q. Zhang:** None. **S. Oku:** None. **P. Zhang:** None. **A. Craig:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.07/WW54

Topic: I.03. Anatomical Methods

Title: Optimizing the Nissl method for cell staining with supervised deep learning

Authors: *S. HAN[1], S. SINGH[2], D. J. MILLER[3];
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Abstract: Although arguably the foundation of comparative brain mapping, Brodmann's pioneering cytoarchitectural map of the distribution of cells in the brain has yet to be directly quantitatively validated by single cell morphometrics. In the current report, we present the results of our two-pronged approach to standardize the Nissl staining method via quantitative histological validation. Specifically, we systematically varied the precipitation steps for the Nissl method to provide a dose dependency curve of cell morphology in rat brain tissue (adults of both sexes, n=6) and acquired 3D whole brain slide image volumes under 100x objective magnification with a focus on the cerebral cortex. We then annotated these image volumes with cell contours and subtype identities via consensus among expert (N = 3) and novice investigators (N = 11) to form the training set (n = 31 volumes of 9,892 cells; evaluation ~ 0.7) for the Nothing New U-Net architecture (Train/Test/Valid Split of 70/20/10%). We provide evidence of robust quantitative metrics (alpha < 0.01) to disambiguate morphological cell types into neurons, astrocytes, endothelial cells, oligodendrocytes, and microglia by size and coloration. We then compared the model's prediction of cell subtype identity, and associated features, across histological conditions as an index of staining quality. In particular, we show the optimal timing for the key precipitation steps are best under ~10 minutes, and demonstrate the loss of morphological details with shorter and longer cycles until morphology is unrecognizable. Standardizing the Nissl staining procedure by validation with a deep learning algorithm provides proof of concept to automate slice-based cell counting methods in histology. Our work sets the stage for quantitative macroscale histology to enable both a higher resolution analysis of neurological disease as well as to achieve the scale of samples needed for studies of brain evolution.

Disclosures: **S. Han:** None. **S. Singh:** None. **D.J. Miller:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.08/WW55

Topic: I.03. Anatomical Methods

Title: High throughput multiplex immunofluorescence staining, imaging, and AI-driven analysis shows differences in neural marker localization across disease models

Authors: *R. CLARY, S. PHANSE, A. CHOLEWINSKI, P. SAVICKAS;
HistoWiz, New York, NY

Abstract: Multiplex immunofluorescence is a technique utilizing multiple antibodies paired with unique fluorophores on the same slide. This increases the information that can be gathered from a single slide, which means that precious tissue from model organisms or human biopsies can be used more efficiently and enables direct co-localization studies. Traditionally, multiplex immunofluorescence is achieved via parallel staining; primary antibodies from different host species are carefully chosen so that a single cocktail of biomarkers can be applied to label multiple targets. However, the determining factor in this approach is host species rather than staining quality or experimental design, so overall staining quality suffers or has to be split across multiple slides if the same host animal is used in multiple primary antibodies. Recently, the second generation of immunofluorescence labeling has utilized tyramide, which forms strong covalent bonds that can withstand rounds of antibody stripping, to transition to sequential staining. Fluorophores that are linked to tyramide form covalent bonds with antibody targets; these bonds are stronger than those between primary antibodies and their target antigens so only the fluorescent label remains after stripping steps. Thus, this labeling technique can be applied to sequential staining paradigms to label desired antigens with the best performing antibodies regardless of whether the host animal is shared with another biomarker in the panel.
We developed multiplex immunofluorescence panels for neural lineage and neuronal disorders. Ongoing studies will apply these panels to tissues from multiple organisms and disease models, for example mouse models of Alzheimer's and Parkinson's Disease as well as human tissue samples. High resolution whole-slide images will be analyzed using Visiopharm software's AI models for automated quantification and analysis of cell types within the brain. We will then compare expression patterns of each biomarker across healthy and diseased samples to quantitatively test whether neuronal labeling and co-expression differ in healthy and diseased states. This system creates a customizable framework that can be applied to similar models for the design and optimization of multiplex panels with subsequent high throughput staining and automated image analysis to provide seamless statistical data for interpretation.

Disclosures: **R. Clary:** A. Employment/Salary (full or part-time):; HistoWiz. **S. Phanse:** A. Employment/Salary (full or part-time):; HistoWiz. **A. Cholewinski:** A. Employment/Salary (full or part-time):; HistoWiz. **P. Savickas:** A. Employment/Salary (full or part-time):; HistoWiz.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.09/WW56

Topic: I.03. Anatomical Methods

Support: Fitzgerald Translational Neuroscience Fund

Title: Investigating the viability of adult organotypic rat brain slices by embedding them with varying protein concentrations of Matrigel

Authors: *E. ANGELOPOULOS[1,2,5], J. T. LIM[3,2,5], D. CULLEN[6,5], M. D. SERRUYA[4,2]; [2]Ctr. for Neurorestoration, [3]Neurosci., [4]Neurol., [1]Thomas Jefferson Univ., Philadelphia, PA; [5]Corporal Michael J. Crescenz Veterans Affairs Med. Ctr., Philadelphia, PA; [6]Ctr. for Brain Injury and Repair, Dept of Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Organotypic brain slice cultures have been widely used as an alternative to primary dissociated cultures due to their preserved three-dimensional architecture and their potential to simulate *in-vivo* like conditions. However, one of the major obstacles to the success of this approach is the difficulty in supporting long term survival of organotypic brain slices. To overcome this problem, we investigated the effect of different protein concentrations of Matrigel on cell viability of adult organotypic rat brain slices using Calcein-AM/Ethidium Homodimer-1 live staining. Matrigel is a basement membrane preparation that has been used as a scaffold for three-dimensional cultures, but its effect on organotypic brain slices has not been extensively studied. Previous studies suggest that the survival and differentiation of differentiating cell populations was higher after they were mixed or coated with Matrigel. This may be due to the gel's biochemical cues and mechanical support. Thus, our hypothesis was that organotypic brain slices embedded with Matrigel will be more viable compared to control slices. Different protein concentrations of Matrigel were prepared by diluting the stock solution with Neurobasal media. Hemisected coronal brain slices from adult female rats at thickness of 400um were obtained by using a Leica vibratome. Each slice was placed inside a well of a 12-well plate and 200uL of one Matrigel solution was deposited above the slice. The embedded slices were placed for thirty minutes in an incubator (37 ℃) with humidified carbogen atmosphere. Then, 3mL of culture media was added in each well and placed back into the incubator. As for the control wells, 3mL of culture media was inserted and were directly placed in the incubator. The culture media was exchanged every two to three days. After performing the live/dead assay, fluorescence images were obtained by using a confocal microscope and cell counting was performed using ImageJ. Compared to control slices, we found that slices embedded with Matrigel have a greater percentage of live cells at early stages (8 days *in vitro*) as well as long term (29 days *in vitro*). Additionally, cell viability may positively correlate with Matrigel protein concentration. Our results show the presence of live cells further away from the borders of Matrigel embedded brain slices, suggesting outward cellular migration into Matrigel-only areas. Thus, Matrigel appears to provide a suitable scaffold that supports survival and cell migration of adult organotypic rat brain slices, which can be used for the construction of slice-to-slice networks for future structural and functional experiments.

Disclosures: E. Angelopoulos: None. **J.T. Lim:** None. **D. Cullen:** None. **M.D. Serruya:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.10/WW57

Topic: I.08. Methods to Modulate Neural Activity

Title: A novel microfluidic platform enabling high throughput electrophysiological recordings of compartmentalized co-culture.

Authors: L. MINY[1], L. DUBUISSON[1], M. HOCHEDEL[1], F. BRELOT[1], A. PONOMARENKO[1], A. WILLSIE[2], D. MILLARD[2], A. PASSARO[2], S. ROUX[1], ***T. HONEGGER**[1];
[1]NETRI, Lyon, France; [2]Axion BioSystems, Atlanta, GA

Abstract: Classic high-throughput in vitro cell culture tools for central or peripheral nervous system applications (CNS, PNS) struggle to accurately replicate physiologically relevant connectivity patterns. This is partly due to the absence of geographical and fluidic isolation between innervated tissues and neurons or between regions of the brain. While conventional organ-on-chip solutions enable compartmentalization, they lack the capacity to record functional activity. In this study, we utilized NETRI's DuaLink MEA, a compartmentalized microfluidic device outfitted with microelectrode arrays (MEA). We created fluidically isolated co-cultures of rodent hippocampal and cortical cells, as well as hiPSC glutamatergic neurons, where we compartmentalized neuron somas and their axonal endings. Using Axion Biosystem's Maestro Pro hardware, we recorded spontaneous effects of compounds on the network activity and compared activity among compartments and microchannels. Microfluidic compartmentalization allowed us to perform compound assays on somas or axonal endings independently, or in combination. For instance, when adding TTX - a sodium receptor blocker - to the somas, mean firing rate was eliminated (~96%) in both the soma compartment and axonal compartment. Adding TTX to the axonal compartment, however, left somatic activity unchanged (~4%). This highlights the ability, in a co-culture context, to independently manipulate and examine detailed subcellular mechanisms like synaptogenesis or plasticity, as well as the effects of therapeutic or toxic compounds. Our findings highlight the potential of this technology for investigating neuronal communication in neurodegenerative diseases. By extension, we can apply this to PNS applications such as pain, innervated skin. or innervated gut. This approach enables a nondestructive assessment of network-level functional responses in CNS and the PNS applications, providing valuable insights for pre-clinical pharmaceutical assays and studying compound-induced effects on advanced multi-cell-type models.

Disclosures: L. Miny: None. **L. Dubuisson:** None. **M. Hochedel:** None. **F. Brelot:** None. **A. Ponomarenko:** None. **A. Willsie:** None. **D. Millard:** None. **A. Passaro:** None. **S. Roux:** None. **T. Honegger:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.11/WW58

Topic: I.03. Anatomical Methods

Support: R35GM138173

Title: Design, development, and validation of a 3D printed microfluidic perfusion system for studying circadian physiology via ex vivo slice culture

Authors: *R. J. ORTIZ, G. G. GUTIERREZ, C. A. BAKER;
Chem. and Biochem., New Mexico State Univ., Las Cruces, NM

Abstract: The suprachiasmatic nucleus (SCN) is a neural region containing about 100,000 cells located within the hypothalamus and functions as the master circadian clock. The SCN receives external stimuli via the retinohypothalamic tract and regulates various physiological and behavioral rhythms in a roughly 24-hour pattern. Disruption of these rhythms are observed in dementia, schizophrenia, Alzheimer's disease (AD) and others. To better understand the neuroendocrine mechanisms that entrain peripheral tissues to the circadian rhythm of the SCN, we have developed a 3D printed microfluidic perfusion device for ex vivo culture of SCN tissue slices. The perfusion system enables temperature control via integrated water circulation chamber, and gas/media exchange with precise transport of chemical stimuli via a "droplet-on-demand" media delivery mechanism. Tissue physiology can be interrogated via epifluorescence imaging and/or via analysis of cellular releasate sampled in the perfusion droplets. Here, we will discuss the design, development, and validation of a perfusion system for studying the circadian physiology of SCN tissue slices. Our previously reported perfusion system was adapted for fabrication by 3D printing, which enables broad accessibility of the technology but also presented substantial challenges associates with materials properties. We will discuss design strategies that effectively addressed reduced thermal conductivity and altered surface tension properties of the 3D printed plastic material as compared to conventional glass microfabrication substrates. To validate the perfusion system for utility in investigating circadian physiology, we first characterized the response of neural activation in the SCN over a 24-hour time period. We utilized intracellular calcium imaging via Fluo-4 AM dye to observe 24-hour patterns of cell activation in response to exogenous stimuli. Fluorescence intensity was monitored vs time after perfusion delivery of potassium chloride stimulation (KCl) to induce widespread cellular depolarization. 60mM KCl stimulus was delivered to SCN slice cultures from male mice (PD: 60-120) twice per hour for 24 hours, and an increase for fluorescence intensity following stimulus indicated Ca2+ influx to induce repolarization of healthy neural tissue. The time-course response of tissue repolarization over the 24-hour monitoring period will be described, and the suitability of the perfusion system for long term (24+ hours) tissue perfusion experiments will be discussed.

Disclosures: R.J. Ortiz: None. **G.G. Gutierrez:** None. **C.A. Baker:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.12/WW59

Topic: I.03. Anatomical Methods

Support: R00CA240681
RF1MH128841

Title: Novel brain clearing for molecular and morphological profiling of neurons and circuits at brain-wide scales

Authors: *K. J. CAO, N. OUELLETTE, M. LOGSDON, J. BAKA, A. GLASER, J. CHANDRASHEKAR;
Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: Examining the diversity of neuronal cells in a given brain region and the complex connectivity between defined cell types of different brain regions is foundational for our understanding of neural activity underlying an organism's behavior. This requires probing circuit elements at multiple scales - in terms of the molecules (channels, receptors, neurotransmitters, mRNA), structure (morphology of dendrites and long-range axonal projections of individual neurons), and connectivity. Selective plane illumination microscopy (SPIM) can achieve centimeters scale imaging of tissues with nanometer or micrometer precision but artifact-free imaging at these brain-wide scale requires spectacularly clear samples.We present here whole brain clearing and processing strategies that achieve optically clear specimens while retaining protein and mRNA. These approaches generate specimens suitable for morphological single neuron reconstructions with transcriptomic information preserved for post hoc molecular identification of cell types. Our modular approach enables sample preparation fit for SPIM-based high-throughput mesoscale anatomy as well as large specimen expansion, including entire mouse brains suitable for high-contrast, high-resolution imaging on a novel expansion-assisted SPIM (ExA-SPIM) system.

Disclosures: K.J. Cao: None. **N. Ouellette:** None. **M. Logsdon:** None. **J. Baka:** None. **A. Glaser:** None. **J. Chandrashekar:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.13/WW60

Topic: I.03. Anatomical Methods

Support: National Institute of Mental Health (BICCN BRAIN Initiative) RF1MH128969

Title: Hub.clear: a simple, adaptable, and scalable clearing method for molecular and cellular profiling of human brain over large volume

Authors: *W. WANG[1], J. XU[1], K. BOWYER[1], K. WHITNEY[2], E. L. THORN[2,3], C. DE SANCTIS[2,3], M. J. CASPER[4], C. PÉREZ CAMPOS[5], W. LI[5], E. M. C. HILLMAN[4,5,6], J. F. CRARY[2,3], Z. WU[1];
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Abstract: Rapid advancements in tissue clearing approaches have greatly expedited systematic investigation of organ-wide cellular compositions and interactions with advanced imaging techniques. Our group has developed several iterations of iDISCO-family tissue clearing protocols (iDISCO[1], iDISCO+[2], and AdipoClear[3,4]) to enable whole mount labeling, imaging, and automated analysis of large intact organs including adult mouse brain.
To extend our approach to clearing and immunostaining the human brain, we have optimized a new protocol, HuB.Clear (Human Brain Clearing), that ensures consistent labeling and clearing of large intact human brain samples. We have developed a suite of techniques to overcome various challenges in human brain tissue applications, including optimized sample collection and storage, lipofuscin blocking, antigen retrieval, and reproducible multiplexed immunolabeling that are compatible and scalable with advanced tissue clearing and imaging of large whole human brain slabs over 8 mm thickness. The clearing and imaging media are aqueous based for convenient implementation to broad microscopy platforms including SCAPE (Swept Confocally-Aligned Planar Excitation)[5]. The entire HuB.Clear protocol is based on robust, affordable, chemical-assisted procedures for easy adaptation and parallel processing desired for large scale projects like human brain profiling.
Our HuB.Clear protocol preserves the performance of most antibodies validated in traditional histological studies. Clearing can also be reversed to perform re-staining or post-hoc analysis and validation with standard histo-pathological assays. Importantly, HuB.Clear's well-preserved tissue morphology facilitates automatic data registration and analysis across multiple imaging modalities to enable brain-wide anatomical and patho-histological profiling at different scales.

Disclosures: W. Wang: None. **J. Xu:** None. **K. Bowyer:** None. **K. Whitney:** None. **E.L. Thorn:** None. **C. De Sanctis:** None. **M.J. Casper:** None. **C. Pérez Campos:** None. **W. Li:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica Microsystems. **E.M.C. Hillman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica Microsystems. **J.F. Crary:** None. **Z. Wu:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.14/WW61

Topic: I.03. Anatomical Methods

Title: Optimizing the Gallyas histology method for 3D myelinated axon fiber length density with supervised deep learning in the mammalian brain

Authors: *S. SINGH[1], S. HAN[2], D. J. MILLER[3];
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Abstract: Magnetic resonance imaging (MRI) has proven to be an important noninvasive tool to study the brain in health and disease, yet because of technical challenges we lack ground-truth histological validation of putative microstructure like myelin. Specifically, traditional stereology is so slow and histology is so tedious that even after a hundred years, we still have no optimal protocol for myelin staining. In this report, we present our work to validate a modified Gallyas silver myelin stain with supervised deep learning. We systematically varied the staining parameters of a modified version of the Gallyas method and measured adjacent series of sections in the rat cerebral cortex (adults of both sexes, n=6) by acquiring 3D whole slide scans (depth 10 micrometers at 100x). We then used our deep learning model (Nothing New U-Net, trained on n= 26 volumes of ~125,000 drawn fragments across 8 users, ~60% accuracy) to estimate the total myelinated fiber length density (MFLD) per unit brain image volume. We demonstrate a dose-dependent curve, which revealed that optimal staining occurred under about 10 minutes when silver impregnation was ~2 minutes longer than the clearing step. We show that varying the incubation time produces changes in MFLD in excess of published differences (~15%) between functionally defined brain regions in mammals. We, therefore, present our optimized protocol to fully reveal neuroanatomical myelin content along with validation by computer vision to facilitate comparisons across users and projects. Ultimately, we can apply this optimized staining procedure to evaluate local and global changes in myelin content across development as well as in the presence of injury or disease.

Disclosures: **S. Singh:** None. **S. Han:** None. **D.J. Miller:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.15/WW62

Topic: E.04. Voluntary Movements

Support: NIH Grant RF1MH114276
NIH Grant U19NS104649
NIH Grant R01NS063226
Leducq 22CVD0
Simons Collaboration on Global Brain: 542991

Title: A Cortical Functional Motor Topography Derived from Awake Behaving Mouse Brain Data

Authors: *W. XU[1,2], A. J. YAGIELSKI[1,2], L. M. CARMONA[1,2], D. N. THIBODEAUX[1,2], K. I. UGORJI[1,2], F. LODGHER[1,2], S. SHAHSAVARANI[1,2,3], E. M. C. HILLMAN[1,2];
[1]Columbia Univ., New York, NY; [2]Mortimer B. Zuckerman Mind Brain Behavior Inst., New York, NY; [3]Section on Functional Imaging Methods, Lab. of Brain and Cognition, NIH, Bethesda, MD

Abstract: Most mouse brain atlases lack detail within the motor cortex. Structural atlases like the Allen Brain Atlas, rely on histology and gene expression patterns, whereas functional mappings of the motor cortex have relied either on complex task paradigms or cortical stimulation. All of these approaches have provided only coarse representations of motor cortex organization.

In-vivo functional imaging techniques such as wide-field optical mapping (WFOM) offer a more direct means of mapping the cortex. However, the complexity of movements, combined with inevitable proprioceptive feedback signals, fails to untangle the complex representation of movements in the cortex.

Here, we present a novel finding that permits precise mapping of somatomotor regions spanning primary sensory and primary and secondary motor cortices. This mapping reveals an intricate topological map that can permit real-time analysis of sensorimotor activity patterns during real time behavior.

Our topographic map was revealed by analyzing activity recorded in head fixed, awake Thy1-jRGECO1a mice. First, we established a topographic sensory map (S1) by delivering sequential tactile stimulation to different body parts of the mouse. We then computed the way in which the activity patterns of specific brain regions were correlated to each other, and how these correlations changed over time. During quiet rest, strong fluctuations in neuronal signals were observed between individual sensory regions and small regions of the medial frontal cortex, which put together formed a clear functional topographic map within the area defined as secondary motor cortex (M2). We also found that during movements like locomotion, these correlation patterns were less clear, but found stronger correlations between paired S1 and M1 regions. The locations of M1 for hindpaw and forepaw were validated using corticospinal tracing.

Utilizing this new S1-M1-M2 topography, we can study neuronal representations of diverse behaviors, finding strong involvement of M2 in complex integrated movements such as grooming, but only transient engagement of M2 at the start of locomotion. This detailed topography of the mouse primary and secondary sensorimotor cortex enables further study of dynamic coupling between M1/M2/S1 in complex behavior.

Disclosures: **W. Xu:** None. **A.J. Yagielski:** None. **L.M. Carmona:** None. **D.N. Thibodeaux:** None. **K.I. Ugorji:** None. **F. Lodgher:** None. **S. Shahsavarani:** None. **E.M.C. Hillman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica Microsystems.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.16/WW63

Topic: E.04. Voluntary Movements

Title: Validation of robot-based kinematic assessment of reach function

Authors: *S. RAO[1], B. BOETTGER[2], D. EVERSELY[2], A. ROY[2], B. HENNESSIE[2], K. WESTLAKE[1];
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Abstract: 75% of individuals post stroke experience persistent Upper Extremity (UE) impairments, which results in inefficient UE use in the Activities of Daily Life (ADL). Reaching is one such essential ADL and hence accurate assessment of reaching deficits is a requisite to provide efficacious UE reach movement rehabilitation. Rehabilitation robots have been developed with the purpose of performing higher resolution objective measurements of kinematic metrics that can be used to assess motor performance over time. Despite of growing interest in the use of robotic devices as an evaluatory tool, only limited number of studies have examined their clinical applicability. It is therefore important to evaluate the robotic measurement system's validity as compared to external gold standard reference systems like Optitrak motion tracking system (i.e., concurrent validity). In this study, we propose to utilize the REACH robotic device, which has embedded sensors that enables it to perform an objective kinematic assessment of the reach movement. The purpose of this study is to investigate the concurrent validity and limits of agreement of the REACH device in assessing the kinematic parameters of reaching movement, by exploring its relationship with kinematic measures from the Optitrak motion tracking system. The hypothesis is that the correlation between the kinematic parameters of Smoothness ($1/s^2$), Peak Speed (cm/sec), and Range of Motion (Flexion and Extension) from the REACH and Optitrak systems (measured by r^2) and accuracy (measured by Root Mean Square error) would demonstrate good concurrent validity and limits of agreement. For this validation, we will recruit a total sample of N=20(n=10 healthy young adults and n=10 individuals with chronic stroke). Kinematic validation will be performed as the participant performs a reach-to-target movement with the REACH device. Simultaneously kinematic parameters will be measured by the Optitrak with help of markers placed on UE. Validating the kinematic parameters from the REACH device, would aid in its use clinically as systematic diagnostic tool and may provide the basis for the planning of an individualized UE intervention

post stroke. This will potentially act as a key step toward integrating technology into clinical practice.

Disclosures: S. Rao: None. **B. Boettger:** None. **D. Eversely:** None. **A. Roy:** None. **B. Hennessie:** None. **K. Westlake:** None.

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.17/WW64

Topic: E.05. Brain-Machine Interface

Title: Towards the development of a digital instrumental activities of daily living scale for the neurological population: systematic review results, methodology and interim results

Authors: A. FRY[1], J. BRANNIGAN[2], ***A. SAWYER**[3], M. NARDOZZI[1], L. SPIELMAN[4]; [1]Synchron, Inc., New York City, NY; [2]Univ. of Cambridge, Cambridge, United Kingdom; [3]Rehabil. and Human Performance, Icahn Sch. of Med. at Mount Sinai, New York City, NY; [4]Rehabil. and Human Performance, Mount Sinai, New York City, NY

Abstract: Instrumental Activities of Daily Living (IADLs) are the activities necessary to function independently in society. Nowadays, many IADLs are accomplished using digital technologies. However, the scales used to measure IADLs predate many technologies and do not reflect this modern reality. Brain computer interfaces (BCIs) have the potential to improve IADLs in people with varying disabilities, but we are without the instruments to accurately capture clinical benefit. This presents a substantial barrier for translation to market and reimbursement. With the BCI landscape expected to grow exponentially in the coming decades, there is critical need develop an IADL scale that reflects modern IADL performance. The objective of this ongoing body of work is to develop a validated digital IADL scale for use in people with various neurological conditions, where IADLs may be accomplished using digital technologies such as BCI. The specific aim of the systematic review was to evaluate existing validated IADL scales available for people with severe paralysis. A systematic review was performed, compliant with the Preferred Reporting Items of Systematic Reviews and Meta-Analysis (PRISMA-SR). The review was ineligible for PROSPERO registration due to the absence of a defined clinical outcome. Search strategies were developed for MEDLINE, Embase, and CINAHL and conducted on 1st November 2022. No limits or restrictions were applied. Studies were eligible if they were validation studies for a scale which included IADL items and published in English. Blinded screening was conducted using Rayyan (Rayyan Systems Inc., Cambridge, USA) (JB, AS). The search yielded 183 studies for full text review, of which 91 were eligible, with 73 individual ADL scales. Independent data extraction and classification of items into basic ADLs, IADLs and cognitive tasks was undertaken (JB, AS). Differences throughout were reconciled through discussion with an occupational therapist (MN). Then, items classified as basic ADLs, those that had no current possibility of being performed digitally, and

purely cognitive tasks were removed from the item pool, resulting in a deductively generated digital IADL bank of 326 items. Results are also presented for: (i) inductive IADL item generation from individuals with lived experience; (ii) a Delphi process sorting (deductive and inductively generated) items into domains; (iii) focus groups with key opinion leaders; and (iv) a Q-sort to determine items most diagnostic of each domain. This methodology provides consensus on the first draft of a long-form digital IADL instrument. Subsequent validation in patient populations will be required.

Disclosures: **A. Fry:** A. Employment/Salary (full or part-time):; Synchron, Inc. **J. Brannigan:** None. **A. Sawyer:** None. **M. Nardozzi:** A. Employment/Salary (full or part-time):; Synchron, Inc. **L. Spielman:** F. Consulting Fees (e.g., advisory boards); Consulting time reimbursed by Synchron, Inc..

Poster

PSTR573. Innovations in Surgical, Histological, and Behavioral Approaches

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR573.18/WW65

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH IRP Grant CL090033
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Title: Prototyping high intensity light for fluorescence-compatible removal of lipofuscin-derived autofluorescence in human nervous tissue

Authors: *D. KING[1], M. SAPIO[1], D. MARIC[1], A. MANALO[1], A. GHETTI[2], M. IADAROLA[1], A. MANNES[1];
[1]NIH, Bethesda, MD; [2]AnaBios, San Diego, CA

Abstract: Autofluorescence in human tissue impedes optimal multiplex fluorescence imaging and accurate quantification of signal. This has been a major barrier to the use of multiplex mRNA-directed detection strategies in particular, as several common chemical methods perform poorly in combination with existing procedures. One pervasive source of autofluorescence is lipofuscin, an age-associated agglomeration of oxidized lipoprotein, which absorbs maximally at around ~400 nm, and emits strongly between 430-650 nm, which includes most of the optimized emission wavelengths of commercial dyes used in multiplex labeling. These characteristics of the endogenous fluorescence strongly interfere with signal detection, particularly of lower-expressed targets. Currently, computational methods and/or development of fluorophores outside this range have been used with some success. However, the removal of the fluorescent pigment from tissue would streamline existing protocols, making them more efficient, reproducible, and easier to deploy in a core facility setting. Formalin-fixed paraffin embedded human dorsal root ganglion (DRG) tissue (healthy controls sourced from AnaBios Corp.) was exposed to high

intensity white light to photobleach the lipofuscin. Based on data collected from previous small-scale prototypes, it was determined that 72 hours of light exposure was optimal for producing near total reduction of lipofuscin signal. Because the samples were paraffin embedded and remained at 2 C for the duration of the exposure, the tissue was minimally impacted, leading to increased signal-to-noise. This was assessed by measuring fluorescence intensity using four Opal dyes in the 4-plex TSA-amplified in situ hybridization protocol (Advanced Cell Diagnostics.) Parameterization was performed using both a small-scale prototype device, and a larger custom device designed and built to specifications for this project. Subsequent to prototyping, we tested the rigor of the technique on brain tissue from patients with Alzheimer's Disease, which presents a particular challenge. In addition to high levels of lipofuscin, the plaque formation in these brain tissues has similar fluorescence emitting properties that pose technical challenges. Reducing lipofuscin and other autofluorescence in tissue compromised by pathology such as Alzheimer's Disease would open up fluorescence in situ hybridization as a viable staining option. Our study provides an effective method for removing interfering autofluorescence, validates the technique in pathological aged brain tissue and enhances multiplex fluorescence in situ hybridization in a wide range of applications.

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Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.01/WW66

Topic: I.04. Physiological Methods

Support: NIH Grant UF1-NS122123
NIH Grant U01-NS103518

Title: Multiregional calcium imaging of neurons in the non-human primate brain

Authors: *B. PESARAN[1], J. CHOI[2], K. WINGEL[1], J. HAGGERTY[1], A. CHARLES[3]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]New York Univ., New York, NY; [3]Johns Hopkins Univ. - Homewood Campus, Lutherville-Timonium, MD

Abstract: Progress in understanding multiregional networks involves multiregional imaging. In smaller organisms, including C elegans, drosophila, and now in mice, cellular multiphoton imaging of different fluorophores,GFP/BFP/RFP, has resolved activity during behavior across large-scale networks. As Multiphoton imaging expands into NHP, e.g., macaque (macaca mulatta) optical imaging of large-scale networks is facing new fundamental barriers. First, large-scale brain windows must be developed to provide sufficient access to large-sale networks (>5cm). Second, hardware and software must be designed to enable cellular-level imaging at scale across large-scale networks in awake, behaving primates. Specifically, instrumentation

must enable experimenters to flexibly target different optically accessible systems in a stable imaging plane and be integrated into an experimental user interface to guide experimentation. Current approaches pre-select an area of interest under the window and finer adjustments to the FOV can be accomplished by optical focusing, e.g., in a mesoscope (sutter-scope). Third, to record activity across cortex, we must be able to elicit large-scale viral expression in vivo. Finally, data processing pipelines must be designed to coregister and identify neurons at scale. Here, we present solutions to each of these challenges. We engineer a multiplanar primate brain window customized to each experimental subject. We develop approaches to reproducibly and flexibly position and reposition the imaging field of a multiphoton microscope. We elicit viral expression of fluorophores and the GECI GCaMP8m across centimeter-scale extents using convection-enhanced delivery. We also present algorithms for building volumetric maps of neurons over large areas, registering fields-of-view, and extracting neuronal activity from functional data. These algorithms are integrated into instrumentation and are tuned to the expression and imaging properties in the primate brain. Finally, we provide a user interface to enable experimentalists to navigate the large areas now visible. The results demonstrate a novel approach toward realizing a cellular-resolution multiregional primate brain observatory for research into the neural mechanisms of behavior and cognition.

Disclosures: **B. Pesaran:** None. **J. Choi:** None. **K. Wingel:** None. **J. Haggerty:** None. **A. Charles:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.02/WW67

Topic: I.04. Physiological Methods

Support: NIH Grant R01 NS111028 to T.J.E
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Academic Investment Research Program (AIRP) grant from UMN to T.J.E.

Title: Stability and flexibility in neocortical functional segmentation revealed by wide-field Ca^{2+}imaging

Authors: *A. NIETZ, M. STRENG, L. S. POPA, R. E. CARTER, E. B. FLAHERTY, J. D. ARONSON, T. J. EBNER;
Neurosci., Univ. of Minnesota - Twin Cities, Minneapolis, MN

Abstract: Understanding how the brain plans, executes, recalls, and modifies information requires a description of neural activity across multiple spatiotemporal scales. Many brain functions including decision-making and behavior acquisition are hypothesized to be emergent

properties where regions acquire new functions that are distinct and separable from the functions of the component parts. We used wide-field Ca^{2+} imaging to investigate the stability and flexibility of neocortical functional segmentation across time and behaviors. We hypothesized that functional segmentations would have both stable and variable components due to many behaviors utilizing similar areas. We performed wide-field Ca^{2+} imaging in mice implanted with a transparent polymer skull that allows for chronic simultaneous visualization of a large region of the dorsal neocortex. Mice were head-fixed to a freely moving disk treadmill which allowed for resting, walking, and grooming behaviors. We used Independent Component Analysis (ICA) to segment the neocortex and identify independent spatial regions (ICs) of unique Ca^{2+} activity across the entire dataset for a mouse. This segmentation produced a template set of ICs. ICA segmentations were evaluated across a wide range of timescales and behaviors. Results showed that template ICs were present across timescales from days to 30 seconds although they occurred with a lower probability at shorter timescales highlighting the stability of select ICs. At shorter timescales, unique ICs also appeared suggesting they may act transiently to refine the cortical network. Behavior-specific analyses revealed both common and unique ICs across behaviors. These data suggest that behaviors utilize a core set of ICs and that cortical networks operate in a semi-stable state retaining the ability to reorganize in a task-dependent manner.

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Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.03/WW68

Topic: I.04. Physiological Methods

Support: NIH UF1 NS108213
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Simons Collaboration on Global Brain: 542991
Research Initiatives in Science and Engineering
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Title: Whole-brain imaging of Drosophila melanogaster with SCAPE microscopy to explore brain-state related perturbations to functional connectivity and behavioral representations

Authors: *E. ÖZEN[1], W. LI[2], E. M. C. HILLMAN[2];
[1]Columbia University, Dept. of Biomed. Engin., New York, NY; [2]Columbia University, Mortimer B. Zuckerman Mind Brain Behavior Inst., New York, NY

Abstract: The brain is a complex, interconnected network of cells whose morphology and function drive all aspects of behavior. The fruit fly Drosophila melanogaster provides a model

system whose brain is small enough to fit within the 3D field of view of imaging systems such as swept confocally aligned planar excitation (SCAPE) microscopy, while providing a repertoire of spontaneous behaviors that permit analysis of real-time neuronal representations of behavior. Spatiotemporal unmixing analysis of real-time pan-neuronal (cytosolic) GCaMP data acquired in adult Drosophila brain at 6-10 volumes per second with SCAPE microscopy reveals complex networks. Each spatiotemporal component represents a functional unit in the dataset comprised of either an individual or group of neurons that can be identified using anatomical maps, or functionally connected regions exhibiting synchronous activity patterns. This unmixing method provides functional network-based representations that can be aligned with specific behaviors of the animal. In head-fixed, behaving flies walking on an air-suspended ball receiving regular odor delivery, these functional networks enable simultaneous observation of odor circuit dynamics and representations of real-time behaviors such as spontaneous locomotion. Structures such as the primary olfactory brain area, antennal lobe, or the higher-order olfactory processing center, lateral horn, can be seen to respond differentially to appetitive and aversive odors, as well as repeated delivery of the same odor. Simultaneously, networks aligned to locomotion include the protocerebral bridge, a substructure of the central complex which integrates various sensory information and motor control in the insect brain.
This real-time, brain-wide recording approach and analysis pipeline then opens up the opportunity to explore how functional circuits and their activity patterns are altered during brain state perturbations. In exploratory studies, we are examining how acute administration of drugs such as alcohol leads to changes in brain-wide responses to repeated stimuli, perturbations in behaviors, and whether we can detect state-dependent changes in functional network and neural representations of spontaneous behavior.

Disclosures: **E. Özen:** None. **W. Li:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica. **E.M.C. Hillman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.04/WW69

Topic: I.04. Physiological Methods

Support: NIH Grant NS110069
NIH Grant AG070507
NIH Grant NS126920

Title: Vascular dynamics contaminate fluorescence imaging by absorbing light in an arterial-diameter-dependent manner

Authors: *P. O'HERRON, K. XIE;
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Abstract: Blood strongly absorbs visible light. This leads to the widely-observed shadows in multi-photon fluorescence imaging data, and makes it difficult to collect fluorescence data from below blood vessels. However, two features of typical experiments make this a more serious problem than is typically considered. One is that emitted photons undergo tremendous scattering, and so fluorescence from cells that aren't under vessels is also reduced by blood absorption. The second is that vessels in the brain - particularly the large pial arteries - dynamically change diameter, and, through experimental manipulations, can have more than two-fold diameter differences. The differences in blood volume generated by these diameter changes has a profound impact on the detected fluorescence in two-photon imaging data. Here we sought to quantify the impact of arterial diameter changes on detected fluorescence signals in the cerebral cortex and explore methods of accounting for and correcting for these effects. We show with inert (non-functional) indicators, that although cells close to or under vessels have the largest fluorescence changes, cells far away from blood vessels also change their fluorescence based on vessel diameter changes. We use sensory stimulus-evoked increases in vessel diameter and optogenetically evoked decreases to show these effects occur for any diameter change and do not depend on neural activity. We also show how the lateral distance from vessels and the depth from the surface affects the amount of vessel "shadowing". We tried multiple corrections to try to extract the true calcium signal from the contaminated signal, including subtraction of signal in the surrounding neuropil and using a second fluorescence channel (with an inert indicator). We also used a ratiometric indicator which in theory should overcome the shadowing effect since it should be equal in the two channels. However, all of these approaches suffer from imperfect corrections. This work shows that the activity of cortical cells (neurons, astrocytes) estimated by fluorescent calcium signals (or other functional indicators - such as glutamate, GABA, and other neurotransmitters) must be carefully interpreted, and the effect of changes in vascular diameter on the detected fluorescence must not be ignored.

Disclosures: P. O'Herron: None. **K. Xie:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.05/WW70

Topic: I.04. Physiological Methods

Title: Effects of reducing axial resolution in two-photon calcium imaging on retrieving functional neuronal activity.

Authors: *H. A. YOON[1], R. G. NATAN[2], A. S. CHARLES[3], N. JI[2];
[1]Mechanical Engin., [2]Mol. and Cell Biol., Univ. of California, Berkeley, Berkeley, CA;
[3]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Two-photon calcium imaging is widely used in neuroscience to record population activity of neurons *in vivo*. In recent development of two-photon microscopy methods, optical resolution is sometimes sacrificed in pursuit of recording activity from ever larger numbers of neurons. We systemically investigated how reducing resolution, especially along the axial direction, impacts the quality of calcium imaging data from the mouse primary visual cortex *in vivo*, by recording visually evoked activity from the same neurons using two-photon excitation foci with axial full widths at half maximum ranging from 3.6 μm to 21 μm. With lower resolution associated with increasing amount of neuropil contamination, we examined whether existing popular calcium imaging analysis pipelines can accurately retrieve the activity pattern of individual neurons from two-photon calcium imaging data acquired at lower resolutions. Our results provide valuable benchmarks and guidelines for calcium data analysis and future microscopy development efforts.

Disclosures: H.A. Yoon: None. **R.G. Natan:** None. **A.S. Charles:** None. **N. Ji:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.06/Web Only

Topic: I.04. Physiological Methods

Support: Cure Alzheimer's Fund 65539
NIH AG064554
NIH P50AA027055

Title: Imaging of evoked cortical depolarizations using either ASAP2s, or chi-VSFP, or Di-4-Anepps, or intrinsic optical signals.

Authors: *S. D. ANTIC, V. O. IVANOVA, B. L. BARBEAU;
Neurosci., UConn Hlth., Farmington, CT

Abstract: Population voltage imaging is used for studying brain function, brain dynamics, and brain circuits. We used two transgenic mice lines, equipped with genetically encoded voltage indicators (GEVIs), dubbed “VSFP” and “ASAP2s”. We also used voltage-sensitive dye, Di-4-Anepps. In the context of population voltage imaging (optical LFP), how do these 3 voltage indicators perform? In brain slices prepared from ASAP-transgenic or VSFP-transgenic mice, we performed multi-site optical imaging of evoked cortical depolarizations - compound excitatory postsynaptic potentials (cEPSPs). We analyzed the cEPSP voltage waveforms obtained with 3 voltage indicators. Optical signal amplitudes (dF/F) were compared using ANOVA followed by unpaired student’s t test (at least 19 data points per one voltage indicator). The ASAP2s signal amplitude (dF/F) was on average 4 times greater than di-4-Anepps, and 6 times greater than VSFP. The optical cEPSP decay (OFF rate) was the slowest in di-4-Anepps and fastest in ASAP2s. When ASAP2s expression is weak, we observe slow intrinsic optical signals

superimposed on the ASAP2s traces. Fast hyperpolarizations that typically follow depolarizing cortical transients were prominent in ASAP2s but obscured in the VSFP and di-4-Anepps experiments. Experimental applications for ASAP2s may potentially include certain systems neuroscience studies that require voltage indicators with large signal amplitude (dF/F), fast decay times (fast response time is needed for monitoring brain oscillations), and/or detection of brain patches in transiently hyperpolarized states (afterhyperpolarization).

Disclosures: S.D. Antic: None. **V.O. Ivanova:** None. **B.L. Barbeau:** None.

Poster

PSTR574. Optical Methodology: Application

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Program #/Poster #: PSTR574.07/WW71

Topic: I.04. Physiological Methods

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Swedish Research Council (2022-01620)
Knut & Allice Wallenberg Foundation (2021.0142)

Title: Voltage-seq: all-optical postsynaptic connectome-guided single cell transcriptomics

Authors: *V. CSILLAG, M. BIZZOZZERO HIRIART, J. NOBLE, B. REINIUS, J. FUZIK; Karolinska Institutet, Solna, Sweden

Abstract: Understanding the routing of neuronal information requires the functional characterization of connections. Neuronal projections recruit large postsynaptic ensembles with distinct postsynaptic response types (PRTs). PRT is probed by low-throughput whole-cell electrophysiology and is not a selection criterion for single-cell RNA-sequencing (scRNA-seq). To overcome these limitations and target neurons based on specific PRTs for soma harvesting and subsequent scRNA-seq we created Voltage-Seq. To test our methodology, we established all-optical voltage imaging and recorded the PRT of 8347 mouse periaqueductal gray (PAG) neurons evoked by the optogenetic activation of ventromedial hypothalamic (VMH) terminals. PRTs were classified and spatially resolved in the entire VMH-PAG connectome. We built an on-site analysis named VoltView to navigate soma harvesting towards target PRTs guided by a classifier which used the VMH-PAG connectome database as a reference. We demonstrated the agility of Voltage-Seq in locating VMH-driven GABAergic neurons in the PAG, solely guided by the on-site classification in VoltView.

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Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.08/WW72

Topic: I.04. Physiological Methods

Title: Using CaMPARI2 to investigate neuronal activity in the mouse dorsal striatum during Pavlovian conditioning

Authors: *R. PASS, K. KURIMA, K. LIYANAGAMA, J. R. WICKENS;
Okinawa Inst. of Sci. and Technol., Onna, Okinawa, Japan

Abstract: Cognitive flexibility is fundamental to adaptive behaviour in response to changing environmental cues. Approaches to understanding the mechanisms through which pivotal brain regions like the striatum achieve this are rapidly progressing. The development of the permanently photoconverting calcium sensor, CaMPARI2 (Moeyaert, *et al* 2018), allows for more controlled interrogation of key neuronal populations at specific time periods during behavioural tasks. Only during user defined periods of exposure to 395-405 nm wavelength light concurrent with high calcium influx result in photoconversion from the green to red form. We present the first reported proof of principle employment of CaMPARI2 to identify active neurons during Pavlovian conditioning. Naive C57BL/6 males (n = 10) received bilateral dorsal striatum non-specific CaMPARI2 (AAV1, 5 & 9) injections and unilateral optic cannula implantation. Twenty-two sessions with 30 pairings of a 2 second audio cue (70db; CS) paired with soy milk reward (US) were conducted in 7 mice. During each trial a UV LED provided 10 seconds of 405 nm light spanning the baseline, cue, reward and post reward period. During the variable ITI there was no light exposure. Fiber optic recordings of green and red channels were conducted prior to conditioning (Day-1, D-1) then every 3 conditioning days. An increased red/green ratio was observed in all mice between D-1 and 9 before reaching a plateau, indicating conversion to the red form. No increase was observed in control mice (n=2) which received no conditioning. Post-mortem immunohistochemistry with FLAG (green form) and specific red CaMPARI2 antibodies confirmed photoconversion only in the implanted hemisphere of conditioned mice. These preliminary data indicate that CaMPARI12 was able to detect increased neural activity in the striatum during a 10 second period including cue presentation and reward. Further work is underway to tease out specific time intervals when neural activity is increased, and to identify the active cells. Limiting light exposure to discrete times will determine when activity is occurring during learning. Non-specific CaMPARI2 co-stained with other markers will elucidate active cell populations when optogenetically manipulating acetylcholine release, a key neurotransmitter modulating striatum-dependant cognitive flexibility.

Disclosures: **R. Pass:** None. **K. Kurima:** None. **K. Liyanagama:** None. **J.R. Wickens:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.09/WW73

Topic: I.04. Physiological Methods

Support: CIHR FDN-143209

Title: Open-source camera system for capture of mouse cortical activity and posture patterns in 3-dimension during stroke recovery

Authors: *T. L. FONG[1], H. HU[2], T. H. MURPHY[3];
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Abstract: Robust characterization of stroke is necessary to understand large-scale remapping of motor functions. Three elements are necessary for the proper characterization of stroke: 1) evaluation of functional circuitry, 2) quantification of movement in three dimensions, and 3) measurement of blood flow. Taking advantage of recent advancements in the open-source libcamera stack, we have developed a suite of tools for biological and behavior assessments using the Raspberry Pi. Currently, most published open-source imaging systems utilize RGB images generated from the onboard image signal processor (ISP) for analysis. Although the data may still hold biological meaning, the underlying ISP are not optimized for customized conditions such as the use of different lenses, filters, and lighting conditions. Our system has the capability to extract the raw unprocessed RGB (Bayer Pattern Data) for analysis of biological activity such as GCaMP6s and blood flow. Regarding movement, precise characterization requires 3D measurements using at least three distinct view angles to resolve posture ambiguities in cases such as visual occlusion. Therefore, our setup can join multiple Raspberry Pi through ethernet connections and GPIO pins to achieve near synchronous recordings using light pulses. Using our system, we have characterized widefield cortical remapping of green fluorescent tetO-GCaMP6s x CAMK tTA and red fluorescent Thy1-jRGECO mice that have received photothrombotic stroke while performing a water-reaching task. Lesion sizes were also quantified using the system with laser speckle contrast imaging. Reach trajectories and fine digit movement were captured using three front view cameras and processed by Deeplabcut for pose estimation followed by triangulation using Anipose. Overall, we present a system to robustly quantify widefield image signals and behaviors in 3D.

Disclosures: T.L. Fong: None. **H. Hu:** None. **T.H. Murphy:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

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Program #/Poster #: PSTR574.10/WW74

Topic: I.04. Physiological Methods

Support: U01EY033001
Glaucoma Research Foundation Schaffer Award
Knights Templar Eye Foundation Career Starter Award
Vision for Tomorrow

Title: Hyperreflective dots in Central Fovea visualized by visible light Optical Coherence Tomography Fibergraphy

Authors: *M. GRANNONICO[1], M. KRAUSE[2], B. P. TYLER[2], D. A. MILLER[3], W. FAN[3], M. LIU[1], R. K. KURANOV[3], H. F. ZHANG[3], P. A. NETLAND[2], X. LIU[1];
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Abstract: Optical coherence tomography (OCT) is a non-invasive imaging technique widely used in ophthalmology to visualize retinal layers and structures. The current clinical technology in OCT field is the near-infrared (NIR) OCT system, which often fails to achieve high axial resolution of anatomical features, limiting the visualization and quantification of subtle changes within retinal layers in disease condition. The recently developed visible light-OCT (vis-OCT) provides a higher resolution compared to the NIR-OCT, which can improve the diagnosis and management of diseases. To directly compare the performance of NIR-OCT and vis-OCT, we acquired vis-OCT images from the same patients immediately following NIR-OCT imaging. Vis-OCT volumes were used to generate fibergrams (vis-OCTF) in order to visualize individual retinal ganglion cell (RGC) axon bundles in the retinal nerve fiber layer (RNFL) around fovea for the first time in patients. Vis-OCT *en face* images consist of a 3 × 3 mm with 1.3-μm axial resolution, compared to the 9 × 9 mm NIR-OCT *en face* with 7.0-μm axial resolution. Interestingly, we observed hyperreflective dots, or bright spots, in central fovea in both NIR-OCT and vis-OCT b-scan images of the patients. The bright spots in central fovea are hypothesized to be linked to several age-related and pathological conditions. Side-by-side comparison revealed that vis-OCTF shows a clear image of the hyperreflective dots and the RGC axon bundles compared to the NIR-OCT. In conclusion, the high-resolution images generated by vis-OCT make possible an accurate characterization of the RNFL and the dots, which helps for a better understanding of ocular diseases in future studies.

Disclosures: M. Grannonico: None. **M. Krause:** None. **B.P. Tyler:** None. **D.A. Miller:** None. **W. Fan:** None. **M. Liu:** None. **R.K. Kuranov:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Opticent Health. **H.F. Zhang:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Opticent Health. **P.A. Netland:** None. **X. Liu:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.11/WW75

Topic: I.04. Physiological Methods

Support: NIH/NEI Grant DP1EY033975

Title: Temporal control of whole-brain GCaMP expression for long-term widefield imaging of cortical networks.

Authors: *L. MUSARAT, M. J. HIGLEY;
Neurosci., Yale Sch. of Med., New Haven, CT

Abstract: Genetically-encoded fluorescent reporters of neural activity are a standard tool for investigating cellular and network function at a range of spatial scales. Several groups have made use of transgenic mouse lines where the calcium indicator GCaMP6 is broadly expressed throughout the brain, although reports of pathophysiological activity as a consequence of transgene expression place limits on the utility of these models. The Allen Institute Ai162 line encodes GCaMP6s at the TIGRE locus, where expression is conditioned on the presence of Cre recombinase and can be suppressed via a Tet inactivator sequence and administration of doxycycline. Suppression of transgene expression during development might serve to ameliorate neural pathology, though the time course of GCaMP6s levels and practical consequences for in vivo imaging have not been explored. Here, we generated mice expressing GCaMP6s in glutamatergic neurons via a cross with the Slc17a7 (VGlut1)-Cre line. We examined a total of 66 male and female mice, divided into two major cohorts: the "On-Dox" group (n = 44) received oral doxycycline from in utero until P40, whereas the "Off-Dox" group (n = 22) received a regular chow diet until P40 and were then switched to a doxycycline diet. Histological analyses revealed minimal GCaMP6 expression at P40 for On-Dox mice that rapidly increased after cessation of doxycycline, reaching a plateau in ~15 days. Reinstatement of doxycycline drove a rapid loss of GCaMP6s expression within ~5 days. We carried out widefield, mesoscopic imaging across the cortical mantle in awake, head-fixed mice, finding that the signal-to-noise for measurements of spontaneous and visually-evoked neural activity tracked the histological expression data. Electrophysiological measurements of cortical activity further support the hypothesis that doxycycline-induced suppression of transgene expression can ameliorate pathophysiological activity patterns in experimental mice. Overall, our results demonstrate the utility of this strategy for rapid temporal control of GCaMP6s expression across development.

Disclosures: L. Musarat: None. **M.J. Higley:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.12/WW76

Topic: I.04. Physiological Methods

Title: High-resolution in vivo fluorescent imaging of neural activity in freely moving animals using multi-camera array microscope.

Authors: *X. YANG[1], A. BÈGUE[2], K. KIM[1], C. COOK[1], M. HARFOUCHE[2], K. ZHOU[3], S. XU[4], R. HORSTMEYER[1];
[1]Duke Univ., Durham, NC; [2]Ramona Optics Inc, Durham, NC; [3]Univ. of California, Berkeley, Berkeley, CA; [4]Duke university, durham, NC

Abstract: High-content imaging of *in vivo* model organisms, such as *C. elegans, Drosophila,* and zebrafish, is critical in this biological discovery process. Model organism imaging yields insights into developmental processes, disease progression, drug susceptibility, and fundamental questions within neuroscience. Of recent interest is the ability to jointly image organism behavior and obtain functional fluorescence measurements of neural activity, which has the potential to offer insights into neurodegenerative diseases (e.g., Parkinson's and Alzheimer's), lead to the discovery of new drugs for neurological disorders (e.g., anxiety), and elucidate key responses to toxins, to name a few applications. Their natural, continuous motion hinders the study of small organisms at high resolution. Current imaging methods either zoom out to capture larger areas at a lower resolution or zoom in to observe a few organisms at high resolution, both presenting limitations. For the first method, despite its utility in tracking two-dimensional moving organisms, the method lacks the precision to capture important phenotypic information or accurately register fluorescence. The alternative approach is to "zoom in" to observe just one or a few organisms at a time within a limited while organisms can easily move out of the restricted viewing area. To overcome the above challenges, we have developed a multi-camera array microscope (MCAM) platform that employs 54 compact lenses and sensors (6x9 array) to synchronously image across a macroscopic area (8x12 cm) at high resolution (4 μm/pixel) to produce videos with nearly 1 gigapixel per image frame. The MCAM is also capable of 3D video recording via a stereoscopic capture and processing strategy that utilizes multiple imagers and records ratiometric fluorescence imaging with suitable excitation and emission filters. This work examines several strategies to apply our novel MCAM architecture to jointly record the behavior and fluorescent neural activity of unconstrained *Drosophila* and zebrafish at near-cellular resolution in 3D. We apply a ratiometric processing strategy to account for the impact of motion and depth by normalizing time-varying GCaMP activity traces with a constant recorded RFP signal. Automated machine learning-based organism tracking, alignment, and behavioral classification are jointly applied to recordings of motor activity and freely moving behaviors within collections of both organisms during unique behaviors. These preliminary experiments demonstrate the MCAM's ability to capture fluorescent neural activity at high resolution across multiple organisms during free movement.

Disclosures: X. yang: None. **A. Bègue:** None. **K. Kim:** None. **C. Cook:** None. **M. Harfouche:** None. **K. Zhou:** None. **S. Xu:** None. **R. Horstmeyer:** A. Employment/Salary (full or part-time):; Ramona Optics Inc.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.13/WW77

Topic: I.04. Physiological Methods

Title: Miniscope-based blood vessel imaging enables a novel assay for migraine therapeutic concept testing

Authors: *K. ZITELLI[1], Z. BALEWSKI[1], P. BOTTA[2], A. A. R. ASUNI[2], B. HALL[2], D. OLLERENSHAW[1], J. NASSI[1];
[1]Inscopix, Inc., Mountain View, CA; [2]H Lundbeck A/S, Copenhagen, Denmark

Abstract: Vascular dynamics play an important role in brain health and CNS pathologies (migraine, stroke, ischemia). The nVue miniscope platform enables simultaneous neuronal calcium imaging alongside visualization of blood vessels and permits longitudinal measurements of vessel diameter and/or red blood cell velocity. Here, we present a newly-developed algorithm that facilitates accurate vessel diameter measurements for a multitude of vessel sizes. Together with our collaborators at Lundbeck, we have applied the capabilities of this platform towards creating an *in vivo* assay for screening therapeutic compounds using preclinical migraine-like hypersensitivity models. The middle meningeal artery (MMA) is a dural blood vessel that is implicated in migraine pathology in patients - dilation of this vessel is often associated with the onset of a migraine episode. In mice, we enabled optical access to the MMA and its secondary and tertiary branches by performing a craniotomy over the vessel and installing a coverslip, creating a cranial window. We then installed a baseplate over the window for docking the miniscope. To visualize the MMA and surrounding vessels with the miniscope, fluorescein isothiocyanate dextran was injected via the tail vein. Levcromakalim, a drug known to elicit migraine attacks in patients, resulted in significant dilation of the MMA compared to vehicle controls, consistent with predicted migraine pathology. Caffeine, administered as a negative control to induce a vasoconstriction response, actually also resulted in significant MMA dilation after a brief period of vasoconstriction, suggesting a more complex effect of caffeine than previously thought. These methods lay the groundwork for a miniscope-based assay for migraine therapeutic efficacy assessments. We will continue to use these methods to evaluate other potential mechanisms and therapeutic targets relevant to migraine pathophysiology, such as CGRP and nitroglycerin, as well as to assess standard-of-care migraine treatments and novel treatment strategies.

Disclosures: **K. Zitelli:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **Z. Balewski:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **P. Botta:** A. Employment/Salary (full or part-time):; H Lundbeck A/S. **A.A.R. Asuni:** A. Employment/Salary (full or part-time):; H Lundbeck A/S. **B. Hall:** A. Employment/Salary (full or part-time):; H Lundbeck A/S. **D. Ollerenshaw:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **J. Nassi:** A. Employment/Salary (full or part-time):; Inscopix, Inc..

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

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Program #/Poster #: PSTR574.14/WW78

Topic: I.04. Physiological Methods

Support: BBSRC Grant BB/V000411/1
NC3Rs PhD studentship NC/R001421/1
NC3Rs Grant NC/W00092X/1

Title: Functional whole brain imaging in the larval zebrafish as a novel approach for profiling neuroactive chemical action

Authors: *M. J. WINTER[1], J. PINION[1], A. PILEHVAR[1], M. GOODFELLOW[2], A. D. RANDALL[3], A. TAKESONO[1], T. KUDOH[1], L. U. SNEDDON[4], C. R. TYLER[1];
[1]Biosci., [2]Mathematics, [3]Med., Univ. of Exeter, Exeter, United Kingdom; [4]Biol. and environmental sciences, Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Genetically encoded Ca^{2+} sensor transgenic zebrafish combined with fluorescence light-sheet microscopy (FLSM) affords a powerful approach for assessing the effect of neuroactive chemicals on the CNS of a relevant vertebrate animal model. Importantly, embryo-larval zebrafish are transparent, have high throughput amenability and an accessible CNS containing around 100000 neurons. Collectively these features allow assessment of regional changes in neuronal activity across the whole brain, as well as alterations in functional connectivity after chemical treatment, which can be used to assess new drug safety liabilities, novel mechanisms of action, and comparative levels of chemical efficacy. Our experimental approach employs 4dpf zebrafish with pan-neuronal expression of GCaMP6s (*elavl3:GCaMP6s* courtesy of Misha Ahrens, Janelia, VA), FLSMs and a custom Python-Matlab image analysis pipeline. We have used this workflow to investigate the effects of multiple chemicals across a range of mechanisms of action (MOAs) including those associated with seizure liability in mammals, and those proposed for use as anaesthetics in fish. Our data show that as early as 4dpf, the larval zebrafish brain is highly responsive to a wide range of neuroactive chemicals including those acting upon NMDA, $GABA_A$ and glycine receptors, along with acetylcholinesterase and monoamine transporters. In addition, brain region-specific analysis of activity has provided insight into the initiation of drug-induced neuronal hyperexcitation, and the efficacy of anaesthetics operating via different primary MOAs. We are continuing to refine our approach to improve specificity and sensitivity, for example by investigating the use of artificial intelligence for individual voxel-based image analysis. The higher throughput amenability compared to traditional rodent based approaches for assessing CNS safety or efficacy, mean that this approach can be deployed at a relatively early stage in drug discovery and development, affording early warning of potential CNS-associated safety labilities, or providing early *in vivo* target validation data to support investment decisions. The use of non-protected 4dpf animals also means there are ethical advantages compared with using invasive approaches in protected rats and mice.

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Poster

PSTR574. Optical Methodology: Application

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Program #/Poster #: PSTR574.15/WW79

Topic: I.04. Physiological Methods

Support: NIH Grant U01NS120820

Title: Employing an improved iSeroSnFR biosensor to track serotonin release in cortical and subcortical circuitry during acute vs. chronic SSRI administration

Authors: *K. R. LONG-IYER[1], E. C. WRIGHT[1], R. R. DALANGIN[2], Y. HUA[1], L. TIAN[1]; [1]Biochem. & Mol. Med., Univ. of California, Davis, Davis, CA; [2]CERVO Brain Res. Ctr., Ville de Québec, QC, Canada

Abstract: Serotonin (5-HT) has been linked to a large array of behavioral and affective states, yet technological limitations have historically constrained interrogation of this circuitry. Originating from the raphe nuclei, the 5-HT system exhibits distinct projections to both cortical and subcortical networks, engaging in widespread communication with downstream targets. Notably, the orbitofrontal cortex (OFC) assumes a pivotal role in top-down modulation of learning, while a separate pathway showcases prominent 5-HT projections to the bed nucleus of the stria terminalis (BNST), influencing anxiety circuitry. In this study, we employed our lab's improved iSeroSnFR fluorescent serotonin biosensor, coupled with in vivo fiber photometry, to characterize endogenous 5-HT release in both OFC and BNST during Pavlovian aversive learning. Additionally, we examined the effects of acute (1 day) and chronic (28 days) oral administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine on 5-HT release in these regions, alongside behavioral measures of aversive learning. Our results indicate that acute fluoxetine treatment enhances 5-HT release in both OFC and BNST to the conditioned stimulus, a tone cue, enhancing aversive learning acquisition. Chronic SSRI treatment caused a mild increase of cue-triggered 5HT release compared to no drug control but less so than acutely treated mice. Our findings contribute to a more thorough understanding of therapeutic interventions that systemically target 5-HT circuitry, elucidating region-specific drug effects on neurochemical release.

Disclosures: **K.R. Long-Iyer:** None. **E.C. Wright:** None. **R.R. Dalangin:** None. **Y. Hua:** None. **L. Tian:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.16

Topic: I.04. Physiological Methods

Support: NIH RO1 (5R01MH129732-02, A-CM)

Title: A pipeline for super-resolution STED imaging and IMARIS analysis of nanoscale synapse organization in cortical brain slices

Authors: *E. KRUZICH[1], R. PHADKE[2], A. BRACK[2], I. PICARD[3], D. STROUMBAKIS[3], A. CRUZ-MARTIN[1];
[1]Neurobio., [2]Mol. and Cell. Biol. and Biochem., [3]Biol., Boston Univ., Boston, MA

Abstract: Recent technological advances in the ability to image molecules in biological specimens have accelerated our understanding of the molecular mechanisms of neuronal function in the healthy and diseased brain. Among these techniques, super-resolution imaging methods have emerged as invaluable tools for studying the intricate details of neuronal structures. We present a comprehensive pipeline for the imaging and analysis of various structures and immunochemical stains in brain slices, utilizing the powerful combination of super-resolution STED microscopy and IMARIS software. We argue that super-resolution imaging in brain slices is an optimal approach because it allows for studying the molecular determinants of physiologically relevant neuronal circuits. By employing STED microscopy in conjunction with IMARIS, we demonstrate a highly accessible approach to unraveling the nanoscale organization of synaptic proteins. Our pipeline begins with STED optimized preparation of mouse brain tissue, followed by imaging methods that enable us to visualize subcellular structures with unprecedented detail. Here, we focus on labeling AMPA receptor subunits and endosomal markers to characterize the trafficking processes within dendritic spines and determine the morphology of various neuronal and glial structures in L1 apical tufts of the medial prefrontal cortex, a neuronal circuit that underlies cognitive processes such as associative learning and attention. Next, we analyze STED images using IMARIS. This powerful 3D image analysis software offers a wide range of tools, including colocalization analysis, object segmentation, and protein density and distribution quantification. We also utilize native IMARIS functions and downloadable extensions paired with custom MATLAB code to determine the subcellular localization of specific proteins in areas including the dendritic shaft, spine head, and spine neck. At each step of the pipeline, we describe potential problems paired with proposed solutions. We aim to make the meticulous examination of the synaptic protein landscape a more accessible endeavor, believing that elucidating the intricate molecular determinants underlying synaptic dysfunction will set the foundation for developing effective therapies.

Disclosures: **E. Kruzich:** None. **R. Phadke:** None. **A. Brack:** None. **I. Picard:** None. **D. Stroumbakis:** None. **A. Cruz-Martin:** A. Employment/Salary (full or part-time):; Molecular and Cellular Biology and Biochemistry, 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 Network Systems Biology,

Boston University, Boston, MA, USA, Department of Pharmacology and Experimental Therapeutics, Boston University, Boston, MA, USA.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.17/WW80

Topic: I.04. Physiological Methods

Title: Near infrared nanosensors to study dopamine neuromodulation across broad spatial scales

Authors: *C. BULUMULLA[1], A. KRASLEY[1], D. ZHANG[1], A. BEYENE[2];
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Abstract: We will describe how an optical dopamine biosensor developed from functionalized single wall carbon nanotubes (SWCNT) can be flexibly deployed to enable measurements of dopamine release at several spatial scales. At the synaptic scale, we demonstrate that the technology can assay the release and spatial propagation of dopamine from a single bouton, with quantal sensitivity. At the scale of neuronal processes, the nanosensors enable measurement of dopamine release simultaneously from hundreds of release sites that are spread across single or multi-neuronal axonal arbors of isolated neurons. Additionally, the nanosensors enable measurement of dopamine release in brain slices as well as *in vivo*. For each spatial domain over which the tool is deployed, we will present data that demonstrates the utility of the tool and highlight novel biological insights obtained from imaging in these domains. Finally, we show that we can take advantage of the unique near-infrared and short-wave infrared emission spectrum (900 - 1400 nm) of SWCNTs to enable dual color imaging via multiplexing with existing sensors that operate in the visible region. Using this approach, we investigate the co-release of glutamate and dopamine from a subset of dopamine neurons that are immunoreactive for the vesicular transporter VGLUT2.

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Poster

PSTR574. Optical Methodology: Application

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.18/WW81

Topic: I.04. Physiological Methods

Support: ERC Starting Grant

Title: Assessment of enteric morphology for guided design of in vivo micro-endoscope

Authors: A. PLANCHETTE, I. GANTAR, J. SCHOLLER, N. MOJTAHEDI, S. BARALE, Y. CABRARA, S. PAGES, A. SOBOLEWSKI, *T. LAABS, G. BARTHET, M. GORA;
Wyss Ctr. for Bio and Neuroengineering, Geneva, Switzerland

Abstract: The involvement of the enteric nervous system (ENS) in regulating health and its relevance to digestive and neurological disorders is widely recognized. This organ system's capacity to regulate relatively distant neurological networks provides an opportunity for new therapeutic avenues, such as secondary neuromodulation by primarily targeting the ENS. However, dynamic in-vivo studies of ENS function are hindered by the lack of advanced tools, whose design is dependent on a well-characterized three-dimensional architecture. Driven by our end-goal to develop an intraluminal in-vivo ENS micro-endoscope with neuromodulation capabilities, we applied state-of-the-art 3D microscopy to characterize relevant structural and functional components of the ENS in healthy mouse and human colon tissues. Key measurable parameters included the cell count, density and spatial distribution of the enteric network within the layers of the gut and longitudinally, nearest distance from the lumen to critical layers of the ENS and more. By applying 2D and 3D segmentation methods, we isolated and quantified signals of interest. In healthy mouse colon, we measured a ratio of HuC/D-positive neurons at 0.31% relative to tissue volume. Per mm3 of colon, 1160 neuronal cells are counted with 2136mm3 median volume per cell. To estimate the distance of structures the micro-endoscope will target, we segmented neurons labelled with Tuj1 and Synapsin 1 in co-stained samples and located the myenteric plexus at a depth of 300mm from the epithelial lining for both labels. The sensory neurons that make up most of the mucosal network were situated in the range 50-70 mm in depth. In addition to elucidating ENS architecture, this method can be combined with the observation of cell types from other physiological systems known to interact with the ENS, such as the immunological compartment. For example, when comparing the CD3-positive cell population in a DSS-induced inflammatory condition versus healthy control, we observed a 48% decrease in number of cells per mm3 and a 35% increase in the size of cell clusters distributed throughout the tissue. With these capabilities, we showcase the usefulness of volumetric quantification of ENS architecture that can be relevant for physiological investigations and for guiding the development of novel research tools.

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Poster

PSTR574. Optical Methodology: Application

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Program #/Poster #: PSTR574.19/WW82

Topic: I.04. Physiological Methods

Support: U19NS128613
R01NS078168
R01NS101353

Title: Investigating the drivers and mechanics of brain motion in behaving mice

Authors: *S. GARBORG[1], Q. ZHANG[2], K. TURNER[8], J. M. RICOTTA[3], N. FRANK[4], M. MOSTAFA[5], F. COSTANZO[1,2,6], P. J. DREW[1,2,7];
[1]Dept. of Biomed. Engin., [2]Dept. of Engin. Sci. and Mechanics, [3]Dept. of Kinesiology, [4]Dept. of Mechanical Engin., [5]Dept. of Biol., [6]Ctr. for Neural Engin., [7]Departments of Biol. and Neurosurg., Pennsylvania State Univ., University Park, PA; [8]Brown Univ., Pawtucket, RI

Abstract: The brain moves within the skull during many behaviors, but the drivers and physiological impact of this phenomenon are unknown. Brain motion is a ubiquitous confound for imaging in both humans and animals, and brain motion may also help remove metabolic waste by displacing and mixing cerebrospinal fluid in the subarachnoid space as the brain shifts within the skull. We visualized brain motion relative to the skull in awake head-fixed mice freely behaving on a spherical treadmill using high-speed, multi-plane two-photon microscopy. Brain motion was directed and stereotyped, and was tightly correlated with locomotion, but not respiration or cardiac pulsations. Electromyography electrodes implanted into the abdominal muscles further revealed an even stronger correlation with observed rostral-lateral brain shift within the skull. Passive pressure application to the abdomen of anesthetized mice drove brain motion of similar magnitude and direction as those observed during locomotion. Our results provide evidence of a mechanical system that links intra-abdominal pressure changes and brain motion within the skull, suggesting that the abdomen may influence brain health.

Disclosures: S. Garborg: None. **Q. Zhang:** None. **K. Turner:** None. **J.M. Ricotta:** None. **N. Frank:** None. **M. Mostafa:** None. **F. Costanzo:** None. **P.J. Drew:** None.

Poster

PSTR574. Optical Methodology: Application

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Program #/Poster #: PSTR574.20/WW83

Topic: I.08. Methods to Modulate Neural Activity

Support: Brain Initiative grant RF1NS113287
Brain Initiative grant RF1NS1260441

Title: Micro camera arrays for ultra-wide multi-site cellular resolution calcium imaging across the dorsal cortex of behaving mice.

Authors: J. HU[1], ***A. CHERKKIL**[1], I. OLADEPO[1], Z. VIAVATTINE[1], S. FAUSNER[2], V. PATHAK[2], R. HOSSAIN[1], K. SAXENA[1], R. HORSTMEYER[2], S. B. KODANDARAMAIAH[1]; [1]Univ. of Minnesota, Minneapolis, MN; [2]Duke Univ., Durham, NC

Abstract: Recording the activities of individual neurons distributed across multiple brain regions can provide new insights into the brain's functions and animal behaviors. Over the last few years, a number of miniaturized devices have been developed to image the neuronal activities in small fields of view in freely behaving animals. Scaling the devices to image larger FOVs results in larger objectives and correspondingly large cameras that are difficult to be miniaturized for studying freely locomoting and behaving animals. Furthermore, such devices need to contend with the complex three-dimensional surface of the brain. We have engineered miniaturized micro-camera array microscopes (mini-MCAM) and large-FOV micro-camera array microscopes (L-FOV MCAM) which have two innovations to enable calcium imaging at cellular resolution across the dorsal cortical surface. First, we engineered a multi-plane transparent skull implant with 4 transparent windows to open up 42mm^2 of the dorsal cortical surface for imaging. This area encompasses most of the bilateral primary and secondary motor cortices, much of the somatosensory cortex areas, the retrosplenial, association, higher visual, and parts of the primary visual cortex. We next built an array of fluorescent micro-cameras to image each of the four planes defined by the windows. The mini-MCAM offers a FOV of up to 11 mm² per camera with a peak resolution of 9.9 µm. Meanwhile, the L-FOV MCAM extends the FOV to 38mm² per camera with a peak resolution of 13.4µm. We imaged neuronal activity using both MCAMs in head-fixed Ai163 x Cux2-creERT2 transgenic mice that sparsely expressed GCaMP6s in layers 2-3 pyramidal neurons in the cortex. We present calcium trace activity and preliminary analysis of spontaneous brain activations in both head-fixed and freely behaving cases for the mini-MCAM system. We showcase preliminary results for spontaneous head-fixed calcium imaging performed using the LFOV-MCAM. This lineup of MCAM technologies could potentially allow neuroscientists to do high-resolution wide-field imaging in behaving mice, opening up capabilities to study neural activity underlying rich behaviors.

Disclosures: J. Hu: None. **A. Cherkkil:** None. **I. Oladepo:** None. **Z. Viavattine:** None. **S. Fausner:** None. **V. Pathak:** None. **R. Hossain:** None. **K. Saxena:** None. **R. Horstmeyer:** None. **S.B. Kodandaramaiah:** Other; Objective Biotechnology Inc., Co-Founder.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.21/XX1

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Margaret Milam McDermott Foundation to Thomas Floyd, MD

Title: Intrinsic biological (HIF-1a, HIF-2a) versus extrinsic chemical indicators of cellular hypoxia

Authors: *H.-K. WU, B. SNYDER, T. F. FLOYD;
Anesthesiol., UTSW (UT Southwestern), Dallas, TX

Abstract: Many studies investigate protective or injurious effects of hypoxia on neural activity, but a standard to measure and directly compare hypoxic values across studies is lacking. Defining cellular hypoxia in cerebral cell cultures is necessary to compare functional activity since brain cells typically reside at oxygen (O_2) concentrations between 3-6% *in vivo* but are cultured at 18-21%. This suggests discrepancies may exist between *in vitro* and *in vivo* experiments. In addition to intrinsic oxygen-sensitive proteins, such as Hypoxia-Inducible Factors-alpha (HIF-1a & HIF-2a), commercially available products have all been used as hypoxia indicators. We hypothesized that both HIFa proteins and a commercially available product, Image-iT Green hypoxia reagent (ITGHR-dye), would be equivalent indicators of hypoxia in a neuron-glial bi-culture. Primary rat hippocampal neurons and astrocytes were co-cultured on coverslips for 13 days at standard conditions. On day 14, cultures were subjected to hypoxia within hypoxic chambers (XVivo System 3, Biospherix) by gradually decreasing O_2 levels from 21% to 10%, 5%, 2.5%, or 1% for 6 hours each. To investigate commercially available ITGHR-dye staining, 85% confluent cultures at each level were treated with 2μM green fluorescent dye for 30 minutes, then rinsed and fixed with 4% paraformaldehyde (PFA) and coverslipped. ICC for HIFa proteins was performed on fixed cells using antibodies for HIF-1a conjugated to Dylight 650 or HIF-2a conjugated to Dylight 550 overnight, followed by DAPI staining. Five 20x images were acquired from each replicate using a Leica DMi8 fluorescence microscope with LAS X software at channels for FITC, Cy3, and Cy5. Results within each culture were averaged and analyzed with GraphPad Prism (v. 9). Intrinsic proteins HIF-1a and HIF-2a showed stabilization at 10% O_2, with their expression increasing as O_2 levels decreased. Similarly, ITGHR-dye exhibited the same expression pattern, although the slope between 10% and 1% O_2 was much steeper for ITGHR-dye compared to HIF-1a or HIF-2a. We compared relative fold changes for HIFa and ITGHR-dye to various O_2 levels when values of 21% O_2 were normalized. On average, HIF-1a intensity was 8-fold higher at 1% O_2 compared to 21% and HIF-2a was 12-fold higher, whereas ITGHR-dye intensity increased by 50-fold (vs. HIF-2a, $p=0.0336$; vs. HIF-1a, $p=0.0067$) at all hypoxic stages. These results indicate that both intrinsic and commercial probes can be used as hypoxic indicators. There is a need to define and standardize functional *in vitro* hypoxia experiments to better model and understand basal *in vivo* mechanisms.

Disclosures: H. Wu: None. **B. Snyder:** None. **T.F. Floyd:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.22/XX2

Topic: I.08. Methods to Modulate Neural Activity

Support: R01 NS12366
R21 EY02945

Title: Illumination methods for voltage imaging with a microLED light source

Authors: *F. SPEED[1], V. KUMAR[2], C. SALADRIGAS[3], S. VIEAU[5], I. KYMISSIS[2], J. GOPINATH[3], V. BRIGHT[4], C. WELLE[5], D. RESTREPO[6], E. GIBSON[7];
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Abstract: Voltage imaging enables the direct recording of individual membrane potentials across large populations of neurons in vivo. However, applications of in vivo voltage imaging are still limited to experiments with head fixed mice, as miniature microscopes are unable to reach the frame rates required for this modality to be used with freely moving animals. To reach this goal, a microLED light source was fabricated that can provide patterned excitation for Structured Illumination Microscopy (SIM). This poster highlights data obtained with this source and the the next steps for implementation with genetically encoded voltage indicators.

Disclosures: F. Speed: None. **V. Kumar:** None. **C. Saladrigas:** None. **S. vieau:** None. **I. Kymissis:** None. **J. Gopinath:** None. **V. Bright:** None. **C. Welle:** None. **D. Restrepo:** None. **E. Gibson:** None.

Poster

PSTR574. Optical Methodology: Application

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR574.23/XX3

Topic: I.08. Methods to Modulate Neural Activity

Support: TKP2021-EGA-42

Title: Acousto voltage imaging in awake mouse with JEDI-2P

Authors: *D. PALFI[1], B. CHIOVINI[2], K. ÓCSAI[3,4], A. MIHÁLY[1], Z. MEZRICZKY[1], G. KATONA[1], B. ROZSA[5,4];
[1]Peter Pazmany Catholic Univ., Budapest, Hungary; [2]Inst. of Exptl. Med. Hungarian Acad. of Sci., Budapest, Hungary; [3]Dept. of Mathematical Geometry, Inst. of Mathematics, Univ. of Technol. and Econ., Budapest, Hungary; [4]Brain Vision Ctr., Budapest, Hungary; [5]Inst.of Exptl. Med., Budapest, Hungary

Abstract: Functional two-photon imaging is a great tool for understanding the activity of neurons in vivo preferably from multiple cortical layers. The technique provides great spatial resolution with low temporal resolution. On the other hand, electrophysiological recording give

high temporal resolution but just in a few points in the brain. To understand the direct communication among neurons, imaging their potential changes is necessary. However, calcium imaging is very popular, it is an indirect tool to detect membrane potential changes. Voltage indicators which are fast dynamic direct tools for monitoring, are quite new in two-photon functional imaging. JEDI-2P is an excellent candidate achieving this goal. Combinating fast acousto-imaging technique with modern voltage indicator grant us to monitor activity of dozens of cells even on dendritic level. Here, I present different applications which demonstrates the power of direct imaging. With ultrahigh temporal resolution even subthreshold signals can be followed during dendritic processes. In vitro and in vivo 3D measurement from multiple cells with up to 50 kHz temporal resolution, by using novel acousto-optical scanning techniques and JEDI-2P indicator. These novel tools can revolutionize our knowledge of neuronal computation science.

Disclosures: D. Palfi: None. **B. Chiovini:** None. **K. Ócsai:** None. **A. Mihály:** None. **Z. Mezriczky:** None. **G. Katona:** None. **B. Rozsa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Femtonics Ltd..

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.01/XX4

Topic: I.04. Physiological Methods

Support: NIH Supplement to Promote Diversity in Health-Related Research to WAT (MH122935-01S1)
GRFP: National Science Foundation - Wendy Torrens
NIMH R15MH122935 to SMH and MEB

Title: The effect of hair type on Electroencephalography: Testing a new clip

Authors: *W. A. TORRENS[1], R. B. YANKAWAY[2], M. RUIZ[2], S. M. HAIGH[3];
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Abstract: Electroencephalography (EEG) is abundantly used in research and applied settings because it has excellent temporal resolution for measuring real-time neural signals, is portable and is inexpensive and non-invasive. EEG is also the standard method used in the diagnosis of several neurological disorders (e.g., epilepsy). However, current EEG methods are inadequate for data collection with coarse and curly hair types. EEG requires direct contact between the electrode and the scalp to detect neural signals. Caps are typically used to hold the electrodes in place to ensure that the same electrode is held securely in the same location. Caps, however, are

not compatible with coarse and curly hair textures because the electrode sits over the hair, not the scalp. This creates a fundamental racial bias in EEG methods. Here, we test clips that we developed to combat the issue of poor electrode-to-scalp contact to collect EEG data across hair types with a simple hair-parting technique. EEG data were collected from 5 individuals: 2 of type 1 hair (very straight), 2 of type 3 hair (wavy), and 1 of type 8 hair (coily). Participants listened to auditory clicks presented at 4 Hz while EEG data were collected using a BioSemi system. The 4Hz clicks generated a steady-state response with high signal-to-noise that can easily be detected at the level of the individual. These data were collected once with the clips and again with the traditional EEG cap. Data from the Fz electrode were processed in MATLAB and run through a Fast Fourier Transform to calculate the power at 4Hz and its harmonics. The root-mean-square signal-to-noise ratios (SNRs) were calculated for the 4Hz signal and the following 4 harmonics for the cap and the clip data separately. For hair type 1, there was no difference in measured power between the traditional cap and the clips. However, we observed a 72% increase in signal power for hair type 3 and a 500% increase in signal power for hair type 8 with the clips compared to the cap. These findings suggest that the clips have the potential to correct racial bias in current EEG methods by ensuring that high SNR is recorded regardless of hair type.

Disclosures: **W.A. Torrens:** None. **R.B. Yankaway:** None. **M. Ruiz:** None. **S.M. Haigh:** None.

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.02/XX5

Topic: I.04. Physiological Methods

Support: EPSRC Grant EP/T020970/1

Title: Development of an electroencephalography device with conductive elastomer electrodes

Authors: *N. STEENBERGEN, B. TAHIRBEGI, J. GODING, R. GREEN;
Imperial Col. London, London, United Kingdom

Abstract: Clinical electroencephalography (EEG), and particularly continuous EEG (cEEG) is underused due to various cost and logistical hurdles. This is mainly due to the use of wet electrodes, which are cumbersome to apply and dry out in 24 hours. 3D dry electrodes that penetrate through hair remove the barrier to clinical implementation. To achieve the optimal blend of electrical and mechanical properties, conductive elastomer electrodes (CEEs) have been investigated as a potential dry electrode material. PEDOT-based CEEs are a point of focus for thin-film electrodes, but few have been adapted into 3D structures. Those that have been produced have various pin structures (pin heights of 100μm-1cm, pin diameters of 100μm-3mm, pin spacings of 200μm-3mm, and electrode diameters of 500μm-3cm) with no parameter evaluation. This study is thus an exploratory investigation of the development of a high-end EEG

device using dry CEEs to lower the time resource burden of current clinical EEG systems. It was hypothesised that dry PEDOT-based CEEs have the optimal blend of electrical and mechanical properties to provide viable signal through hair whilst maintaining comfort.
The aim of this work is to produce a long-term dry EEG headcap with similar function to existing clinical EEG. This was achieved by determining optimal electrode parameters via electromechanical and electrochemical testing on an agar model and in different hair types. Then, the dry EEG headcap was tested against standard Ag/AgCl electrodes in a 30-minute study analysing somatosensory event-related potentials and participant satisfaction.
3D CEEs behaved similarly to their dry flat-film counterparts and Ag/AgCl control electrodes in PBS solution and on agar (at 100 Hz, 96.3 ± 4.5 Ω, 70.1 ± 5.9 Ω, and 101.1 ± 21.2Ω, respectively), indicating CEE functionality in a 3D pin structure. Then, wet and dry electromechanical testing was performed on electrodes with pin heights of 2-10mm, pin diameters of 1-3mm, pin spacings of 1-3mm, and electrode diameters of 1-2.5cm to confirm which parameter combinations worked best in different hair types. Here, an optimal candidate was found for each hair type. The resulting dry EEG headcap performed similarly to conventional Ag/AgCl electrodes with a slightly lower SNR but similar time-domain and frequency-domain features. The dry headcap was also quicker to apply and preferred by the participants.
These results are a promising platform for the development and soft and dry electrodes. Translation of this technology could aid in the more widespread adoption of clinical EEG.

Disclosures: **N. Steenbergen:** None. **B. Tahirbegi:** None. **J. Goding:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Polymer Bionics Ltd. **R. Green:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Polymer Bionics Ltd.

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.03/XX6

Topic: I.04. Physiological Methods

Support: NeuroOne Medical Technologies

Title: Evaluation of a new stereoelectroencephalography-guided radiofrequency ablation system in an in vivo swine model

Authors: C. DIAZ-BOTIA[1], M. VOMERO[2], **M. PORTO CRUZ**[3], M. MCNEIL[1], S. ONG[1], D. KRIDNER[3], S. MERTENS[3], H. HARIS[3], D. ROSA[3], R. E. GROSS[5], A. VISHWADEEP[6], G. A. WORRELL[7], J. VAN GOMPEL[7], S.-Y. CHANG[7], I. KIM[7], F. MIVALT[7], *A. KULLMANN[4]; [1]NeuroOne, Los Gatos, CA; [2]NeuroOne, New York, NY; [3]NeuroOne, Eden Prairie, MN;

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Abstract: Stereoelectroencephalography (sEEG)-guided radiofrequency (RF) ablation is a procedure in which sEEG electrodes implanted for seizure evaluation are used for tissue lesioning. This is accomplished by delivering electric current at high frequency (above 250 kHz), to raise the temperature between the active contacts sufficiently to destroy the epileptogenic tissue. The procedure has been shown to reduce seizure frequency with minimal risks. Presently, the temperature at which sEEG-guided RF ablations are performed cannot be monitored. This study evaluates a new RF ablation system, which uses FDA-cleared sEEG electrodes equipped with a unique temperature control accessory designed to monitor and maintain the temperature at which ablations are performed, in an in vivo swine model. sEEG electrodes (n=13) were implanted into the brain of two pigs. In one animal sEEG positioning was verified using computer tomography imaging. RF energy was delivered for specific durations (30-300 s) and temperatures (50-90°C) in monopolar and bipolar (between two adjacent sEEG contacts) configurations. For most lesions, size, evaluated from the MRI scans, was proportional to temperature and time. Histological examination of tissue showed a necrotic center surrounded by a ring of pallor composed of neuropil vacuolation and intramyelinic edema (n=4 ablations). The adjacent neural tissue was intact. Comparison of sEEG impedance pre- and post-ablation indicated that continuation of electrophysiological recordings is feasible. In summary, this innovative sEEG-guided RF ablation system delivered clinically relevant RF energy to ablate porcine brain tissue in vivo. The system offers several advantages, including ability to precisely monitor and regulate temperature during the ablation, ability to continue intracranial sEEG recordings pre- and post- ablation, and potential to reduce the number of surgical procedures by providing treatment using the same electrodes used for diagnostic purposes.

Disclosures: C. Diaz-Botia: A. Employment/Salary (full or part-time):; NeuroOne. **M. Vomero:** A. Employment/Salary (full or part-time):; NeuroOne. **M. Porto Cruz:** A. Employment/Salary (full or part-time):; NeuroOne. **M. McNeil:** A. Employment/Salary (full or part-time):; NeuroOne. **S. Ong:** A. Employment/Salary (full or part-time):; NeuroOne. **D. Kridner:** F. Consulting Fees (e.g., advisory boards); NeuroOne. **S. Mertens:** A. Employment/Salary (full or part-time):; NeuroOne. **H. Haris:** A. Employment/Salary (full or part-time):; NeuroOne. **D. Rosa:** A. Employment/Salary (full or part-time):; NeuroOne. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne. **R.E. Gross:** F. Consulting Fees (e.g., advisory boards); NeuroOne. **A. Vishwadeep:** None. **G.A. Worrell:** None. **J. Van Gompel:** F. Consulting Fees (e.g., advisory boards); NeuroOne. **S. Chang:** None. **I. Kim:** None. **F. Mivalt:** None. **A. Kullmann:** A. Employment/Salary (full or part-time):; NeuroOne.

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.04/XX7

Topic: I.04. Physiological Methods

Support: AMED Grant 22be1004203h0001.
collaborative project with Sony semiconductor solutions Inc.

Title: A novel field potential imaging technology for analyzing neural electrical function at a single-neuron resolution using a large area CMOS-MEA with 236,880 electrodes

Authors: *N. MATSUDA, X. HAN, N. NAGAFUKU, I. SUZUKI;
Electronics, Tohoku Inst. of Tehchnology, Sendai, Japan

Abstract: The technology for measuring the electrical activity of the nervous system is essential for understanding higher functions and neurological diseases, drug discovery development, and toxicity evaluation of compounds. In the recent years, microelectrode array (MEA) technology has been largely developed for electrical measurement of neural activities. Especially MEAs with large amounts of electrodes at a high density provides a high spatio-temporal resolution at the single-cell level, and noninvasive measurements of large areas which increase insights on underlying neuronal function. In the present study, we used a complementary metal-oxide semiconductor (CMOS)-microelectrode array (MEA) that uses 236,880 electrodes each with an electrode size of 11.22 × 11.22 μm and 236,880 covering a wide area of 5.5 × 5.7 mm in presenting a detailed and single-cell-level neural activity analysis platform. This live field potential imaging method was applied for measurements of brain slices, human iPS cell-derived cortical networks, peripheral neurons, and human brain organoids. As results, detailed propagation pattern was detected between brain regions. For cultured neurons, the synaptic strength was influenced by compounds based on single-cell time-series patterns. Furthermore, we classified DRG neurons based on single neuron firing patterns and related compound responses, and verified axonal conduction characteristics and changes to anticancer drugs in cultured peripheral neurons. Finally, network activities and transition to compounds were successfully extracted for brain organoids. These results provide new understanding of the basic mechanisms of brain circuits *in vitro* and *ex vivo* on human neurological diseases, and show the possibility of the current field potential imaging technology utilization for drug discovery, and compound toxicity assessment.

Disclosures: N. Matsuda: None. **X. Han:** None. **N. Nagafuku:** None. **I. Suzuki:** None.

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.05/XX8

Topic: I.04. Physiological Methods

Support: U01-NS099697
Muri 68984-CS-MUR.01

Title: Characterization of platinum iridium coated microelectrocorticography signals using electrodes in the non-human primate

Authors: *K. WINGEL[1], A. DUBEY[1], J. CHOI[2], K. BARTH[3], C. SCHMITZ[3], J. VIVENTI[3], B. PESARAN[1];
[1]Univ. of Pennsylavnia, Philadelphia, PA; [2]New York Univ., New York, NY; [3]Duke Univ., Durham, NC

Abstract: Brain-machine interfaces (BMI) depend on the ability to obtain high-quality neural recordings. Electrocorticography (ECoG) uses electrodes placed on the cortical surface to record neural activity in the form of field potentials and decode the neural network of interest during behavior. Surface electrodes are commonly manufactured from tin and gold then coated in Platinum-Iridium (Pt/Ir) to improve recording and stimulation performance. In human subjects, ECoG is regularly performed using macroelectrodes with diameter ~2.3mm . The use of smaller diameter electrodes, diameter 200um or less, in microelectrocorticography (uECoG) arrays may allow for the capability to perform more localized neural recording and to detect features of underlying neural activity not observed using macroelectrodes. Electrode array recording properties depend on multiple factors such as electrode contact material, contact size, contact pitch, and electrode location with respect to the neural sources. The motivation behind the use of Pt/Ir coated uECoG arrays in BMIs is the ability to more-precisely sense neural activity due to lower electrode impedance but this has not been previously tested in NHP. To characterize Pt/Ir coated uECoGs we recorded neural signals using two variants of a 244-contact liquid crystal polymer (LCP) uECoG array in two non-human primates (NHP). Version 1 of the uECoG array consists of a 2 layer design with 195 um contacts at a pitch of 762 um, while version 2 of the uECoG array consists of a 3 layer design with 195 um contacts at a pitch of 900 um. In each array, the contacts are either gold or have an Electrodeposited Platinum-Iridium Coating (EPIC; Platinum Group Coatings LLC). We tested coated and uncoated uECoG array contacts by recording electrode impedance and signal, both on the bench and in vivo in Prefrontal Cortex (PFC), motor cortex (M1), and posterior parietal cortex (PPC). As expected, the contacts coated with Pt/Ir displayed lower electrode noise and lower impedance values on the bench and in vivo. Uncoated electrodes had an impedance of ~50 ohms and the coated electrodes had an impedance of ~5 ohms when sampled at 1000 Hz. We consistently observed higher quality recordings on coated contacts compared with uncoated contacts on the bench and in vivo. We resolved signal changes at higher frequencies with better signal to noise ratio (SNR) in Pt/Ir coated electrodes. The use of Pt/Ir coating supports the door for the future of bidirectional BMIs to perform as neuroprosthetics.

Disclosures: K. Wingel: None. **A. Dubey:** None. **J. Choi:** None. **K. Barth:** None. **C. Schmitz:** None. **J. Viventi:** None. **B. Pesaran:** None.

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.06/XX9

Topic: E.05. Brain-Machine Interface

Title: High-density sEEG array for directional sensing of brain signals

Authors: *I. RACHINSKIY[1], C.-H. CHIANG[1], K. BARTH[1], C. WANG[1], M. TRUMPIS[2], S. DURAIVEL[1], S. SINHA, 27708[6], B. FRAUSCHER[3], D. G. SOUTHWELL[4], G. B. COGAN[5], J. VIVENTI[1];
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Abstract: *Objective:* Stereoelectroencephalography (sEEG) is an intracranial recording technology for localizing epileptogenic zones (EZ) in patients with drug-resistant epilepsy. In contrast to surface recordings, sEEG can access deep brain regions and has low morbidity and mortality outcomes. In addition, recent studies using sEEG have demonstrated potential neuroprosthetic applications for restoring speech and limb movements in patients with loss of neurologic function. However, current sEEG devices have 8 to 18 ring electrodes spaced five millimeters apart, thereby limiting the ability to record highly resolved sub-millimeter scale neural activations. In this study, we have leveraged thin-film fabrication to develop a high-density and high-channel sEEG array on liquid crystal polymer (LCP) substrate for high-fidelity neural recordings. *Methods:* We established a two-metal layer photolithography process on LCP in the university cleanroom to fabricate the micro-sEEG grids. A rolling methodology transforms the planar device to a cylindrical form factor with ~900µm diameter to match currently used clinical devices. We validated our devices in vivo by performing neural recordings from rat's auditory cortex and intra-operatively in the hippocampus of a surgical resection patient. *Results:* We overcame the opaqueness and low metal adhesion of the LCP material to achieve a fabrication yield of over 80%. We used 17µm trace/space to fit 61 channels in four columns along the circumference of the electrode. A tone decoding task in the rat auditory cortex demonstrated successful tone prediction with an accuracy of 69% and evoked signal to noise ratio of ~15dB. Intraoperative recordings from the human hippocampus captured over 300 interictal discharges and demonstrated ability to sense directionally specific epileptic signatures that provided more intricate potential field mapping compared to standard ring electrodes. *Significance:* This work establishes a methodology for developing LCP devices within the university cleanrooms rather than outsourcing to the sparse companies capable of thin film neural device fabrication. Our in-house fabrication reduced the time and cost of iterative device development process while still adhering to designs and manufacturing schemes for future large throughput. Overall, high density sEEG grids hold promise for more effective localization of EZ from deep-brain regions as well as neuroprosthetic applications through high resolution and directional sensing of neural activity.

Disclosures: I. Rachinskiy: None. **C. Chiang:** None. **K. Barth:** None. **C. Wang:** None. **M. Trumpis:** None. **S. Duraivel:** None. **S. Sinha:** None. **B. Frauscher:** None. **D.G. Southwell:** None. **G.B. Cogan:** None. **J. Viventi:** None.

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.07/XX10

Topic: I.04. Physiological Methods

Support: Startup funding from MCW

Title: A novel setup for 24/7 simultaneous acquisition of intracranial EEG, single neuron, and behavioral data, from humans implanted with depth electrodes for seizure monitoring

Authors: H. NAGHSHBANDII[1,2], F. J. CHAURE[3], S. DOMINGUEZ ZESATI[1], M. MEYERINK[1], S. CORNELL[1], ***H. G. REY**[1,2];
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Abstract: Stereo EEG (sEEG) is a clinical procedure where electrodes are implanted in deep structures of the brain of patients with drug resistant epilepsy to identify the areas where seizures originate. Signals from these electrodes (iEEG) are recorded 24/7 at the epilepsy monitoring unit (EMU) for ~7-10 days. A modified probe allows us to include microwires protruding from the tip that sense field potentials and single neuron activity. Advances in electrophysiology hardware and computing power push us to revise the way in which we exploit data acquisition in the EMU. Here we present a novel setup for data acquisition during sEEG that offers transformative capabilities for research in clinical and cognitive neuroscience. iEEG is split into two parallel streams, one for the clinical system and one for the research system. The latter will simultaneously acquire the signals from the microwires. All signals are acquired through fully digital front ends, providing improved signal to noise ratio, and enabling 24/7 recordings (as the cables carrying digital signals can be very long). The setup is also capable of delivering controlled electrical stimulation while recording brain activity. In addition, analog and digital inputs are available, being important to synchronize behavior and brain signals. A custom-made cart hosts the acquisition system along with the hardware used during behavioral experiments. One computer saves data for offline processing (including backups to remote servers), while another computer can analyze the data online to support dynamic experiments, such as closed-loop stimulation. Another computer delivers stimuli in a controlled way (e.g, using Psychtoolbox or PsychoPy) using different modalities (screen/speakers for visual/ auditory stimuli) while the patient behavior can be recorded with different devices (camera, microphone, gamepad). Moreover, a PlayStation setup is synched with the acquisition system, allowing us to record behavior and brain activity while patients watch movies/TV shows, play videogames, or listen to music/podcats/audiobooks. Importantly, this can be initiated by the patient at any point without the need for the experimenter to set up things in the room, as the custom cart holds the screen and speakers in an arm that the patient can move away from/towards them. The proposed setup offers the chance to acquire neural data from the human brain in novel and unique ways. It can be used to improve the choice of treatment in epilepsy, but also to study other severe conditions such as depression and obsessive compulsive disorder. In addition, non-clinical research can benefit in unique ways in fields like memory, language, and sleep.

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Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.08/XX11

Topic: I.04. Physiological Methods

Support: R01NS121219

Title: Dry MXene electrodes for EEG recordings: fabrication of human-scale headsets and benchmarking against clinical gelled electrodes

Authors: *S. SHANKAR, D. XU, M. MOJENA, F. CIMINO, K. A. DAVIS, F. VITALE; Univ. of Pennsylvania, Philadelphia, PA

Abstract: Electroencephalography (EEG) is a valuable tool for non-invasive monitoring of electrical activity from the scalp for clinical monitoring of neurological disorders. Clinical EEG typically involves gel-based electrodes, whose application is time-consuming and requires skin-irritating abrasives and pastes. Recently, we have introduced dry EEG electrodes based on Ti_3C_2 MXene materials. These electrodes offer enhanced comfort and ease of use, requiring minimal skin preparation. Furthermore, they are conformable to the scalp, providing a comfortable fit for users. Here, we advance this technology towards research and clinical use by fabricating two different configurations of EEG headsets. The first configuration is a reduced-montage headband consisting of 8 channels placed at equal spacing on FP1, FP2, F7, F8, T3, T4, T5. The second configuration is a standard 10-20 montage with 21 recording sites at the canonical scalp locations. In both devices, the dry electrodes are fabricated from porous pillars infiltrated with Ti_3C_2 MXene (diameter: 8 mm, height: 6 mm), enabling access to the scalp through hair without the need for gel or pastes. The electrodes are connected to the recording amplifiers via snap connectors attached to snap leads. Owing to the high electrical conductivity ($155 \pm 4\ \Omega$, n= 5 electrodes) and surface area, the average 10 Hz impedance with the scalp of these dry porous MXene-infused electrodes is $2.1 \pm 1.8\ k\Omega$ (n=5 subjects). To further validate the dry MXene EEG technology, we have benchmarked it against clinical gelled cup Natus electrodes. Briefly, we have recruited patients in the outpatient epilepsy clinic at the Hospital of the University of Pennsylvania. For each participant, we recorded EEG with the reduced-montage headset and MXene electrodes for 20 minutes prior to clinical EEG. We recorded EEG in the following conditions: resting state, eyes open/closed, and sleeping, which corresponded to the same tasks used during clinical EEG acquisition. A preliminary analysis of the recordings shows comparable quality of the EEG signals acquired with the dry MXene and clinical gelled electrodes, while the duration of the skin prep and electrode placement operation reduced by ~2X. In conclusion, we have developed and validated a novel dry EEG technology that can improve user comfort, reduce electrode placement and skin preparation time, and reliably transmit signals of interest, providing a comfortable and efficient solution for EEG monitoring.

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Poster

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Program #/Poster #: PSTR575.09/XX12

Topic: I.04. Physiological Methods

Support: Supported by NIDEK Co., Ltd.

Title: Development of low-resistance flexible multilayer micro array for wide-area neural stimulation

Authors: *T. KONO[1,2], Y. TERASAWA[3,4], H. TASHIRO[5,2], J. OHTA[2,4];
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Abstract: In the brain stimulation device, a flexible electrode board requires the ability to fit over a wide area of the brain surface. The ISO14708-1 requires the temperature rise during device operation to stay below 2 °C. The temperature rises of the device result from the Joule heat generated during operations, a keeping the wiring resistance low is critical. In addition, in the case of wireless power transfer from external sources, devices have a limit to the amount of power available for operation due to the relationship between power transmission efficiency and heat generation. Therefore, wiring resistance must be low to reduce the extra electric power consumed by the conductive lines. Increasing metal circuit width and thickness is one technique to reduce circuit resistance. However, increasing the metal thickness by the thin-film process induces cracks due to residual stress. Therefore, the maximum available metal thickness is limited. Another requirement for a flexible circuit board is multilayer interconnection to reduce the total circuit length. However, conventional multilayer interconnect technology mainly applies to thin-film processes. Therefore, application to thick films presents challenges. In this study, we aimed to fabricate low-resistance multilayer circuit boards using polydimethylsiloxane (PDMS), a flexible material with biocompatibility, and Pt foil. To fabricate the pattern of a circuit, we used a femtosecond laser to microfabricate a Pt foil into an arbitrary shape. We achieved multilayer interconnection by stacking metal wiring on a staircase called stacked vias. The connection between stacked vias and interconnections uses micro-welding technology. The electrodes for micro-welding use a copper-tungsten electrode with a tip diameter of less than 250 μm square by machining, and the parallel gap method performed welding on the PDMS. The flexible multilayer Pt-foil circuit board had a circuit resistance of less than 1 Ω and enabled

curving. The technique applies to any layout of circuits and is applicable as a common technique for fabricating various devices.

Disclosures: **T. Kono:** A. Employment/Salary (full or part-time):; NIDEK Co., Ltd. **Y. Terasawa:** A. Employment/Salary (full or part-time):; NIDEK Co., Ltd. **H. Tashiro:** Other; grant from NIDEK Co., Ltd. **J. Ohta:** Other; grant from NIDEK Co., Ltd..

Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR575.10/XX13

Topic: I.04. Physiological Methods

Title: Quantitative detection of neurotransmitters on boron doped diamond and carbon fiber electrode arrays using multichannel fast-scan cyclic voltammetry and machine learning algorithms

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Abstract: The incidence of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease is steadily increasing in industrial nations. In order to fully understand these diseases and advance treatment approaches, a better understanding of the neural circuitry and the involved neurotransmitters is needed. One, commonly utilized measurement to quantify neurotransmitter release is fast-scan cyclic voltammetry (FSCV), an electrochemical technique that can measure electroactive neurotransmitter concentrations on a sub second time scale. Traditionally FSCV is performed using carbon fiber microelectrodes (CFME) which yield a low limit of detection (LOD). However, boron-doped diamond microelectrodes (BDDME) promise advantages of a wider electrochemical window, improved stability, resistance to biofouling and production scalability through wafer processing. While FSCV yields high spatial resolution, typical measurements are made on a single fiber, and analyzed by hand. As multi-channel arrays of both CFME's and BDDME's are developed, these by hand-measurements become cumbersome and data intensive due to the quantity of electrochemical data produced per channel measured. Herein, we propose that machine learning approaches can be used to automatically detect and quantify concentration changes and facilitate discrimination of neurotransmitter mixtures. In this study, we gathered FSCV data utilizing custom made CFME- and BDDME arrays. Using these arrays, we measured varying concentration of both dopamine, serotonin and relative pH changes. To the collected data, we implemented a gaussian fitting approach after conventional linear time- invariant (LTI) filtering of the signals as feature extraction and

compared it to commonly used principal component analysis (PCA) of the data. Subsequently we applied and optimized various machine learning techniques:
•linear regression
•k-nearest neighbors
•support vector regressor
•decision tree regressor
•Bayesian ridge regressor
The chosen models were utilized to investigate whether simpler models are sufficient in contrast to Deep Learning, and thus very complex models. Here, we show how machine learning can be accomplished on a simple lab PC and be utilized to autonomously analyze FSCV data for both electrode materials and therefore making multichannel measurements more feasible.

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Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

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Title: Development and Validation of a 4096 channel Electrocorticography (ECoG) Grid in the Pig Brain

Authors: ***J. LEE**[1], T. S. PORTER[2], K. LEE[2], P. BOTROS[2], L. LIU[2], A. MUNK[2], H. U[2], D. ROTH[3], P. PIZARRO[3], Y. TCHOE[5], J. GU[2], S. FISHER[2], R. VATSYAYAN[2], A. M. BOURHIS[2], W. JEON[2], A. PAULK[6], S. S. CASH[6], E. HALGREN[7], S. BEN-HAIM[4], A. M. RASLAN[8], D. HALL[2], S. A. DAYEH[2];
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Abstract: Epilepsy is one of the most prevalent neurological diseases and over a quarter of all epilepsy patients experience drug-resistant epilepsy where surgical interventions or electrical

stimulation become the most effective paradigms for treatment. Electrocorticography (ECoG) is the gold-standard mapping technique to identify epileptogenic zones and to delineate boundaries between pathological and healthy tissue. High spatiotemporal resolution with a broad cortical coverage is crucial for precise localizations to enhance our understanding of epilepsy, improve patient outcomes and reduce functional impairments and potential side effects. However, current clinical ECoG electrodes have low spatial resolution and channel count where individual contacts are from 2 to 3 mm in diameter and up to 10 mm in intercontact spacing. Building upon our first-in-human intraoperative brain mapping with 1024-channel microelectrode arrays on pathological tissues, we have designed and scaled ECoG grids up to 6.4 x 6.4 cm^2 area with 4096 recording channels. Novel platinum nanorods (PtNRs) were utilized as electrode contacts for low impedance and long-term stability with parylene-C as the insulating material, which is transparent to the brain surface and conformal to its movements. By utilizing advanced multi-layer parylene-C fabrication methods, we achieved over 90% yield of recording contacts, with average impedance of 20 kΩ for 30 μm diameter contacts. As an intermediate transition towards wireless human implantation and to demonstrate stable, high-channel recording, we used our 4096-ch grid in the anesthetized pig brain model. The grid dimension was optimized to be 3.2 x 1.3 cm^2 to cover a single hemisphere of the pig brain, with contacts spaced 250 μm horizontally and 400 μm vertically. The acquisition system and software were custom-designed through IMEC chips and Open Ephys respectively. Contralateral sensory mappings were obtained through somatosensory evoked potential measurements by electrical and air-puff stimulations. We also artificially induced epileptic seizures by sub-cortically injecting 4-Aminopyrodine neurotoxin through a perfusion hole on the grid and recorded epileptiform cortical activities and patterns. Sensory mapping measurements were also repeated to observe compounded sensory effects after neurotoxin application. Overall, our results advance the scaling and development of high-channel clinical grids for a semichronic epilepsy monitoring platform with fully wireless data and power transfer, and also pave the way toward other applications in responsive neurostimulator systems and brain-machine interfaces.

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Poster

PSTR575. Electrophysiology Techniques: Advances in EEG and Related Techniques

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Topic: I.04. Physiological Methods

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Title: Histological Response of Neural Tissue to Pulsed Electrical Stimulation: A Safety Study

Authors: *R. VATSYAYAN[1], K. TONSFELDT[2], A. M. BOURHIS[3], H. U[4], S. DAYEH[5]; [1]Electrical And Computer Engin., Univ. of California San Diego, San Diego, CA; [3]Electrical and Computer Engin., [2]Univ. of California San Diego, La Jolla, CA; [4]Univ. of California San Diego, San Diego, CA; [5]Electrical and Computer Engin., UCSD, San Diego, CA

Abstract: Introduction: With the introduction of sophisticated neuromodulation devices to clinical practice, electrical stimulation is increasingly being utilized for diagnostic and therapeutic applications. The emergence of novel high geometrical surface area electrode materials such as Poly(3,4-ethylenedioxythiophene)-polystyrenesulfonate (PEDOT:PSS), Platinum-Iridium (PtIr) and Platinum Nanorods (PtNR) have enhanced the ability to deliver targeted electrical stimulation through low impedance micro-contacts. Subsequently, there is a need to evolve our understanding of the thresholds for safe electrical stimulation, building upon the empirically determined limits established by the Shannon's equation. **Methods:** We fabricate Parylene-C based flexible microelectrode arrays with PtNR and Planar Pt stimulation contacts. The contact diameters were varied from 30µm to 1000µm to capture the effect of size on the tissue response. We first establish the electrochemical safety limits for stimulation in benchtop measurements in saline by performing Electrochemical Impedance (EI), Cyclic Voltammetry (CV) and Voltage Transient (VT) measurements. We then repeat these measurements for a smaller subset of parameters *in vivo* on the rat brain. Using the EI measurements, we model the resistive and capacitive elements of the electrochemical interface between the electrode contact and the surrounding media. We correlate the interface parameters to the Charge Injection Capacity (CIC) established from the VT and CV measurements. Next, we measure the response of neural tissue to pulsed electrical stimulation in acute and chronic implantations. We deliver biphasic, bipolar stimulation of varying amplitudes at 50Hz for 7 hours. At the end of the stimulation session, the tissue is marked with tissue-staining dyes and extracted. The tissue is then fixed and sliced for histology. We stain the tissue to measure 4 parameters for determining damage to tissue: Neuronal size and shape, DNA integrity, dendritic density and early apoptosis. **Results:** Using histological measures for tissue damage, we correlate the electrochemical and neural stimulation safety limits across electrode contacts of different sizes and materials, for multiple pulse widths of stimulation. We observe significant damage to tissue for current levels exceeding the electrochemical safety limits. We further compare these safety limits to those established by the Shannon's equation and observe that damage can occur below these pre-established limits. Finally, we demonstrate the dependence of the safety limits to the choice of the stimulation parameters and electrode design.

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Poster

PSTR576. Network Models

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Program #/Poster #: PSTR576.01/XX16

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

Support: Swiss government's ETH Board of the Swiss Federal Institutes of Technology

Title: Using the Neocortical Microcircuit as a Realistic Ground Truth To Map Spike Sorting Biases

Authors: *S. LAQUITAINE, M. IMBENI, J. THARAYIL, M. REIMANN;
Blue Brain Project, Swiss Federal Inst. of Technol., Geneva, Switzerland

Abstract: Accurate spike sorting on extracellular recordings, the correct assignment of spikes to their neuron sources, is critical to understand the neural codes underlying brain functions. The development of spike sorting algorithms is supported by evaluation against a known ground truth, provided by hybrid synthetic simulation data sets. Generated by adding a limited set of spike waveforms sorted from real recordings to a simulated background, they may underestimate the diversity of biological spike shapes. Additionally, strong assumptions about firing rate statistics are made during their generation. Here, we pursue a complementary approach, evaluating state-of-the-art spike sorters against data from simulations of a recently published, large scale model of rodent neocortical circuitry. The model comprises 30,190 morphologically detailed neurons, spanning all six cortical layers. It captures the biological diversity in 60 morphological and 11 electrical neuron types and features realistic connectivity. We simulated extracellular recordings, sampling potentials at the coordinates of 32 contacts separated by 40 microns of a virtual neuropixels 1 probe. The resulting traces were analogous to real recordings in that they were composed of three components: overlapping spikes with high signal-to-noise-ratio, low amplitude multi-unit activity and background noise. In accordance with (Schomburg et al., 2012), spike shapes varied with cell positions relative to contacts. We then evaluate the Kilosort3 spike sorter (Pachitariu et al., 2023), against the true spike trains of all 534 modeled neurons located within 50 μm of the contacts as well as a number of existing synthetic data sets. In accordance with (Buzsaki & Mizuseki, 2014), our model had a long-tailed firing rate distribution with a peak below 1 Hz. After confirming published accuracies on existing data sets, we found a significantly lower performance of Kilosort3 on our simulated data, specifically for neurons with low firing rates (1.3% detected cells with accuracy over 80%), leading to an overestimation of the mean population firing rates. This bias resulted from several mechanisms. Kilosort3 missed 15% (n=78/534) of the ground truth units, which all fired sparsely (firing rate < 0.2Hz) and were equally distributed across morpho-electric types or cortical layers. Other sparse firing units (n=292/534) were detected, but merged with other units. Altogether our results indicate that tuning spike sorters on high firing rate simulations can lead to undersampled and poor sorted sparse firing units.

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Poster

PSTR576. Network Models

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Title: Impact of inter-areal connectivity on sensory processing in a biophysically-detailed model of neocortical micro- and meso-circuitry

Authors: *S. BOLAÑOS PUCHET, A. ECKER, D. EGAS SANTANDER, J. B. ISBISTER, C. POKORNY, M. W. REIMANN;
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Abstract: Connections between areas of the neocortex exhibit regularities in their layer termination patterns. These provide a basis for establishing cortical hierarchies, as a way to explain and predict information flow across areas. In the classical picture, based on the primate visual system, lower areas send feedforward projections primarily to layer 4 in higher areas, and these send feedback projections primarily to layers 1 and 6 in lower areas. Further studies using genetically-encoded tracers in mice have greatly expanded on this view, exhibiting a variety of layer termination patterns and establishing a hierarchical organization of all cortical and thalamic regions. In the context of sensory perception, bottom-up feedforward signals are thought to carry sensory inputs or sensory-derived prediction errors, while top-down feedback signals convey context information or internal predictions. However, the mechanisms through which these processes could happen and their relation to the underlying connectivity are not completely understood. Here we use a computational model of neocortical micro- and meso-circuitry to study the impact of inter-areal connectivity on sensory processing. The model integrates a large body of data from rodent primary somatosensory cortex and reproduces biological features across multiple scales: from a range of ion channel models combined to produce diverse electrical types, to millions of morphologically detailed neurons filling an atlas-based volume, to local and long-range networks mediated by billions of stochastic synapses. Notably, long-range connectivity in the model incorporates layer termination patterns associated with feedforward or feedback pathways in the literature. We used the model as a starting point to simulate and study an idealized case of two isolated areas interacting only through long-range connectivity. First, we extracted from the model two spatially separate - but reciprocally connected - subvolumes that served as putative cortical areas. Second, we provided sensory inputs to one of the areas through the activation of thalamic afferents with different spatio-temporal patterns, both separately and in combination. Background inputs were also provided to attain an *in vivo*-like network state in both areas. Third, we analyzed the responses in both areas while searching for signatures of sensory discrimination (stimulus identity) and integration (stimulus composition). Finally, in order to assess the impact of inter-areal connectivity, we selectively blocked or adjusted different

pathways between the two areas and measured the change in the responses, finding a range of pathway-specific effects.

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Poster

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Title: Multiscale data integration to generate atlas-based biophysical modeling of first- and higher-order mouse thalamic nuclei

Authors: *A. E. SOPLATA[1], E. IAVARONE[1], P. LITVAK[1], H. DICTUS[1], V. R. MUDDAPU[1], A. ROMANI[1], H. MARKRAM[1], S. L. HILL[1,2,3,4];
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Abstract: The role of the thalamus in sensory and cognitive processing has drawn renewed attention in recent research (Halassa, 2023). Notable differentiation in connectivity is observed in "first-order" nuclei, such as the ventral posteromedial nucleus (VPM), and "higher-order" nuclei like the posterior medial nucleus (POm), providing unique pathways for sensory information relay and inter-region communication (Roy et al., 2022). To further investigate these phenomena, we developed an atlas-based, anatomically-constrained model of the somatosensory mouse thalamus.
Our model of VPM, POm, and RT integrates several layers of data including cellular, synaptic, electrophysiological, and morphological information. Building upon our previous thalamoreticular microcircuit (Iavarone et al., 2023), the model includes data-driven updates to the Blue Brain Cell Atlas (Erö et al., 2018) including regional cell density and interneuron differentiation (Rodarie et al., 2022), validation of cell placement, region-specific core and matrix structural and functional synaptic connectivity from cortex, region-specific structural connectivity from lemniscal and paralemniscal sensory inputs, and single-cell electrophysiological and morphological types.
Our goal is to provide a comprehensive computational tool for exploring how thalamocortical

phenomena differ between first- versus higher-order nuclei, including arousal-dependent depolarization, sleep stages, slow-wave oscillations, and spindles and their coupling with slow-wave oscillations, and whisker-touch responses and processing. . Following rigorous validation, we believe our model paves the way for in-depth exploration of the nuanced function of the thalamus in neural processes.

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Poster

PSTR576. Network Models

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Program #/Poster #: PSTR576.04/XX19

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

Title: Exploring the effect of cortical feedback on reticular inhibition in a model of the rodent thalamocortical loop

Authors: ***P. LITVAK**[1], J. HERTTUAINEN[1], H. MARKRAM[1], A. ROMANI[1], S. L. HILL[2,1]; [1]Blue Brain Project, EPFL, Geneva, Switzerland; [2]Ctr. for Addiction and Mental Hlth., Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Thalamocortical circuitry is central to the interplay between cognitive and sensory processes such as the ability to allocate attentional resources to salient cues yet the contribution and interplay of its building blocks is poorly understood. In vitro work shows that top-down cortical feedback into the thalamic reticular nucleus (TRN) can modulate thalamocortical information flow through dynamic excitatory-inhibitory balance which is dependent on the rate and time course of corticothalamic activation (Crandall et al., 2015). Computational studies suggest that although at a mechanistic level it is not known how top-down attentional control via the TRN, compared to direct projections to thalamus may differentially regulate information processing, thalamic gain is more effectively regulated by top-down regulation of the TRN rather than direct modulation of thalamic neurons (Gu et al., 2021). To gain insight into the multi-scale representation and processing of sensory information in thalamocortical loops, we coupled an anatomical somatosensory thalamoreticular model (Iavarone et al., 2023) to non-barrel primary somatosensory cortical microcircuitry (Reimann et al., 2022), extending a previously established pipeline for a model of an isolated patch of neocortical tissue (Markram et al., 2015). The combined circuit comprises over 957K cortical and thalamic neurons connected via 66 million synapses and captures the electrical behaviour of 212 morpho-electrical types. It recreates within-region biological synaptic connectivity as well as realistic spontaneous and evoked activity, allowing dissection of cellular and synaptic contributions.Although the TRN can be recruited by multiple brain structures to integrate cognitive and sensory information, with this study we explore the hypothesis that TRN implements an attentional filter controlled by top-down cortical signals by investigating corticothalamic feedback over different time scales and

modes of activation, establishing experimentally testable circuit mechanisms for top-down attentional allocation via the TRN.

Disclosures: **P. Litvak:** None. **J. Herttuainen:** None. **H. Markram:** None. **A. Romani:** None. **S.L. Hill:** None.

Poster

PSTR576. Network Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR576.05/XX20

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

Support: This study was supported by funding to the Blue Brain Project, a research center of the École polytechnique fédérale de Lausanne (EPFL), from the Swiss government's ETH Board of the Swiss Federal Institutes of Technology.

Title: Time-warped spike timing: in vivo evidence and a large-scale in silico cortical model

Authors: *J. B. ISBISTER, M. W. REIMANN;
EPFL, Lausanne, Switzerland

Abstract: We present analysis of in vivo spiking activity and emerging in vivo-like dynamics within a new large-scale biophysically-detailed model of the somatosensory cortex [1]. The model comprises 8 subregions and 4.2 million morphologically-detailed neurons connected through 13.2 billion local and long-range synapses. Precise spike time patterns are rarely reported but maintain their appeal, due to the sensitivity of neural integration, and their potential speed and efficiency. We previously showed how cortical state affects spike time patterns in vivo [2]. When the cortex was less excited, precise spike sequences were stretched: evolving slowly in time. This stretching is referred to as "time-warping" and may explain why precise spike sequences are rarely reported. As spike time patterns were co-modulated over cortical layers and columns, we suggested that decoding neurons might also be modulated and could respond in a state dependent manner, which would confer a higher coding capacity. We present new visualizations of time-warped multi-neuron single spike patterns (for up to 12 neurons), which could be a fundamental form of representation, and make estimates of cortical state from spike timing information. We show that trial-to-trial changes in shared excitability are low-dimensional, increasing the possibility that decoding neurons are also modulated. We also explore such representations in the model. We developed an efficient calibration technique which brings the model into an in vivo-like regime by accounting for missing synapses from other brain regions. The model displays fluctuating spontaneous activity and highly realistic stimulus responses, and shows how white matter connectivity shapes emergent dynamics. The automated calibration technique closes the loop for iterative refinement of cortical models. [1] Isbister, Ecker, Pokorny, Bolaños-Puchet, Egas Santander, ⋯, Markram, Ramasaway, &

Reimann (2023). Modeling and Simulation of Neocortical Micro-and Mesocircuitry. Part II: Physiology and Experimentation. bioRXiv. [2] Isbister, Reyes-Puerta, Sun, Horenko, & Luhmann (2021). Clustering and control for adaptation uncovers time-warped spike time patterns in cortical networks in vivo. Scientific Reports, 11(1), 1-20.

Disclosures: J.B. Isbister: None. **M.W. Reimann:** None.

Poster

PSTR576. Network Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR576.06/XX21

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

Support: NIMH109548

Title: Mesoscopic Dynamics in Large Neuronal Populations: Insights from Simulations and Statistical Physics

Authors: *A. SHEREMET, Y. QIN;
Univ. of Florida, Gainesville, FL

Abstract: This study investigates the collective dynamics of large populations of Hodgkin-Huxley neurons, employing large-scale simulations to gain a understanding of mesoscopic collective dynamics in the cortex. The primary focus is on uncovering the intricate mesoscopic oscillatory patterns that manifest as a macroscopic representation of neural activity in these populations.
Our approach integrates computational simulations with a statistical physics-based population model, providing a valuable framework for exploring mesoscopic dynamics comprehensively. This integration acts as a vital link between individual neuronal behaviors and collective neural activity.
The study places significant emphasis on unraveling the complexities of collective dynamics, including the emergence of oscillations and non-linear phenomena like cross-frequency coupling. These insights not only yield a comprehensive understanding of the collective behavior of large neuronal populations but also enhance our knowledge of large-scale neural populations in the brain.
The findings of this study present novel insights into mesoscopic neural dynamics and underscore the importance of further validation and ongoing research. Moreover, they have the potential to inform innovative therapeutic strategies for neurological disorders. This work reinforces our understanding of collective neural activity at the mesoscopic level, emphasizing the necessity of continuous validation and exploration of these intricate dynamics.

Disclosures: A. Sheremet: None. **Y. Qin:** None.

Poster

PSTR576. Network Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR576.07/XX22

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

Support: NIMH109548

Title: Exploring Collective Neural Activity: Mesoscopic Dynamics and Implications for Neurological Phenomena

Authors: *Y. QIN, A. SHEREMET;
Univ. of Florida, Gainesville, FL

Abstract: This study provides a theoretical and numerical examination of mesoscopic collective neural activity, specifically the spatio-temporal oscillatory patterns within the cortex. We present a novel statistical model using the principles of statistical physics, where we consider individual Hodgkin-Huxley neurons as discrete particles. By integrating the microscopic descriptions of cell dynamics detailed by the Hodgkin-Huxley equations with statistical physics, we derive governing equations for collective activity. Our study shows that collective activity emerges as a macroscopic expression of neural activity.
Our findings reveal intricate characteristics of mesoscopic dynamics, including oscillations and wave propagation, cross-frequency coupling due to nonlinearity, and the potential for Turing patterns based on wave dispersion/dissipation analysis. Although further validation of the proposed model is required, the results are in line with known features of brain activity.
In addition, our work provides fresh insights into complex neurological phenomena, such as the role of nonlinearity in brain rhythm modulation, the interactive dynamics between calcium and sodium channels in working memory, and memory formation through the reinforcement of inhomogeneity via Turing instability. These insights may lay the foundation for novel therapeutic strategies for related neurological disorders.

Disclosures: Y. Qin: None. **A. Sheremet:** None.

Poster

PSTR576. Network Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR576.08/XX23

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

Support: NIH Grant 1RF1DA055665

Title: Combined mechanistic and input-output modeling of the hippocampus: latent dynamic analysis of hippocampal spiking activities during a spatial delayed nonmatch-to-sample task

Authors: *C. XU, Z. LU, D. SONG;
Biomed. Engin., USC, Los Angeles, CA

Abstract: We aim to combine mechanistic and input-output modeling techniques to build a full-scale realistic model of the rat hippocampus that is functionally indistinguishable from the real hippocampus using generative adversarial network approach. To implement this approach, it is essential to develop a statistical method that allows the comparison of population-level neural dynamics between the real and the simulated neuronal populations. Here, we utilize three dimensionality reduction techniques, kernel smoothing (KS), principal component analysis (PCA), and canonical correlation analysis (CCA), to estimate and compare latent dynamics (LDs) of neuronal populations using spike trains recorded from hippocampal CA3 and CA1 regions in rats performing a spatial delayed nonmatch-to-sample (DNMS) task. First, we extract spike patterns (-2 to 2 sec) from CA3 and CA1 spike trains during eight different DNMS events involving different spatial locations (left vs. right), task phases (sample vs. response), and behavioral outcomes (correct vs. error). Second, spike patterns are smoothed with Gaussian kernels with different standard deviations (STDs) to obtain continuous firing rates. Third, PCA is performed on the firing rates to extract the principal components (PCs) during DNMS events. LDs are then calculated by projecting the population-level neural activities onto PCs. Fourth, to compensate changes in LDs projected to different PCs across different animals and sessions, CCA is applied to align LDs by finding the linear combinations of LDs with maximal correlation. Correlation coefficients are then calculated to quantify the similarities between LDs within and across different PCs. Lastly, the optimal STD of the Gaussian kernel is chosen as the STD that maximizes the difference between the correlation of aligned LDs in the real data and the randomly generated surrogate data. With this method, a single-value metric of the similarity between two neural population dynamics involving different sets of neurons and DNMS events is obtained. We have analyzed LDs between different DNMS sessions within the same animal and between different animals. Results show a high degree of similarities between LDs despite the high variability of neural dynamics at the individual neuronal level. Results suggest that LDs are largely preserved during different sessions within the same animals and across different animals. Therefore, the profiles of latent neural dynamics can be used to compare the neural population dynamics and further validate the mechanistic model of the hippocampus with spike trains recorded from the real hippocampus.

Disclosures: **C. Xu:** None. **Z. Lu:** None. **D. Song:** None.

Poster

PSTR576. Network Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR576.09/XX24

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

Support: NIH/NIDA, Brain Initiative 1RF1DA055665

Title: Combined mechanistic and input-output modeling of the hippocampus: Deep multi-input multi-output model of spike train transformations

Authors: *B. MOORE[1], **D. SONG**[2];
[1]Biomed. Engin., USC, Los Angeles, CA; [2]USC, Univ. of Southern California, Los Angeles, CA

Abstract: The goal of this research is to build a biologically realistic large-scale model of the hippocampus that is functionally indistinguishable from the real hippocampus. We use an innovative modeling approach inspired by generative adversarial networks (GANs) that combines mechanistic and input-output modeling techniques. The focus herein is on a novel multi-input multi-output (MIMO) model that yields population-level spatial-temporal invariant representation of the input-output nonlinear dynamics responsible for neural spike transformations across different hippocampal regions during a spatial navigation task. The MIMO model has shallow convolution layers to capture shared temporal dynamics across input and output neurons. The convolution layers are followed by carefully designed fully-connected layers that benefit from latent representations and multi-task learning to capture shared nonlinear transformations across input and output neurons and across different MIMO systems. The result is Deep-MIMO, a model that scales with superior efficiency to our previous shallow (double-layer) MIMO model as recorded input neurons and output neurons increase, while also creating a population-level invariant representation of input-output transformations. Deep-MIMO can capture arbitrary forms of temporal dynamics and high-order nonlinearities, whereas a shallow MIMO model is only able to capture multiplicative nonlinearity of prespecified order. Deep-MIMO permits the comparison of nonlinear dynamics between different neural datasets, e.g., dataset recorded from animals and dataset simulated with the full-scale mechanistic model, without relying on a one-to-one correspondence between recorded and simulated neurons. This produces a powerful tool for functional validation of the full-scale mechanistic model with experimental data as the ground truth. Deep-MIMO has been validated with a second-order Volterra kernel-based spiking neuronal network model. Results show that the deep-MIMO can accurately recover the ground-truth kernels, probability of spiking in output neurons, and output spiking activity. This model will be further applied to experimental data and used to validate the large-scale mechanistic model of the hippocampus.

Disclosures: **B. Moore:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bryan Moore MD has small stock positions in Medtronic, Johnson & Johnson, and Thermo Fisher Scientific. **D. Song:** None.

Poster

PSTR576. Network Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR576.10/XX25

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

Support: BYU CURA Grant

Title: Identifying neural circuits implicated in neurogenic stuttering through the lesion network mapping method

Authors: *O. BENZLEY[1], A. BREWER[1], E. BAUGHAN[1], F. SCHAPER[2], M. A. FERGUSON[3], J. NIELSEN[1];
[1]Neurosci. Ctr., Brigham Young Univ., Provo, UT; [2]Neurol., Brigham and Women's Hosp., Boston, MA; [3]Neurol., Harvard Med. Sch., Boston, MA

Abstract: Neurogenic stuttering is a distinctive subtype of acquired stuttering which arises from strokes, encephalitis, tumors or traumatic brain injuries. It is characterized by speech disruption, elongation of syllables, sound repetition, and altered speech rhythm. Previous research using voxel-wise lesion symptom mapping methods has failed to identify a specific neural network responsible but has identified certain regions that could be relevant, including a variety of regions associated with the cortico-striato-thalamocortical loop. The primary objective of this study is to confirm the involvement of this network while also identifying other potentially implicated networks in the pathophysiology of neurogenic acquired stuttering. To achieve these objectives, we used the lesion network mapping method, which includes brain regions functionally connected to the lesion site, in contrast to voxel-wise lesion symptom mapping, which relies only on regions at the lesion site. A systematic literature review was conducted to identify relevant case studies involving patients exhibiting neurogenic stuttering (n=29). A majority of cases included in the analysis were attributed to ischemic stroke (n=24), with the others resulting from a tumor, a traumatic brain injury and hemorrhagic stroke. Eligibility criteria for case study selection included temporal association between onset of the symptom and stroke occurrence, availability of clear neuroimaging data, and participants being of adult age. Lesion network mapping analysis was performed on the 29 lesions, with a large cohort of healthy control resting-state scans (n=1000). Following completion of the analysis, lesion networks exhibited functional connectivity to the cortico-striato-thalamocortical loop, primarily in the left striatum (n=25), thalamus (n=25), and motor cortex (n=23). Furthermore, an area in the left inferior parietal lobule was also functionally connected to the lesion networks (n=23). Brain regions that were found to be negatively correlated with the lesion networks included the orbitofrontal prefrontal cortex, the dorsolateral prefrontal cortex, and the inferior temporal lobe. Our results align with previous reports that disruptions in the cortico-striato-thalamocortical loop contribute to neurogenic stuttering. It also reveals novel regions, particularly the left parietal region, that may also contribute. Further research is warranted to determine the specific roles that these networks play, as well as how they interact with each other in the context of neurogenic stuttering.

Disclosures: O. Benzley: None. **A. Brewer:** None. **E. Baughan:** None. **F. Schaper:** None. **M.A. Ferguson:** None. **J. Nielsen:** None.

Poster

PSTR576. Network Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR576.11/XX26

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

Title: Identifying neural circuits associated with dysarthria through the lesion network mapping method

Authors: *A. WARNICK[1], O. BENZLEY[1], F. SCHAPER[3], M. A. FERGUSON[4], J. A. NIELSEN[2];
[2]Psychology, [1]Brigham Young Univ., Provo, UT; [3]Maastricht Univ. Med. Ctr., Maastricht, Netherlands; [4]Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: Dysarthria is a common symptom of speech impairment, due to neuromuscular disruptions that impede regular movement used to produce organized sound. The variety of ways dysarthria manifests include slurred speech, an inability to modulate voice volume, disruption of pronunciation, and slowed speaking rate caused by a weakness in the muscles used for speech. This disorder is frequently experienced after brain trauma such as ischemic or hemorrhagic stroke, tumors, encephalitis, or other severe head traumas. Previously, brain regions thought to be associated with dysarthria include bilateral superior hemispheres of the cerebellum, posteromedial or paravermal regions of the cerebellum, and the thalamocortical network. The purpose of this study is to identify what networks of the brain are involved in the symptom dysarthria when caused by stroke or other brain injuries. A literature review was used to find case studies of patients who presented with acquired-brain-injury-induced dysarthria, a majority of which involved ischemic stroke as the cause of lesion. Inclusion criteria for case studies included temporal association between developing dysarthria and experiencing a stroke, and an image of the brain slice that displays lesion location. The lesions in these cases (n=56) were then traced onto a standard brain atlas (MNI), after which a lesion network mapping analysis (Fox et al., 2018) was performed using a cohort of 1,000 healthy control resting-state fMRI scans. We completed a lesion network mapping analysis and found that more than 90% of the patients had brain lesions that were functionally connected to the thalamus (n=51) and the cerebellum (n=51). Cerebellar connectivity was found bilaterally, with higher correlation in the left hemisphere. We also found that the lesions were negatively correlated to the lateral temporal lobe of the left hemisphere (n=48). Our results align with previous findings of cerebellar and thalamic involvement in the brain networks associated with dysarthria. Further research in this area could help in determining which symptoms a patient will experience after a lesion, such as predicting whether they will develop dysarthric speech.

Disclosures: A. Warnick: None. **O. Benzley:** None. **F. Schaper:** None. **M.A. Ferguson:** None. **J.A. Nielsen:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.01/XX27

Topic: B.07. Network Interactions

Support: NIMH grant 1R01MH135565

Title: Cholinergic regulation of network activation mediates switching between different memory formation/consolidation modes during wake, NREM and REM sleep.

Authors: *Z. NOUREDDINE[1], M. R. ZOCHOWSKI[2];
[1]Univ. of Michigan, Ann Arbor, ann arbor, MI; [2]Univ. of Michigan, Univ. of Michigan, Ann Arbor, MI

Abstract: The mechanism in which the brain switches its dynamics from online activation and storage of incoming information to later offline reactivation and consolidation of the acquired information has been an outstanding question in system's neuroscience. Acetylcholine is a potent neuromodulator that plays a role in the control of vigilance states in the brain. It acts on network dynamics via various synaptic and cellular mechanisms. ACh levels are typically high during active waking and REM sleep, whereas cholinergic levels are low during quiet waking and specifically during NREM sleep. Here, using in-silico biophysical network model of interacting excitatory and inhibitory neurons, we investigate the potential role of acetylcholine, and specifically M1 receptor pathway, in modulating activation and reactivation patterns in a memory network and subsequent differential binding of memory representations that are being consolidated. We found that during high ACh states, observed during active waking and REM sleep, only local sub-populations of cells that are actively driven by external stimulus representing specific features of a memory are coactivated. The sequences of these features are activated sequentially in a temporally ordered fashion. In contrast, when ACh is low, during quiet waking or NREM sleep, the network becomes disinhibited, leading to synchronous reactivation of multiple feature representations and formation of cohesive memory trace via spike timing dependent plasticity STDP. In all, our results indicate ACh may play a critical role in controlling interplay between online memory storage and offline consolidation during various vigilance states.

Disclosures: Z. Noureddine: None. **M.R. Zochowski:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.02/XX28

Topic: B.07. Network Interactions

Support: Israel Science Foundation Grants 1745/18, 1978/13
European Research Council Synergy Grant 951319
Gatsby Charitable Foundation

Title: Noise resilience of memory stored in low-dimensional neural manifolds through multiple synaptic timescales

Authors: *G. CHECHELNIZKI[1,2], N. SHAHAM[2,3], Y. BURAK[2,1,3];
[1]ELSC, Jerusalem, Israel; [2]Hebrew Univ. of Jerusalem, Jerusalem, Israel; [3]Racah Inst. of Physics, Jerusalem, Israel

Abstract: Short term memory in the brain is theorized to often be implemented by continuous attractor networks, which represent stored variables in persistent neural activity. Since neurons are noisy, the variability in their activity degrades the memory, which can manifest as random diffusion (Burak and Fiete 2012). Derivative feedback is known from control theory to increase robustness against various common perturbations, and it was shown in Lim and Goldman 2013 that it can be implemented via slow excitatory and fast inhibitory synaptic timescales, decreasing memory drift due to weight mistuning. In this work we show that the utility of derivative feedback in neural networks is not limited to synaptic mistuning, but in fact can greatly reduce the degradation of memories due to ongoing neural noise. We start by examining the simple case of a linear attractor network, and then demonstrate that the principles that lead to the mitigation of diffusion generalize to a far more general class of nonlinear networks. To demonstrate this, we first derive a general expression for the diffusion coefficient of a stored variable in an attractor network of Poisson neurons with arbitrary connectivity and synaptic timescales as a generalization of Burak and Fiete 2012. We successfully test our theory on ring networks, inspired by the representation of heading in the central complex of insects. Since the number of neurons in these networks is small, noise driven diffusion is naively expected to be prominent. We find that our theory correctly predicts the increase of memory stability as a function of synaptic timescale differences in such models, when they are endowed with negative derivative feedback. Furthermore, we identify how to engineer the network connectivity such that the stability of the bump position along the attractor is enhanced, yet perturbations to the bump's structure are not slowed down by the derivative feedback mechanism. Thus, neural activity remains tightly confined to a one dimensional manifold. Insights from our theory allow us to conclude that neurons in head direction cell networks that are commonly thought to be utilized for velocity integration can also act as stabilizers against noise-driven motion.

Disclosures: G. Chechelnizki: None. **N. Shaham:** None. **Y. Burak:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.03/XX29

Topic: B.07. Network Interactions

Support: CIHR Postdoctoral Fellowship (202210MFE-491650-71993)
CIHR Foundation Grant (167276)

Title: Disruptions to gamma oscillations caused by depolarizing shifts in the GABA reversal potential are mitigated by noise

Authors: *S. RICH, S. A. PRESCOTT;
The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Fast synaptic inhibition through $GABA_A$ receptors depends on the chloride reversal potential (E_{GABA}). Abnormal chloride regulation can cause a depolarizing shift in E_{GABA}, compromising inhibition. This occurs in many neurological disorders, with diverse functional consequences. We studied effects of changing E_{GABA} in a computational model of gamma oscillations. Such oscillations are thought to be important for neural processing and, ultimately, for cognition. While the computational study of microcircuit oscillations has a rich history, there has yet to be rigorous study of the effects of changes in E_{GABA} on the oscillatory dynamics of biophysically-motivated networks of excitatory and inhibitory neurons (E-I networks).
Here, we describe the interacting effects of E_{GABA} and noisy input on not only the capacity for an E-I neuronal microcircuit to exhibit stable oscillatory dynamics, but also to encode relevant information in those dynamics. Unsurprisingly, a depolarizing shift in E_{GABA}, and the resulting reduction in inhibition, leads to increased network activity and to less coherent network rhythmicity. Surprisingly, we also found that very minor changes E_{GABA} can cause otherwise stable network rhythms to abruptly collapse and that the effects of depolarizing shifts in E_{GABA} on network coherence were non-monotonic. However, addition of noisy input to the system widened the range of E_{GABA} values in which stable synchronous oscillations could be maintained, both by minimizing the potential for a collapse in rhythmicity and instantiating a more monotonic relationship between E_{GABA} and network coherence. Most interestingly, though, the addition of noise also increased the capacity of oscillations to encode information in the spiking rates of individual neurons, even in the face of substantial shifts in E_{GABA}.
Taken together, these findings present important new insights into the pathological effects of the depolarized GABA reversal potential observed in many neuropsychiatric disorders, while simultaneously highlighting the importance of studying such effects under realistically noisy conditions. This research was supported by a postdoctoral fellowship and foundation grant from the Canadian Institutes of Health Research.

Disclosures: S. Rich: None. **S.A. Prescott:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.04/XX30

Topic: B.07. Network Interactions

Support: NIMH grant 1R01MH135565

Title: Cholinergic modulation of E/I balance and its effects on network dynamics

Authors: *B. E. FRY, M. ZOCHOWSKI;
Physics, Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Various vigilance states - such as waking, quiet waking, NREM and REM sleep - are controlled through changing neuromodulatory milieu in the brain. Acetylcholine (Ach) is one of the critical neuromodulators that dramatically changes cellular and therefore networkwide dynamical properties of the brain, and is known to regulate information transmission and storage. Here we investigate how changes in cholinergic levels, acting specifically through the activation of M1 receptors and the associated m-currents on both excitatory pyramidal neurons and inhibitory interneurons, affect excitatory/inhibitory balance in biophysical in-silico network models. We monitor emerging E/I balance as well as activation patterns and synaptic currents throughout a small-world network of Hodgkin-Huxley neurons. We show that in networks expressing M1 receptors on inhibitory interneurons, high ACh leads to competitive activation among excitatory neurons due to strong inhibition from the interneuron population. This also creates interacting theta/gamma band oscillations through a PING-like mechanism. Conversely, low levels of ACh lead to disinhibition of the excitatory cell population and formation of global slow oscillatory patterns. These effects of ACh on network dynamics are in part determined by the connectivity within and between excitatory and inhibitory populations, which further impacts E/I balance. We hypothesize that the observed neuromodulation of E/I balance and consequent control of network activation patterns will lead to distinct roles of the aforementioned vigilance states in memory storage and consolidation.

Disclosures: B.E. Fry: None. **M. Zochowski:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.05/XX31

Topic: B.07. Network Interactions

Support: NSF Grant 1651396

Title: Modelling naturally occuring long-term changes in hippocampal excitatory synapses using in-vivo spiking activity

Authors: *A. MANKILI[1], N. REN[4], I. H. STEVENSON[1,2,3];
[1]Dept. of Psychological Sci., [2]Dept. of Biomed. Engin., [3]Connecticut Inst. for Brain and Cognitive Sci., Univ. of Connecticut, Storrs, CT; [4]Allen Inst., Allen Inst., Seattle, WA

Abstract: Many studies have shown that long-term plasticity can be induced in behaving animals using electrical or optogenetic stimulation. During natural, ongoing brain activity long-term plasticity is also expected to occur spontaneously and to generate predictable fluctuations in synaptic strength over time. Here we examine to what extent fluctuations in putative synaptic efficacy can be explained by a model of activity-dependent long-term plasticity. Using large-

scale spike recordings in mice from the Allen Institute Neuropixels dataset, we first detect putative excitatory synaptic connections within the hippocampus based on cross-correlations between the spike trains of millions of pairs of neurons. Majority of these putative excitatory connections have a broad presynaptic neuron spike waveform and a narrow postsynaptic neuron spike waveform suggesting a large proportion of putative excitatory-inhibitory synapses. For the subset of pairs (~500) where a transient, excitatory effect was detected, we use a model-based approach to track fluctuations in synaptic efficacy. Previous work found that these fluctuations can be partially predicted from pre- and postsynaptic firing rates and models of short-term plasticity. Here we additionally model naturally occurring long-term potentiation and depression using the Bienenstock-Cooper-Munro (BCM) rule. We find that modeling the covariance of pre- and postsynaptic activity improves prediction of efficacy fluctuations, and we use this finding to interpret synaptic changes associated with the hippocampal theta rhythm and sharp-wave ripples.

Disclosures: **A. Mankili:** None. **N. Ren:** None. **I.H. Stevenson:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.06/XX32

Topic: B.07. Network Interactions

Support: NIH NIBIB
NIH NIMH
NIH NEI
Simons Foundation
NIH R01NS121913

Title: Interpreting area-to-area differences in spiking variability using spiking network models

Authors: *S. WU[1], A. C. SNYDER[3], C. HUANG[4], M. A. SMITH[2], B. M. YU[2], B. DOIRON[5]; [1]Neurosci. Inst., [2]Dept. of Biomed. Engin., Carnegie Mellon Univ., Pittsburgh, PA; [3]Dept. of Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; [4]Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [5]Grossman Ctr. for Quantitative Biol. and Human Behavior, Univ. of Chicago, Chicago, IL

Abstract: Different brain areas are involved in different brain functions and show different spiking variability. How does the different spiking variability emerge as the result of the differences in the underlying biological circuits? It is difficult to experimentally measure the anatomical properties of the biological circuits. A commonly-used approach in theoretical neuroscience is to use a network model with biologically interpretable parameters as a mathematical abstraction of the biological circuits. We fitted a spiking neural network (SNN) model to macaque V4 and PFC population activity to understand how the different model circuits underlie different spiking variability in the two areas. This could help explain how the

difference in the biological circuits across brain areas gives rise to different spiking variability. We analyzed Utah array recordings in V4 and PFC of rhesus monkeys performing a spatial attention task. We used activity statistics, including the firing rate, Fano factor, spike count correlation between pairs of neurons, and population-level statistics based on factor analysis to characterize the spiking variability of the neural recordings (Williamson et al, 2019). Across the two monkeys, V4 showed a lower firing rate, a higher spike count correlation, and a larger percentage of variance shared across the neuronal population than PFC. To interpret such differences in spiking variability between V4 and PFC, we fitted a large-scale spiking network, the spatial balanced network (SBN, Huang et al, 2019), to reproduce the recorded spiking activity. The fitted models reproduce the aforementioned activity statistics well. We observed consistent differences in the fitted circuit models between V4 and PFC across the two monkeys: V4 models have more widely spread neuronal connections and weaker feedforward connections to inhibitory neurons than PFC. Such differences in the model circuits suggest similar differences in the underlying biological circuits of V4 and PFC that underlie the higher co-fluctuation and lower firing rate of V4 activity. Our work advances the mechanistic understanding of the heterogeneity of spiking activity patterns of different brain areas.

Disclosures: **S. Wu:** None. **A.C. Snyder:** None. **C. Huang:** None. **M.A. Smith:** None. **B.M. Yu:** None. **B. Doiron:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

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Program #/Poster #: PSTR577.07/XX33

Topic: B.07. Network Interactions

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N00014-21-1-2290
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Title: Spiking lateral-inhibition circuitry explains disparate cortico-striatal physiological, decision-making, and learning phenomena.

Authors: *A. PATHAK[1,2], S. BRINCAT[3], S. SENNEFF[4], L. R. MUJICA-PARODI[4], E. K. MILLER[3], R. GRANGER[2];
[1]Dept. of Psychological and Brain Sci., [2]Dartmouth Col., Hanover, NH; [3]MIT, Cambridge, MA; [4]Stony Brook Univ., Stony Brook, NY

Abstract: How do brain cell signals (spikes; field potentials) encode information (perceptual and memorial) and compute outcomes (actions, decisions)? Artificial “neural networks” are often used as replacements for neural circuitry; here we incorporate only well-studied physiological characteristics of neurons, within known anatomical circuit organizations. The resulting

simulation is not “trained” on any experimental data sets; it solely engages in voltage-based activity (transients and spikes) in response to both internal (tonic) and external (sensory) stimuli. Distinctive cortical and striatal circuitry incorporates differential excitatory-inhibitory numbers and axon radii; lateral inhibition; transmitters and their time courses; and three distinct localized forms of synaptic change (beta-gamma cortico-cortical LTP; beta-spike LTP reversal; cortico-striatal potentiation via synapse-specific DA and ACh afferents from SNc and TANs respectively). Simulated rhythmic oscillatory activity is driven in part via ascending ACh (basal forebrain), NE (locus coeruleus), and DA (VTA) afferents to particular subclasses of cortical cells. The simulation’s activity is compared side by side with empirical data from macaques, both in response to a designed set of category-learning visual stimuli.
i) Local spike summation patterns in the model approximate specific aspects of empirical field potential measures.
ii) Synchrony between cortical and striatal firing occurs in both model and experimental data.
iii) Experimental learning-related, error-related, and category-related changes to synchrony are replicated in the model, and explained by tests of model parameters.
iv) Novel condition-specific spiking is identified in the simulation, and subsequently confirmed in the empirical data, unveiling a previously unrecognized internal encoding.
These and several additional findings represent a rare instance in which a model generates novel predictions of telencephalic activity which are then confirmed empirically.

Disclosures: **A. Pathak:** None. **S. Brincat:** None. **S. Senneff:** None. **L.R. Mujica-Parodi:** None. **E.K. Miller:** None. **R. Granger:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.08/XX34

Topic: B.07. Network Interactions

Support: ERC Advanced Grant No. 694829
Swiss Data Science Center Project Grant No. C18-10

Title: Ensemble learning and ground-truth validation of synaptic connectivity inferred from spike trains

Authors: C. DONNER[1], J. BARTRAM[2], P. HORNAUER[2], T. KIM[2], D. ROQUEIRO[2], A. HIERLEMANN[2], G. OBOZINSKI[1], ***M. SCHRÖTER**[2];
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Abstract: Probing the architecture of neuronal circuits and the principles that underlie their functional organization remains an important challenge of modern neurosciences. This holds true, in particular, for the inference of neuronal connectivity from large-scale extracellular recordings. Despite the popularity of this approach and a number of elaborate methods to

reconstruct networks, the degree to which synaptic connections can be reconstructed from spike-train recordings alone remains controversial. Here, we provide a framework to probe and compare connectivity inference algorithms, using a combination of synthetic and empirical ground-truth data sets, obtained from simulations and parallel whole-cell patch-clamp and high-density microelectrode array (HD-MEA) recordings in vitro. We find that reconstruction performance critically depends on the regularity of the recorded spontaneous activity, i.e., their dynamical regime, the type of connectivity, and the amount of available spike train data. We find gross differences between different algorithms, and many algorithms have difficulties in detecting inhibitory connections. We therefore introduce an ensemble artificial neural network (eANN) to improve connectivity inference. We train the eANN on the validated inputs of six established inference algorithms, and show how it improves network reconstruction accuracy and robustness. Overall, the eANN performed well across different dynamical regimes, with fewer data, and was able to infer connections reliably. Results indicated that the eANN also improved the topological characterization of neuronal networks. The presented methodology contributes to advancing the performance of inference algorithms and facilitates our understanding of how neuronal activity relates to synaptic connectivity.

Disclosures: **C. Donner:** None. **J. Bartram:** None. **P. Hornauer:** None. **T. Kim:** None. **D. Roqueiro:** None. **A. Hierlemann:** None. **G. Obozinski:** None. **M. Schröter:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.09/XX35

Topic: B.07. Network Interactions

Support: NIH NINDS K22NS104187
SRI Barnard College

Title: Characterizing the oviIN connectome using agglomerative clustering

Authors: *R. A. WEBER LANGSTAFF[1], S. C. THORNQUIST[3], G. J. GUTIERREZ[2];
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[3]Rockefeller Univ., New York, NY

Abstract: The oviposition inhibitory neurons (oviINs) of the Drosophila melanogaster brain are a pair of GABAergic neurons speculated to prevent oviposition, but the recently published connectome suggests a more complex story. The neuron forms bidirectional connections with many neurons from neuropil across the brain, most of which are not sexually dimorphic, is not sexually dimorphic itself, and is one of the most interconnected neurons in the entire nervous system, receiving input from or projecting to 1379 cell types out of the roughly 4000 distinct cell types in the hemibrain. We used the Janelia FlyEM Hemibrain Neuprint database to characterize the circuitry interacting with the oviIN. We found that the oviIN tends to form reciprocal

synapses, with many of its inputs receiving output of similar measure, comparable to the other giant inhibitory interneurons of the mushroom body (MB). We retrieved the oviIN's complete network defined by its immediate neighbors (pre- and post-synaptic to the oviINs) and one step further back to connections between the neurons connected to the oviIN - over 600,000 connections in total. We applied an agglomerative clustering algorithm to the oviIN's complete network which revealed groupings of neurons that had a similar number of connections to specific areas with a high level of reciprocal connectivity. We used this same method on the oviIN's immediate neighbors and found two distinct groupings in the superior medial protocerebrum (SMP), a grouping in the lateral accessory lobe and one in the mushroom body output neurons for the oviIN's pre-synaptic connections. In contrast, the post-synaptic targets showed the SMP cell types being categorized very close to each other and possibly into several distinct subclusters, leading us to hypothesize that the oviIN's function could encompass a sort of normalization as seen in the MB. These analyses have provided a characterization of the oviIN's connectivity and continued analysis will allow us to address whether the oviIN serves as an intermediary linking various sensory and behavioral areas in the hemibrain.

Disclosures: R.A. Weber Langstaff: None. **S.C. Thornquist:** None. **G.J. Gutierrez:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.10/XX36

Topic: B.07. Network Interactions

Title: Cortico-hippocampal wave interaction: Insights from neural mass models

Authors: A. BEHLER[1], S. SONKUSARE[2], L. COCCHI[3], *M. BREAKSPEAR[1];
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[3]Queensland Brain Inst., Brisbane, Australia

Abstract: Understanding the dynamics of the brain's cognitive functions involves unraveling the intricate interactions between its various regions. The interplay between the hippocampus and the cortex is paramount to memory encoding and retrieval. Both structures exhibit waves propagating oscillatory activity. The focus of this study is to investigate the role of wave-wave interactions underlying the integration of these two brain areas.The theory of cortico-hippocampal interactions suggests that they align along principal gradients, with the primary hippocampal gradient functionally embedded within a principle gradient of cortical structure and function. This study explores new perspectives on cortico-hippocampal interactions using nonlinear neural mass models. The results provide new insights on the complex nature of phase-phase interactions, revealing a clear emergence of preferred wave directions and the disrupting effect of local impurities on cortico-hippocampal wave interactions. The computational results yield specific interactions that could be tested with empirical data, such as intracranial data acquired while epilepsy patients undertake perceptual; and cognitive tasks.By investigating the

emergence of wave directions, wave-wave interactions, and the influence of wave dephasing on underlying spiking, our research will offer new insights into the nature of cortico-hippocampal interactions and their implications for understanding cognitive function.

Disclosures: **A. Behler:** None. **S. Sonkusare:** None. **L. Cocchi:** None. **M. Breakspear:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.11/XX37

Topic: B.07. Network Interactions

Support: (NIH) U01 MH114829: Anatomical characterization of neuronal cell types of the mouse brain
NINDS (NIH) Grant R01NS39600: Generation and description of neuronal morphology and connectivity
(DOE) DE-SC0022998: CRCNS22 Learning Rules in the Hippocampus and their Mapping to Neuromorphic Systems

Title: A full-scale CA3 spiking neural network model of cell assembly formation and retrieval

Authors: *J. D. KOPSICK[1,2], G. A. ASCOLI[1,2];
[1]George Mason Univ. Interdisciplinary Neurosci. Phd Program, Fairfax, VA; [2]Bioengineering Dept. and Ctr. for Neural Informatics, Structures, & Plasticity, George Mason Univ., Fairfax, VA

Abstract: While the neuron is typically thought of as the functional unit of neural computation, seminal theories and recent experiments suggest that cell assemblies are the entities encoding for cognitive content. In the hippocampus, these cell assemblies are assumed to reflect the integrated spatial, temporal, sensory, and emotional content of episodic memories, formed by organisms to remember prior experience for future utilization. The formation and retrieval of cell assemblies has been extensively researched theoretically in Cornu Ammonis area 3 (CA3), the hippocampal subregion crucial for the auto-associative operation. However, it is not well understood how cell assemblies are formed and recalled in an actual CA3 circuit, with its observed diversity of glutamatergic and GABAergic neurons, sparse connectivity, and synaptic plasticity. Therefore, we have leveraged the knowledge base Hippocampome.org to build and simulate a full-scale spiking neural network (SNN) of the mouse CA3 for a data-driven modeling investigation of cell assembly formation and retrieval. Importantly, we have developed new and enhanced previous metrics to quantify assembly formation and retrieval. We found that a realistic CA3 SNN, with neuron types and numbers, input-output functions, synaptic signaling, and connection probabilities all derived from experimental data, can robustly store and reliably retrieve cell assemblies, even when only 20% of an assembly is activated. This model can help further understand the contributions of distinct CA3 neuron types in the successful formation and retrieval of cell assemblies, as well as the network dynamics affecting storage capacity.

Disclosures: **J.D. Kopsick:** None. **G.A. Ascoli:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.12/XX38

Topic: B.07. Network Interactions

Support: FRS-FNRS

Title: Unraveling the role of collective bursting neurons, quiet waking, and structural plasticity in memory consolidation using a computational approach

Authors: *K. JACQUERIE, E. KELLENS, J. MAGIS, P. SACRÉ, G. DRION;
Univ. of Liege, Liege, Belgium

Abstract: When memorizing new information, it is commonly accepted that breaks associated with brain rest can improve performance. We investigate this hypothesis using a computational approach. Our neural network, composed of conductance-based model neurons, simulates brain states, transitioning from active learning to quiet waking. It corresponds to a neuronal switch from *tonic firing* to *collective bursting* orchestrated by neuromodulators. Simultaneously, the network modifies synaptic weights through plasticity to encode new memories. Recent findings reveal a *homeostatic reset* induced by collective bursting across various traditional synaptic plasticity rules (pair-based, triplet, or calcium-based rule). Unintuitively, strong weights depress, and weak weights potentiate during bursting until a set point is reached, causing forgetting but also restoring synaptic weights and facilitating new memory formation. We propose a *structural plasticity* rule that complements traditional synaptic plasticity rules governing early-stage Long-Term Potentiation (E-LTP) and provides insights into late-stage Long-Term Potentiation (L-LTP). In our study, we demonstrate the efficacy of this novel mechanism across diverse memory tasks. Initially, we observe that quiet waking underlying collective bursting enhances the Signal-to-Noise Ratio in a pairing memory task. Moving on to a pattern recognition task, the network adeptly learns to identify small patterns, whether overlapping or not. We thoroughly analyze the evolution of receptive fields, represented by pattern-associated weight matrices, during switches from active learning to quiet waking states. Remarkably, during quiet waking periods, memory consolidation occurs without any pattern recall. Extending this approach to the MNIST recognition task leads to notable improvements in performance. In all tasks, blocking quiet waking states decreases the ability to consolidate memory. In conclusion, combining quiet waking with bursting neurons and structural plasticity improves learning and memory consolidation. This research aims to inspire investigations into the biophysical mechanisms of quiet waking in memory and the potential integration of resting states in machine learning algorithms for artificial intelligence.

Disclosures: **K. Jacquerie:** None. **E. Kellens:** None. **J. Magis:** None. **P. Sacré:** None. **G. Drion:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.13/XX39

Topic: B.07. Network Interactions

Title: Feedforward and feedback inhibition support classification differently in a spiking neuronal network

Authors: *D. MÜLLER-KOMOROWSKA, T. FUKAI;
Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: Biological neuronal networks can learn throughout their lifetime from few data points using little energy. Because of these strengths, some biological features could prove useful in artificial neuronal networks. Inhibition is one such feature that is nearly ubiquitous across brain areas and species. We incorporate feedforward and feedback inhibition into a layer of spiking point neurons and use the resulting spike trains to classify samples from machine learning tasks. Our preliminary data show that inhibition can improve classification from spike trains. For tasks with temporal structure (e.g. speech classification) inhibition is also energy efficient. Based on previous work we hypothesize that inhibition converts temporal information into sparse rate information, which makes its overall effect dependent on the information encoding at the input. We also discuss the general rational of transmitting information by inhibiting rather than eliciting spikes. Our results contribute to the effective usage of inhibitory microcircuits in spike-based algorithms.

Disclosures: **D. Müller-Komorowska:** A. Employment/Salary (full or part-time):; Okinawa Institute of Science and Technology. **T. Fukai:** A. Employment/Salary (full or part-time):; Okinawa Institute of Science and Technology.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.14/XX40

Topic: B.07. Network Interactions

Support: Grant GR43782

Title: Modeling the role of ketamine on excitation-inhibition balance in the olfactory bulb

Authors: *E. A. SMITH[1], K. E. DOXEY[1], J. BIRGIOLAS[2], R. C. GERKIN[1], S. M. CROOK[1]; [1]Arizona State Univ., Tempe, AZ; [2]Ronin Inst., Phoenix, AZ

Abstract: Ketamine is an NMDA receptor (NMDAR) antagonist used for anesthesia and pain management that has been gaining attention for its use as an antidepressant. Ketamine elicits rapid-acting antidepressant effects, which are mediated by complex interconnected mechanisms and alter the excitation-inhibition balance in networks of neurons. One mechanism of action of ketamine thought to explain its antidepressant effect is that ketamine modulates inhibitory GABAergic interneuron activity by disinhibiting excitatory neurons. In transgenic mice that lack functional NMDARs on GABAergic interneurons, ketamine fails to induce antidepressant effects, suggesting that ketamine selectively blocks NMDARs on inhibitory interneurons. Ketamine disrupts the excitation-inhibition balance in hippocampus by disinhibiting pyramidal cells - specifically, by reducing IPSP amplitude and increasing synaptic-driven action potentials without affecting intrinsic excitability. Ketamine has been found to impact dynamics in the olfactory bulb (OB) by regulating synchronous activity of neuron populations. In this project, a biophysically realistic computational model of a local subnetwork of rodent OB is used to investigate how ketamine impacts network dynamics. This network model includes 10 mitral cell (MC), 23 tufted cell (TC), and 170 granule cell (GC) models, and is used to measure OB activity in response to simulated odor inputs. We simulate the action of ketamine by blocking NMDARs at the synapses between GABAergic GCs and glutamatergic MC/TCs. Additionally, we simulate ketamine's effect on AMPARs by increasing synaptic AMPAR activity due to extra glutamate in the synapse. In the presence of ketamine, due to blocked NMDARs, GCs receive less excitatory input from MC/TCs, causing decreased inhibition from GCs onto MC/TCs. Local Field Potentials (LFPs) are generated from the model to predict how ketamine changes population activity. With ketamine, the OB model exhibits greater overall excitatory MC/TC activity due to ketamine-induced disinhibition. As expected, ketamine decreases IPSP amplitudes and increases the number of action potentials in response to simulated odor inputs. With this detailed model, we can better understand the excitatory-inhibitory interactions modulating network activity in the OB that are thought to initiate ketamine's rapid antidepressant effects.

Disclosures: E.A. Smith: None. **K.E. Doxey:** None. **J. Birgiolas:** None. **R.C. Gerkin:** None. **S.M. Crook:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.15/XX41

Topic: B.07. Network Interactions

Support: NIH grant R01MH112746

Title: Spectral states in human cortex exhibit global shift in bimodal dynamical regimes

Authors: *H. M. LEFCOCHILOS-FOGELQUIST[1], J. D. MURRAY[2], A. ANTICEVIC[3], M. HELMER[2];
[2]Dept. of Psychiatry, [3]Psychiatry, [1]Yale Univ., New Haven, CT

Abstract: There is increasing evidence that large-scale cortical dynamics can exhibit marked variation in resting-state over a range of timescales – the association of such variation being putative state changes related to arousal, perceptual state, and so forth. Such putative state changes have primarily been documented in FMRI signals, particularly FC measures, but less so in electrophysiological data. Here, we adopt a methodological approach which can account for dynamic changes in spectral signals of electrophysiological data by explicitly using a measure of spectral variation over time, for which we can generate single-variable time courses that give insight into the real-time variation of spectral dynamics across various timescales. Using data from eyes-open resting-state MEG recordings of 89 subjects – composed of three 6-minute sessions per subject – from the Human Connectome Project (HCP), we identify a dominant mode of spectral variation (encompassing the range 4-40Hz) across cortex, which can exhibit slow-timescale bimodal modulations that are synchronous across all cortical areas. Notably, these cortex-wide modulations can be absent, arrhythmic, or oscillatory for single sessions, and thus correspond to distinct dynamical regimes. Using a canonical model of nonlinear dynamics with stochastic noise and adaptation we are able to recapitulate the major qualitative features of these observed asynchronous unimodal and rhythmic bimodal dynamics as well as transitions between them. We propose that these distinct dynamical regimes in spectral dynamics corresponds to putative state changes, and hypothesize that their appearance is an index of arousal and may have possible implications for other psychological and physiological states. Future studies can explore how these dynamical regimes are impacted by pharmacological manipulation and relate to non-stationarity in task performance. Additionally, our non-linear canonical model imparts insight into the mechanism underpinning these dynamical regimes, and could serve as a basis for large-scale cortical models.

Disclosures: H.M. Lefcochilos-Fogelquist: None. **J.D. Murray:** None. **A. Anticevic:** None. **M. Helmer:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.16/Web Only

Topic: B.07. Network Interactions

Support: FRIPRO grant #324239

Title: Dendritic spine morphology modulates extracellular and post-synaptic electrodiffusion

Authors: *E. HAUGE[1], M. HERNANDEZ-MESA[1], P. RANGAMANI[2], M. E. ROGNES[1]; [1]Simula Res. Lab., Oslo, Norway; [2]UCSD, La Jolla, CA

Abstract: Changes in spine morphology represent an integral part of spine plasticity [1] and play a key role in long-term potentiation in part by modulating excitatory postsynaptic potentials (EPSPs) [2]. However, the dynamics of local variations in the ion concentrations and electric potentials within the spine head, neck and dendritic branch, as well as in the extracellular space (ECS), are poorly quantified. Taking advantage of high-fidelity computational modelling and simulation, here, we estimate the impact of changes in dendritic spine morphology; i.e. changes in their shape and size, on postsynaptic potential dynamics. Our approach builds on an electrodiffusive Extracellular-Membrane-Intracellular framework, coupled with the Kirchhoff-Nernst-Planck approximation for electroneutrality [3] and Hodgkin-Huxley-type descriptions of membrane ion channels. We predict the evolution of concentration distribution of key ion species (Na^+, K^+, Cl^-) in addition to the electric potential intracellularly, across the membrane and in the surrounding ECS in response to excitatory and inhibitory membrane currents. The resulting spatio-temporal fields then yield key quantities of interest such as e.g. the spine neck resistance and EPSP shape and intensity. By systematically comparing idealised and microscopy-based spine geometries [4], we evaluate the ephaptic and self-ephaptic effects as well as the magnitude and directionality of induced electric drift. These findings answer important questions regarding the role of electrodiffusion and electric drift on intracellular signalling, and provide a basis for more accurate predictive biophysical modelling of intracellular signalling pathways in general and Ca^{2+} dynamics in particular [4] under physiological and pathological conditions. Such models have the potential to generate experimentally testable predictions for voltage propagation in the plasma membrane of dendritic spines [5].
1. Tønnesen et al. Spine neck plasticity regulates compartmentalization of synapses. Nat Neurosci 17, 678-685 (2014).
2. Lagache et al. Electrodiffusion models of synaptic potentials in dendritic spines. J Comput Neurosci 47, 77-89 (2019).
3. Ellingsrud et al., Finite Element Simulation of Ionic Electrodiffusion in Cellular Geometries. Front Neuroinformatics 14. (2020).
4. Bell et al., Dendritic spine morphology regulates calcium-dependent synaptic weight change. J Gen Physiol, 154(8):e202112980 (2022).
5. Peterka et al., Imaging Voltage in Neurons. Neuron. (2011). 13;69(1):9-21.

Disclosures: E. Hauge: None. **M. Hernandez-Mesa:** None. **P. Rangamani:** None. **M.E. Rognes:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.17/XX42

Topic: B.07. Network Interactions

Support: ERC Synergy Grant 951319
The Gatsby Charitable Foundation
The Kavli Foundation

Title: Uncovering functional connectivity in continuous attractor network models

Authors: *I. A. DAVIDOVICH[1,2], S. GONZALO COGNO[2], Y. BURAK[1];
[1]Edmond and Lily Safra Ctr. for Brain Sci. and Racah Inst. of Physics, Hebrew Univ. of Jerusalem, Jerusalem, Israel; [2]Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway

Abstract: Recent technologies that enable large scale recordings offer new ways to test theoretical predictions on the relationship between network connectivity, neural activity, and computational functions. Among the most widely studied neural network models are those of continuous attractor networks (CANs). CAN models have been used to model a wide range of brain functions, ranging from visual orientation tuning to decision making, and are thought to underlie the firing patterns of head-direction and grid cells. The availability of simultaneous recordings from hundreds or thousands of cells, all participating in the same putative CAN network, raises the question of whether neural connectivity can be inferred in such networks from the spiking activity. This task is challenging, however, because correlations between neurons might arise in the absence of direct connections and lead to wrong estimates of connectivity. Thus, it remains to be determined if connectivity can be reliably inferred in CAN models. Here we explore different approaches to connectivity inference in simulated CANs and show that accounting for the patterns of neural covariation encoded in the low-dimensional attractor manifold can reveal features of the true connectivity. We also highlight the importance of evaluating the credibility of the inference process. Finally, we analyze the problem of finding an ordering for the neurons where the signature characteristics of CAN connectivity can be revealed and show that this is a non-trivial problem due to the presence of mathematically equivalent solutions. Our inference methods suggest that some features of CAN connectivity could be tested experimentally. Moreover, our methods could be generalized to other systems operating on low-dimensional manifolds.

Disclosures: I.A. Davidovich: None. **S. Gonzalo Cogno:** None. **Y. Burak:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

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Program #/Poster #: PSTR577.18/XX43

Topic: B.07. Network Interactions

Support: NIH NBIB Grant U01GM104604
NIH NBIB Grant U01EB025830
Army Research Office Grant W911NF2110091

Title: Network size and sparsity of input activity affect the stability window for synaptic connection strength required for pattern recall in a simulated CA1 network

Authors: *Z. Z. CHOU[1], J.-M. C. BOUTEILLER[2], T. W. BERGER[2];
[2]Biomed. Engin., [1]USC, Los Angeles, CA

Abstract: It is widely accepted that the hippocampal circuitry responsible for storing and recalling memories can be modeled as an attractor network. Theoretical investigations on such networks have revealed that the sparseness of spatiotemporal input patterns, and by extension the sparseness of associational network connectivity, plays an integral role in the storage capacity of the network. Sparser connectivity allows a greater number of patterns to be stored and recalled with higher fidelity. In simulated models of the CA3 and CA1 subfields, it has been demonstrated that the strength of the connections between different cell types, particularly the inhibitory interneurons, must remain within specific ranges to maintain network stability so that stored patterns can be retrieved correctly, or even for pattern encoding to occur at all. The interplay between the required connection strength, sparseness of input pattern activity, and network size has not yet been well characterized, as large-scale investigations on memory capacity have largely been conducted using binary firing neurons that do not take connection weights into account, whereas studies on the effect of synaptic weights have primarily been conducted at a fixed small-scale. This study investigates the impact of network size and the sparsity of input activity on the optimal synaptic conductance range necessary for achieving high fidelity pattern recall in a simulated CA1 neuronal network. The network was initialized to store multiple patterns of a given sparsity. The number of patterns was increased until the relative recall error for the stored patterns exceeded a tolerance threshold, thus indicating the storage capacity of the network. We observed that while increasing network size changed the optimal pattern sparsity to achieve the maximal storage capacity, it also decreased the stability window of the synaptic connection strengths to the point where the effective memory capacity of the network was lower than the predicted maximum. Our results provide a functional implication as to why certain levels of sparsity and network connectivity are observed in biology. They also propose limitations to memory capabilities based on the physical constraints, whether structural or pathological in nature, of a given network.

Disclosures: **Z.Z. Chou:** None. **J.C. Bouteiller:** None. **T.W. Berger:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

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Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.19/XX44

Topic: B.07. Network Interactions

Support: NIH NINDS K22NS104187

Title: Characterizing fan-shaped body connections with oviIN neuron using the Drosophila connectome

Authors: *D. SPIRA[1], S. THORNQUIST[2], G. J. GUTIERREZ[1];
[1]Barnard Col., New York, NY; [2]Rockefeller Univ., New York, NY

Abstract: The *Drosophila* fan-shaped body region in the central complex is highly connected and is thought to have a role in context-dependent behaviors. One neuron, known as the oviposition inhibitory neuron (oviIN), has previously been implicated as an inhibitory neuron type involved in oviposition, or egg-laying. However, the oviIN receives a large number of its inputs from fan-shaped body neurons which are seemingly unrelated to oviposition; additionally, the neuron's morphology is the same in both male and female flies, implying that it influences behaviors outside of the egg-laying process. The online mapping of the Drosophila connectome allows for computational analysis of neuronal connections in a detailed reconstruction of the brain. We aim to produce testable hypotheses about the behaviors that oviIN influences by using connectome data to characterize its connectivity to the central complex. To this end, we have quantified the distributions of connectivity strengths and overall connectivity network between fan-shaped body-type neurons and oviIN. We have used these patterns to determine the nature of the fan-shaped body inputs and the prominence of those connections to oviIN relative to other connections made by fan-shaped body neurons. The data revealed that one fan-shaped body cell type, the FS1As, is the largest input cell type to oviIN, leading to further investigation as to whether FS1A's connectivity patterns to oviIN are different from other connections made by fan-shaped body neurons or made to oviIN in general. These cells are also exceptional in that they show the greatest asymmetry between input to the oviINs and input from the oviINs: the majority of neurons innervating the oviINs receive heavy reciprocal innervation, but the FS1As receive nearly none. In the fan-shaped body, the connectivity of FS1As is similarly distinct, pooling across many more fan-shaped body cell classes than any other output population of the fan-shaped body. None of the other major inputs to oviIN seem to display this same "hub" phenomenon as FS1A. As oviIN's largest input, FS1A's consolidation may have a significant effect on oviIN's activity. From these results, we hypothesize that FS1A may play a unique role in consolidating information from the fan-shaped body that it passes along to oviIN.

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Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.20/XX45

Topic: B.07. Network Interactions

Support: R01 NS128713-01

Title: Understanding variations in MGB tone responses due to TRN synaptic connectivity

Authors: *A. MENDOZA[1], S. ROLÓN-MARTÍNEZ[2], M. N. GEFFEN[3], J. HAAS[1]; [1]Lehigh Univ. Biol. Sci., Bethlehem, PA; [2]Neurosci., [3]Otorhinolaryngology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Ascending auditory information transmits to the medial geniculate body (MGB) en route to the cortex. The thalamic reticular nucleus (TRN) sends feedback inhibition to thalamic nuclei and is thought to provide attentional control over sensory responses through its inhibitory signaling. Recently, molecular and circuit-specific TRN neuronal subtypes within TRN have been described (Clemente-Perez et al., 2017; Martinez-Garcia et al., 2020), though the specific roles these distinct cell types play during sensory relay remains unknown. To explore this circuitry we optogenetically silenced specific TRN cell subtypes while recording tone-evoked responses in vivo from MGB with a silicon microelectrode array. Selective silencing of parvalbumin (PV) or somatostatin (SOM) TRN cell subtypes resulted in surprisingly diverse responses: facilitation, suppression, or no change in individual MGB cell tone responses. To investigate the circuitry that may underlie variability in MGB responses, we constructed a model of two thalamic relay columns, consisting of a primary thalamic relay cell reciprocally connected to a PV TRN cell and a higher-order thalamic cell reciprocally connected to a SOM TRN cell. Model cells were single-compartment Hodgkin-Huxley models. We then varied the connectivity between these columns. We found that intra-TRN lateral inhibition, or varied reciprocal feedback inhibition between TRN and thalamus, could explain the seemingly opposing effects of TRN silencing on MGB tone responses. Electrical synapses between TRN cells alone did not produce substantial changes in MGB responses, but together with chemical synapses could modulate MGB responses. These results show TRN cell subtypes can exert varying, opposing effects on tone responses of MGB and those differences may result from heterogeneity of connectivity within TRN, or through feedback connectivity across thalamic relay columns. Our models provide specific predictions for future experiments into TRN-thalamic connectivity.

Disclosures: A. Mendoza: None. **S. Rolón-Martínez:** None. **M.N. Geffen:** None. **J. Haas:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.21/XX46

Topic: B.07. Network Interactions

Support: NIH Grant R01 EB034143

Title: Modeling transcranial magnetic stimulation evoked neural responses in a cortical circuit with biophysically realistic neurons

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Abstract: Noninvasive brain stimulations, such as transcranial magnetic stimulation (TMS), offer promising avenues for modulating brain activity in clinical and research domains. However, comprehensively understanding the precise mechanisms underlying TMS remains challenging. To address this, researchers have turned to computational models of neural microcircuits to elucidate the effects of TMS. Previous studies have employed point-neuron networks neglecting neuronal morphology or focused on synaptically isolated neurons exhibiting realistic morphologies. In this study, we present a novel computational cortical circuit model to investigate and quantify the effects of TMS parameters within the primary motor cortex (M1) at both the single-cell and population levels. Our cortical circuit comprises thousands of multicompartmental biophysically realistic neurons interconnected by over 300,000 intra- and interlayer synaptic connections (glutamatergic and GABAergic). These neurons are spatially arranged in six layers to mimic the cytoarchitecture of a cortical column of the cerebral cortex. We integrated the cortical column model into a finite element method (FEM) head model, enabling us to couple the TMS-induced electric fields. In this study, we measured the spike activities of individual single neurons and the local field potential (LFP) of the populations. The validity of our model was established by successfully reproducing several key findings from existing experimental literature, including the timing and dose-response relationship of TMS-evoked short-latency responses. Preliminary results suggest that 1) the majority of L5 pyramidal cells are directly activated by single-pulse TMS, and 2) L2/3 and L6 pyramidal cells are indirectly activated. In contrast, L1 inhibitory cells are unlikely to be directly affected by TMS. Our findings highlight that direct neural activation is predominantly influenced by the magnitude of the electric field. Additionally, based on previous literature indicating the involvement of power and phase of intrinsic neural oscillations in mediating motor cortical output, we tested this hypothesis by stimulating the cortical column at different phases of the LFP signal. We observed increased excitability of L5 pyramidal neurons during the trough of the LFP signal. Overall, our comprehensive model provides insight into the neuronal and synaptic activities evoked by TMS. Our computational approach creates a powerful tool for researchers to investigate the physiological effects of TMS on individual neurons and neuronal assemblies, thereby enabling exploration of a wide range of parameters.

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Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.22/XX47

Topic: B.07. Network Interactions

Support: Epilepsiforbundets forskningsfond
Research Council of Norway grant #324239

Title: An electrodiffusive network model with multicompartmental neurons and synaptic connections

Authors: *M. SÆTRA[1], Y. MORI[2];
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Abstract: Most computational models of neurons assume constant intra- and extracellular ion concentrations over simulated time, which is generally reasonable due to the small number of ions involved in generating action potentials and the regulatory mechanisms maintaining long-term ion homeostasis. However, during periods of intense neuronal activity or pathological conditions like epilepsy and spreading depression, ion concentrations can vary significantly. To capture such scenarios, we require models that account for dynamic changes in ion concentrations.

A key challenge when modeling ion concentration dynamics is keeping track of all ionic movement in a biophysically consistent manner that accurately represents the electrodiffusive nature of ion transport within and between cells. In previous work, we have presented biophysically consistent models of single-cell neurons [1, 2] and space-averaged neural tissue [3] based on the Kirchoff-Nernst-Planck equations. However, the neuroscience community still needs a comprehensive modeling framework to investigate the impact of ion concentration changes on networks of neurons connected by chemical synapses.

In this work, we propose an electrodiffusive, ion-conserving model of a neuronal network that extends prior research on single-cell modeling [2]. The model incorporates multicompartmental neurons comprising soma and dendrites, astrocytes, and extracellular space, and keeps track of ion concentrations (Na^+, K^+, Cl^-, and Ca^{2+}), electrical potentials, and volume fractions in all compartments. Neuronal communication occurs via chemical synapses, while astrocytes are interconnected through gap junctions. We envision the model to make a significant contribution to the field of neuroscience. It provides a valuable tool for investigating the influence of ion concentration dynamics on network behavior and offers insights into various pathological conditions associated with altered ion concentrations.

References

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Disclosures: M. Sætra: None. **Y. Mori:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

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Program #/Poster #: PSTR577.23/XX48

Topic: B.07. Network Interactions

Support: DC015543
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NARSAD Young Investigator Grant to MNI
DC009635
DC012557

Title: Contributions and synaptic basis of diverse cortical neuron responses to task performance

Authors: *J. TOTH[1], B. F. ALBANNA[2], B. DEPASQUALE[4], S. FADAEI[5], O. LOMBARDI[3], T. GUPTA[3], K. KUCHIBHOTLA[6], K. RAJAN[7], R. C. FROEMKE[5,8], M. INSANALLY[3]; [2]Otolaryngology, [3]Otolaryngology, Neurobio., [1]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [4]Biomed. Engin., Boston Univ., Boston, MA; [5]Skirball Inst. for Biomolecular Med., New York Univ. Grossman Sch. of Med., New York, NY; [6]Psychological and Brain Sciences, Neurosci. and Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [7]Dept. of Neurosci. & Friedman Brain Inst., Icahn Sch. of Med. At Mount Sinai, New York, NY; [8]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Neuronal responses during behavior are diverse, ranging from highly reliable 'classical' responses to irregular or seemingly random 'non-classically responsive' firing. While a continuum of response properties is frequently observed across neural systems, little is known about the synaptic origins and contributions of diverse response profiles to network function, perception, and behavior. Here, we combined in vivo cell-attached, extracellular, and whole-cell recordings during behavior with a novel task-performing spiking recurrent neural network (RNN). We recorded from the auditory cortex (AC) of rats and mice during a go/no-go auditory recognition task (rats: d' = 2.8±0.1, N = 15; mice: d' = 2.5±0.1, N = 7). In both species, we observed a wide range of single-unit response types from classically responsive cells that were highly modulated relative to pre-trial baseline to non-classically responsive cells with relatively unmodulated firing rates. To relate synaptic structure to spiking patterns over the response-type continuum, we developed a spiking RNN model incorporating both excitatory and inhibitory spike-timing-dependent plasticity trained to perform a similar go/no-go stimulus classification task as behaving animals. This model captured the distribution of heterogeneous responses observed in the AC of behaving rodents. Detailed inactivation experiments revealed that classically responsive and non-classically responsive model units contributed to task performance via output and recurrent connections, respectively. Excitatory and inhibitory plasticity independently shaped spiking responses to increase the number of non-classically responsive units while keeping the full network (all units) engaged in performance. Local patterns of synaptic inputs predicted spiking response properties of network units as well as the responses of auditory cortical neurons from in vivo whole cell recordings during behavior allowing us to predict the functional role of a neuron from the pattern of synaptic inputs.

Strikingly, only non-classically responsive units altered network dimensionality by inducing correlations suggesting these units play a privileged role in determining the scale of network dynamics. Moreover, excitatory and inhibitory STDP had complementary effects on network dimensionality. While each mechanism independently increased the fraction of non-classically responsive units, both rules must be active to preserve high-dimensional activity. Thus, a diversity of neural response profiles emerges from synaptic plasticity rules with distinctly important functions for network performance.

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Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.24/XX49

Topic: B.07. Network Interactions

Title: Unveiling the Neural Architecture of Olfactory Associative Learning in the Fly Brain

Authors: *C.-C. CHARNG, L. CHENG, R.-H. CHEN, K.-L. FENG, C.-C. LO, T.-K. LEE, A.-S. CHIANG;
Natl. Tsing Hua Univ., Hsinchu City, Taiwan

Abstract: Understanding the neural architecture that governs information flow is essential for elucidating the mechanisms underlying learning and memory. This study focuses on the mushroom body (MB), the primary learning center in the fly brain. Olfactory information is conveyed from the antennal lobe (AL) to the MB and the lateral horn (LH) through projection neurons (PN). A central question in the interplay between coding and olfactory associative learning is how PNs project to the coding cells, referred to as Kenyon cells (KCs), within the MB. Previous studies have proposed two competing hypotheses: random and stereotypic projection patterns. Our investigation reveals a hidden structure suggesting that PNs originating from different glomeruli in the AL may exhibit a preference for connecting to specific classes of KCs. Specifically, we observe that non-food-related odors tend to be transferred to γ KCs, while food odors are routed to α/β KCs. This preference leads to distinct activation profiles of KCs in response to different odors. Anatomically, PN cluster 1, which exhibits a preference for γ KCs, densely occupies the dorsal region of the calyx, whereas PN cluster 3, which favors α/β KCs, innervates the ventral region of the calyx to a greater extent. Furthermore, our simulation using DoOR database predicts the empirical response of KCs. Additionally, our simulations demonstrate a tradeoff between odor memory capacity and the ability to generalize. These findings provide insights into the neural processes and network architecture of olfactory learning in fruit fly brains.

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Poster

PSTR577. Computational Modelling of Synaptic Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR577.25/XX50

Topic: B.07. Network Interactions

Title: Global inhibition in head-direction neural circuits

Authors: *C. NING[1], H.-P. HUANG[2], C.-C. LO[2];
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Abstract: Navigation of insects is supported by the central complex which consists of extremely complex neural circuits. Previous studies of the *Drosophila melanogaster* (fruit fly) central complex reported that the head direction, essential information for navigation, is encoded by localized activity (termed activity bump) in two subregions, the ellipsoid body (EB) and the protocerebral bridge (PB). Moreover, detailed connectomic analyses of EB and PB revealed that they form attractor circuits, a network architecture that can support activity bumps based on theories of neural networks. These theories further suggest that feedback inhibition is crucial for the stability of activity bumps. However, several different sets of inhibitory neurons innervate the EB-PB circuits, and their roles and relative contributions to bump stabilization are still not fully understood. To address this issue, we constructed and systematically investigated several variants of biologically realistic neural circuit models based on the recently published EM (electronic microscopy) connectomic data. The circuit models share the basic EB-PB recurrent circuits, which maintain and update the active bump. The differences are the inhibitory mechanism, which is either by ring neurons (ER1) or by delta 7 neurons. We further analyzed the differences between the R models (with ring neurons) and delta models (with delta 7 neurons) in different test conditions. First, we tested the robustness of each model by scanning the available parameter in a large range. Second, we tested the persistency of the bump. Third, we tested the maximum rotational speed that can be supported by the models. Last, we tested how multiple visual stimuli affect bump formation. Our study showed that the R models are more robust while the delta models are more persistent. We also constructed a hybrid model which contains both R and delta 7 neurons and found out that the hybrid model was more stable and better performed than other models in various tests, suggesting the possibility of combined inhibition mechanisms in the head direction circuit.

Disclosures: **C. Ning:** None. **H. Huang:** None. **C. Lo:** None.

Poster

PSTR577. Computational Modelling of Synaptic Networks

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Topic: B.07. Network Interactions

Support: NIH Grant R01NS119520

Title: Evoked Neural Activity: Enhancing Electrode Targeting in Deep Brain Stimulation for Parkinson's Disease

Authors: *M. S. NOOR[1], C. C. MCINTYRE[1,2];
[1]Dept. of Biomed. Engin., [2]Dept. of Neurosurg., Duke Univ., Durham, NC

Abstract: Background: High-frequency Deep Brain Stimulation (DBS) in the Subthalamic Nucleus (STN) is an effective treatment for late-stage Parkinson's disease. Precise targeting of the dorsolateral STN is crucial for therapeutic benefits, but accurate electrode implantation is challenging due to the nucleus's small size. DBS surgeries are typically performed on awake patients, allowing surgeons to evaluate behavioral responses to acute electrical stimulation for target confirmation. This approach offers a reliable method to guide targeting, alongside microelectrode electrophysiology and magnetic resonance imaging. While many patients prefer asleep DBS surgery over traditional awake procedure, challenges arise from the inability to measure behavioral responses and the suppression of intrinsic neural activity due to anesthesia. STN DBS is known to elicit Evoked Neural Activity (ENA) and/or Evoked Resonant Neural Activity (ERNA) in the nuclei. These responses are most prominent in the dorsolateral STN and remain present in asleep patients, suggesting their potential to enhance electrode targeting. Analyzing these responses may also provide insights into the mechanism of action of DBS. **Methods**: The purpose of this work is to understand the origin of ENA, and for this, ENA was reconstructed using a computational model of STN Local Field Potential (LFP). The LFP model incorporated a patient's head, a clinical DBS lead, and approximately 220,000 multicompartment STN model neurons. The neural compartments receive excitatory (AMPA) and/or inhibitory (GABAa) synaptic inputs, mimicking the corticosubthalamic and pallidosubthalamic inputs, respectively. The synapses were modeled using the Tsodyks-Markram synapse model, which can simulate short-term synaptic plasticity. **Results**: ENA consists of two positive (P1 and P2) and one negative peak (N1). P1 appears around ~4 ms, followed by N1 at ~5 ms, and finally, P2 at ~7 ms. Our model demonstrates that P1 is generated by direct activation of pallidosubthalamic fibers, resulting in synchronous inhibition of multiple STN cells. This inhibition causes negative ion flow into the cells, leading to a positive extracellular potential as P1. N1 is produced by subsequent excitatory input through hyperdirect pathway stimulation, while P2 is generated by excitation of GABAergic GPe neurons through subthalamopallidal fiber activation. **Impact**: These findings highlight that ENA arises from the activation of multiple fiber pathways near the STN, emphasizing axon activation as the primary effect of STN DBS. These results have implications for DBS in other diseases and targets.

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come to an institution.; NINDS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hologram Consultants, Neuros Medical, Ceraxis, Qr8 Health, Surgical Information Sciences, CereGate, Cardionomic, Enspire DBS. F. Consulting Fees (e.g., advisory boards); Boston Scientific.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.01/Web Only

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant 1926990
Florida High Tech Corridor Grant 20-10
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Title: Stereology, Deep Learning and Hand-Crafted Algorithms For Quantification Of Neuron Number In Mouse Brains

Authors: *G. DENHAM[1,2], S. S. ALAHMARI[2,3], P. DAVE[2], H. MORERA[2], A. ANDERSON[1], K. SANCHEZ[1], D. DAG[1], P. DELGADO[1], J. RIANO[1,2], L. O. HALL[2], D. B. GOLDGOF[2], P. R. MOUTON[1,2];
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Abstract: Clinical and experimental studies of successful aging, neuroinflammation, neurotoxicology, neurodegenerative disease and other neurological disorders and mental illnesses require reliable methods for quantification of stained neurobiological objects in tissue sections. For decades manual stereology has been the state-of-the-art approach for neuron counts due to avoidance of known sources of systematic error (bias) and the ability to target desired levels of precision through flexible sampling parameters. Limitations of this subjective manual counting approach include the potential for inter-rater error and the need for counting [clicking] over hundreds of cells in each case by a well-trained data collector. Artificial intelligence and hand-crafted algorithms can overcome these obstacles with less tedious, less labor-intensive, and less subjective methods for data collection. Here we assess the performance of the manual optical fractionator method, the gold standard for stereology cell counts, and three automatic and one semi-automatic methods for quantifying the total numbers of Neu-N immunostained neurons [Total NNeuN] in neocortex [NCTX] of male mouse brains (B6, n=7). From one hemisphere of each brain, we sampled NeuN-immunostained cryostat sections cut in a coronal plane (40-µm) in a systematic-random manner through the entire NCTX. All image and data collection were done with assistance from a computerized stereology system (Stereologer®, SRC Biosciences, Tampa, FL). We tested a fully automatic hand-crafted segmentation algorithm called Adaptive Segmentation Algorithm [ASA]; a semi-automatic version where ASA counts were manually corrected for false positives and negatives; and the fully automatic deep learning (DL)-based

Multiple Input Multiple Output [MIMO] approach trained using two different network architectures: image detection using You Only Look Once [MIMO-YOLO]; and image segmentation by U-Net [MIMO-UNET]. All methods were applied to the same stacks of serial z-axis images [disector stacks] through volumes of NCTX collected using the fractionator sampling scheme. Performance metrics were accuracy [% error], reproducibility [Test-Retest] by two blinded data collectors [inter-rater error] and time for supervised data collection. There was comparable accuracy to the gold standard (manual stereology) for all tested methods with lower inter-rater error, higher throughput efficiency and less training time. We also contrast each method in terms of image collection requirements, ease-of-use and technical expertise for data collection.

Disclosures: **G. Denham:** A. Employment/Salary (full or part-time):; SRC Biosciences. **S.S. Alahmari:** A. Employment/Salary (full or part-time):; USF. **P. Dave:** A. Employment/Salary (full or part-time):; USF. **H. Morera:** A. Employment/Salary (full or part-time):; USF. **A. Anderson:** A. Employment/Salary (full or part-time):; SRC Biosciences. **K. Sanchez:** A. Employment/Salary (full or part-time):; SRC Biosciences. **D. Dag:** A. Employment/Salary (full or part-time):; SRC Biosciences. **P. Delgado:** A. Employment/Salary (full or part-time):; SRC Biosciences. **J. Riano:** A. Employment/Salary (full or part-time):; SRC Biosciences. **L.O. 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.B. 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. **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.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.02/XX52

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant MH130472

Title: Neuroimagereg: a web-based platform for enhanced 3d serial-section image registration

Authors: S. HAMZEHEI, R. TRIPP, C. CIANCI, G. RAIMONDI, *L. OSTROFF, S. NABAVI;
Univ. of Connecticut, Storrs Mansfield, CT

Abstract: Image registration is essential for a range of volume-reconstruction approaches in neuroscience, from in vivo imaging to serial-section microscopy methods, and accessible tools for accurate and efficient registration of these complex image sets are needed for many

applications. We have developed a new image registration algorithm along with a user-friendly web-based platform that makes advanced image registration available to all. Our method combines convolutional feature extraction with a Scale-Invariant Feature Transform (SIFT) and a fine-tuned deep learning model called Residual Network with 50 layers (ResNet50) to extract high-level features for image registration. Additionally, we considered a maximum likelihood estimation sample consensus (MLESAC) estimation method for robust outlier rejection, further improving the accuracy of alignment. The web-based platform, developed using a lightweight modern framework, enables users to upload, analyze, and register their images with ease and enhanced precision. Comparative evaluations demonstrate our method's ability to handle various types of distortion and align images more accurately than conventional registration algorithms such as ORB, SIFT with RANSAC, and software packages like bUnwrapJ and TurboJ in ImageJ. Additionally, our tool's powerful feature extraction enables users to build a solid foundation for subsequent analytical processes. We demonstrate our method's ability to register two disparate types of image stacks: serial ultrathin (50 nm) sections stained with fluorescence and MRI images. Our web interface also allows users to apply calculated transforms to all channels of multi-channel images, which is required for serial multiplexing strategies including ultraplex microscopy. Future plans include incorporating user feedback for ongoing refinement of the platform's efficacy and user-friendliness.

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Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.03/XX53

Topic: I.07. Data Analysis and Statistics

Title: Evaluating Spine Identification in Dendritic Spine Counter for ImageJ

Authors: *M. VOLOSHIN[1], A. COMINCINI[2], **J. PARATO**[4,3];
[1]Mighty Data, Inc., Asheville, NC; [2]Columbia Univ., Brooklyn, NY; [3]Columbia Univ., New York, NY; [4]SUNY Empire State, Brooklyn, NY

Abstract: Dendritic Spine Counter is an ImageJ plugin that provides a user interface for performing semi-automatic counts of dendritic spines from minimum or maximum projection images. Dendritic Spine Counter's image recognition algorithms are implemented using hard-coded heuristics based on non-stochastic statistical analysis of adjacent pixel similarities. Here, we test the plugin's ability to identify spines in the following scenarios: Golgi impregnated mouse cortex and hippocampus, DiOlistically labeled cultured rat hippocampal neurons, and DiOlistically labeled mouse hippocampus.

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Poster

PSTR578. Software Tools: Anatomy and Morphology

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Chan Zuckerberg Initiative (2021-240444)
Chan Zuckerberg Initiative (2021-240447)
Chan Zuckerberg Initiative (2022-309537)

Title: The BrainGlobe initiative: an open source neuroanatomy platform for the 21st century

Authors: *A. L. TYSON[1], F. CLAUDI[1], L. PETRUCCO[2], R. PORTUGUES[3], T. BRANCO[1], T. W. MARGRIE[1];
[1]Sainsbury Wellcome Ctr., Univ. Col. London, London, United Kingdom; [2]Inst. Italiano di Tecnologia, Roveretto, Italy; [3]Tech. Univ. Munich, Technische Univ. München, Munich, Germany

Abstract: Neuroanatomy is key for understanding the brain, but software to analyse the data are often single purpose, for one model species and suffer from lack of support following publication. We have established the BrainGlobe Initiative (BGI) - an international, distributed team of users and developers working towards the goal of creating open-source, interoperable and easy to use tools for the analysis of all types of neuroanatomical data.
To analyze data from many samples, it is critical to map individual datasets onto a standard anatomical reference atlas, but neuroscience relies on many animal model species. The BrainGlobe toolkit is therefore not built around a specific brain atlas but rather a generalised atlas framework (the BrainGlobe Atlas API) which is regularly updated with new brain atlases from multiple species.
We have initially focussed on the analysis of whole-brain microscopy data (e.g. from tissue clearing or blockface methods). The BGI software suite includes brainreg, a 3D registration tool for mapping data to an atlas space. Novel tools have also been developed to map specific structures or objects within the brain, such as the volume and location of virus-labelled cells and the location of implanted devices such as recording electrodes. Lastly, brainrender can be used for the visualisation of any data registered to the reference atlas. This includes all data from BGI

software, but importantly, data from other software packages and from large-scale efforts such as the Allen Brain Institute.

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Poster

PSTR578. Software Tools: Anatomy and Morphology

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Program #/Poster #: PSTR578.05/XX55

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant MH125479
NIH Grant EB008374

Title: Subcortical brain segmentation across the human lifespan

Authors: *S. AHMAD, P.-T. YAP;
Dept. of Radiology and Biomed. Res. Imaging Ctr. (BRIC), The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Automated segmentation of subcortical structures from brain MRIs across the human lifespan is crucial for studying the morphological traits of deep brain structures that are associated with development, aging, and diseases. Existing segmentation methods cater to specific life periods (e.g., childhood, adolescence, and adulthood) and are not generalizable to data acquired across the lifespan, resulting in discordant lifespan analysis. Here, we introduce a subcortical segmentation method for brain MRIs acquired from birth to 100 years of age. We aggregated structural MRI data from five lifespan Human Connectome Projects (HCPs): Developing HCP (dHCP), Baby Connectome Project (BCP), HCP Development (HCPD), HCP Young Adult (HCPYA), and HCP Aging (HCPA). We propose a human-in-the-loop subcortical segmentation method as automatically-generated good-quality annotations for subcortical structures across the lifespan are unavailable for neural network training, especially for neonates and infants, whose MRIs exhibit poor and rapidly-changing tissue contrasts. Network training is performed in two stages: (i) Labels of subcortical structures are manually delineated by experts in MRIs of random subjects for different life periods and are used for training the segmentation network (nnUNet). (ii) The predicted subcortical labels from the trained network are refined by experts and pooled with the initial training data to retrain the segmentation network, which is eventually used to segment the subcortical structures in all the MRIs across the human lifespan. We compared our method with the population-specific segmentation methods (i.e., the dHCP pipeline for neonates, atlas-based labeling using iBEAT v2.0 segmentation for infants and children, and FreeSurfer for adolescents and adults). The volumetric trajectories of the subcortical structures segmented with our method vary smoothly across the lifespan, presenting greater consistency than the population-specific methods (Fig. 1).

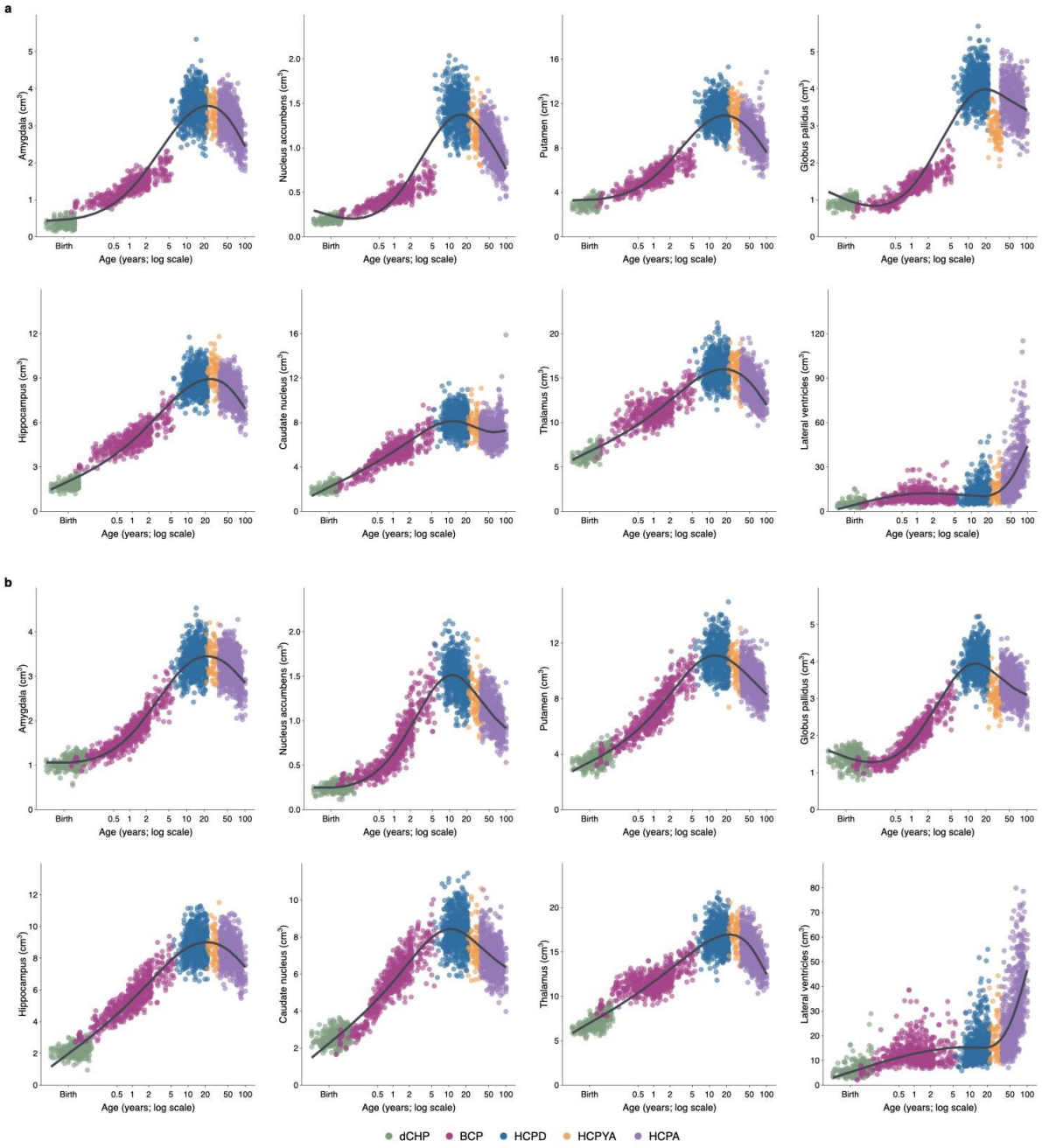

Figure 1: Volumetric trajectories of the subcortical structures segmented with (a) population-specific methods and (b) the proposed method.

Disclosures: S. Ahmad: None. **P. Yap:** None.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.06/XX56

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1RF1MH123402
NIH Grant 1RF1MH124611

Title: Making neuron reconstruction more portable using video compression algorithms

Authors: *L. A. WALKER[1], B. DUAN[2], D. CAI[3];
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Abstract: Several microscopy technologies now enable researchers to routinely capture microscope images in the terabyte-to-petabyte scale. The cost of storing, sharing, and manipulating these images, however, can be significant. Lossless compression methods such as GZIP, LZ4, and ZSTD are common tools for reducing data size on disk but are limited to <4X compression in most microscopy applications, which does not satisfy many data storage requirements. To address this, we propose using video compression using the new Alliance for Open Media Video 1 (AV1) compression codec for storage of microscopy data. AV1 achieves high data compression by using a novel collection of data transforms which are optimized for retaining data quality at low bitrate. We perform benchmarking of several video compression methods and show, depending on quality settings, AV1 allows data compression in excess of 100-fold, reducing whole-brain images to a size storable on a standard desktop. Using a HDF5 filter plugin we developed for AV1, we have integrated support for these new standards into our lab's neuron tracing and annotation software, nTracer2, to allow cloud-based annotation of PB-scale data with low backend cost. Data decompression is GPU accelerated to provide visualization at high speed. We demonstrate that neuron tracing can be performed using image data that has been compressed by >30X with little quality impact on the tracing results of fluorescent reporter mice by performing such tracing on Brainbow microscopy data. By making these tools available to the scientific community, we will improve the ability to share large neuroimages across long distances and potentially reduce the cost for data storage on public data repositories.

Disclosures: L.A. Walker: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LAW and DC have applied for a patent related to this work.. **B. Duan:** None. **D. Cai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LAW and DC have applied for a patent related to this work..

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.07/XX57

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant 1916518
NSF Grant 1912270
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NIH R01MH126699
NIH R01EB030896
NIH R01EB029272
Wellcome Trust Award 226486/Z/22/Z

Title: Visual meridian asymmetry in the structural connectivity of early visual cortical maps.

Authors: *B. CARON[1], F. PESTILLI[2];
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Abstract: It is known that visual field location is preserved through the retina to the brain, a process known as retinotopy(Dumoulin & Wandell, 2008; Holmes & Lister, 1916; Inouye, 1909), allowing for a coherent visual representation. This information can include how far away the stimuli is from the highly sensitive fovea of the retina (i.e. eccentricity), and what quadrant of the visual field the stimuli is coming from (i.e. polar angle).
Recent investigations suggest that asymmetries in visual functionality exist along the vertical and horizontal meridians of the visual cortex. Specifically, contrast sensitivity increases when viewing stimuli along the horizontal meridian as opposed to on either the upper or vertical meridian in a process called horizontal-vertical anisotropy (HVA)(Carrasco et al. 2002; Purokayastha et al. 2021; Himmelberg et al. 2020). HVA has also been identified in cortical surface area estimated using structural magnetic resonance imaging (MRI). However, no investigations have attempted to identify the HVA using structural connectivity patterns of the visual white matter estimated using diffusion MRI.
We evaluated this potential asymmetry in over 1600 healthy participants across a wide age range using automated methods implemented via brainlife.io. Specifically, we developed FAIR (findable, accessible, interoperable, reusable) processing pipeline to estimate polar angle and eccentricity on the cortical surface using a publicly available population receptive fields (pRF) toolbox (Benson et al., 2012, 2014). We used the pipeline to subdivide the visual maps into sub-maps by eccentricity and polar angles. The sub-maps representing the horizontal and vertical meridians were then used to identify the white matter bundles between meridians in pairs of visual maps. The connectivity patterns for 12 visual areas were analyzed.
Asymmetries in connectivity (fiber count density) between visual meridians were identified. More specifically, higher connectivity was estimated between horizontal meridians across areas and smaller counts for vertical meridians. This is consistent with previous results related to function, and structure of the visual system as well as behavioral asymmetries(Benson et al., 2021; Himmelberg et al., 2022). The asymmetries were consistent in 2 independent datasets.
In conclusion, we developed a series of cloud computing applications that can automate the process of tracking and segmenting visual white matter by visual field properties including eccentricity and polar angle. These methods were successfully used to identify the known HVA of the visual system, matching with known visual function and structure.

Disclosures: **B. Caron:** None. **F. Pestilli:** None.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.08/XX58

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

Support: VA SPiRE award 1 I21 RX003728-01A1
Paralyzed Veterans of America (PVA),
Department of Veterans Affairs (VA) Medical Research Service and Rehabilitation Research Service
The Taylor Foundation for Chronic Disease

Title: Open-source virtual reality platform for FAIR and efficient dendritic spine analysis

Authors: *M. REIMER[1,2,4], S. KAUER[3,4], C. BENSON[3,4], S. PATWA[3,4], S. FENG[3,4], M. A. ESTACION[5], L. BANGALORE[3,4], A. TAN[4,2], S. WAXMAN[3,4];
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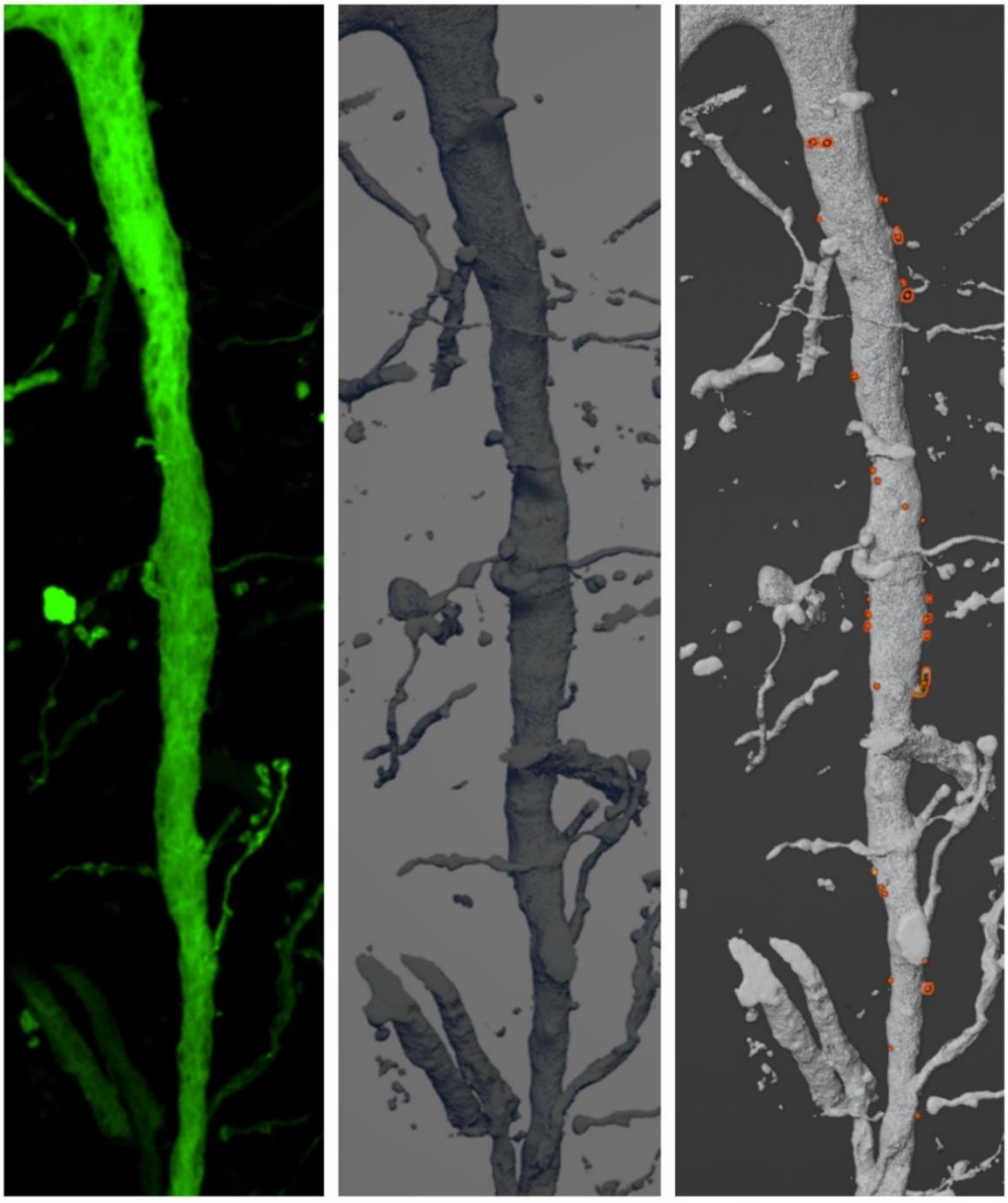
Abstract: Accurate analysis of dendritic spines is crucial for understanding neural connectivity, however existing software tools have limitations in usability, accessibility, and scope of analyses. We present VR-SASE, an open-source software platform using virtual reality (VR) technology for expedited spine analysis. Researchers interact with neural models in a VR environment, enhancing precision, visualization, and control. VR-SASE calculates spine densities and generates morphological measures (length, volume, surface area). Validating our method using drosophila neurons from the DIADEM challenge, we surpassed the gold standard in accuracy. We demonstrated the utility of VR-SASE for studying spine changes in spinal cord injury, supporting the use of romidepsin as a therapeutic agent for spasticity. Incorporation of Neurodata Without Borders and DataJoint software ensures data interoperability, promotes FAIR compliance, and streamlines data organization. Although there are opportunities for refinement, VR-SASE represents a significant advancement in spine analysis with broad applications for neuroscience.



Disclosures: **M. Reimer:** None. **S. Kauer:** None. **C. Benson:** None. **S. Patwa:** None. **S. Feng:** None. **M.A. Estacion:** None. **L. Bangalore:** None. **A. Tan:** None. **S. Waxman:** None.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.09/XX59

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1R24MH114785
NIH Grant R01MH126684

Title: Looking Forward with BossDB: Modern Metadata Solutions, Dynamic Graph Querying, and Streamlined Ingest for Volumetric and Connectomics Datasets

Authors: *H. GOODEN, D. XENES, J. MATELSKY, N. STOCK, S. HIDER, T. GION, E. JOHNSON, W. GRAY-RONCAL, B. WESTER;
Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: As the field of connectomics grows into a critical research area that illuminates the circuitry of the brain, it yields complex multi-modal datasets which require curated solutions for storage and analysis. BossDB has acted as an archive for large-scale electron microscopy (EM) and x-ray microtomography datasets for the past five years and has recently undergone significant development to improve its performance and usability in handling the next generation of datasets. Key advancements include tightly integrated graph querying capabilities via DotMotif and NeuPrint, an improved metadata service, and a streamlined data ingest process. Representing a connectome as a mathematical graph unlocks powerful insights into how the structure and function of a brain may intersect. With DotMotif, our bespoke domain-specific query language for large neural networks, BossDB users can coregister motif occurrence data with anatomical or functional data sources. We provide online query capability with MotifStudio, a scalable connectome query tool for a wide variety of connectome graphs currently hosted in BossDB. Additionally, NeuPrint, a graph querying tool and web-based user interface developed by Janelia Research Campus, gives the user the ability to run complex graph queries on supported datasets. Collaborators can now provide individual synapses, regions-of-interest (ROIs), and neuron information for end-users to query and analyze with a robust GUI. In support of FAIR data principles, BossDB has adopted a standardized metadata schema that adheres to the latest BICCN community guidelines. Metadata preparation now involves a robust data validation and quality-control pipeline to reduce data management overhead, allowing researchers to devote more time to scientific discovery. Our associated metadata portal provides an intuitive interface for any user to browse and view metadata enabled by a RESTful API. Finally, we have developed a streamlined ingest process that simplifies the addition of new datasets into the platform. Infinitely-scalable containerized tools for precompute ingests, downsampling, meshing, and skeletonization have been tightly integrated with our existing cloud-stack to enable extremely high data throughput and processing speeds. With these new developments, BossDB democratizes and enables connectomics research at every stage of the data pipeline, from the first imaged tissue slice to interrogating the connectome network.

Disclosures: H. Gooden: None. **D. Xenes:** None. **J. Matelsky:** None. **N. Stock:** None. **S. Hider:** None. **T. Gion:** None. **E. Johnson:** None. **W. Gray-Roncal:** None. **B. Wester:** None.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.10/XX60

Topic: I.07. Data Analysis and Statistics

Title: The next generation of unbiased 3D stereology for cell counting: Automatic cell counts generated by Cellairus's artificial intelligence technology are analogous to manual cell counts

Authors: M. L. BAINBRIDGE, A. L. WILSON, ***D. PERUZZI**, A. E. SULLIVAN, N. ROUSSEL, A. D. LEDUC, B. S. EASTWOOD, P. J. ANGSTMAN, J. GLASER;
MBF Biosci - MicroBrightField, Inc., Williston, VT

Abstract: With the advent of artificial intelligence, automated stereology is rising to the forefront of unbiased cell counting as it dramatically reduces the labor and expertise involved in manual cell counting. The manual aspects of stereology have been a barrier to wider utilization of design-based stereology, the gold standard for unbiased cell counting. Cellairus utilizes deep learning to replicate expert human observer judgments about recognizing cells, their location and size, dramatically accelerating the process of stereological cell counting. Once the deep learning algorithms are trained, Cellairus identifies cells in 3D volumes throughout 3D brain regions using the same observer criteria as a human expert, resulting in accurate estimates of a cell population within regions of interest. Additionally, automation avoids user fatigue and subjectivity by consistently applying the same cell counting criteria throughout an entire study, and series of studies. The results can be audited and validated for every counting frame site. Cellairus makes stereology easier and faster to perform, without sacrificing the accuracy of the data. In Cellairus, 3D image volumes are analyzed using the Optical Fractionator probe combined with novel 3D detection methods to ensure accurate cell detection and unbiased population estimates. Cellairus can differentiate between different cell types, sub-cellular objects and non-cell objects. In our study, to accommodate varying neuron densities in different brain regions we trained deep learning classifiers on both dense and sparse neuron populations. Cellairus uses deep learning to perform true 3D stereological analysis rather than other automated methods that analyze 2D images collapsed from 3D volumes, which are inaccurate and biased. We validated the cell counts from the Cortex and Caudate-Putamen in mouse brains by comparing automated stereology results with data collected by experts in manual stereology. Coronal brain sections were prepared with two fluorescent labels, DAPI and NeuN. Manual and automated stereology were performed for both wide-field fluorescence and structured illumination microscopy to assess the performance of Cellairus across multiple imaging technologies. Population estimates, coefficients of error, false positive, false negative, and true positive detection rates were quantified and compared between cell counting methods and imaging modalities. In conclusion, stereological results generated by Cellairus are unbiased, accurate, repeatable, and comparable to that of an expert human.

Disclosures: **M.L. Bainbridge:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A.L. Wilson:** A. Employment/Salary (full or part-time):; MBF Bioscience. **D. Peruzzi:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A.E. Sullivan:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N. Roussel:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A.D. LeDuc:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B.S. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **J. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.11/XX61

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

Support: NIH 1R01MH126699-01

Title: The BIDS connectivity project - Developing a practical standard to report brain connectivity data

Authors: P. HERHOLZ[1], A. HEINSFELD[2], B. CARON[3], *K. RAY[2], J. KENT[2], A. SALA[4], D. HERMES[5], C. EIERUD[6], V. CALHOUN[7], R. BLAIR[8], H. RADHAKRISHNAN[9], S. COVITZ[9], A. LAIRD[11], J. A. PERAZA[11], A. DE LA VEGA[2], C. GRATTON[12], C. PERNET[13], R. A. POLDRACK[8], E. P. DUFF[14], D. D. SATTERTHWAITE[10], O. ESTEBAN[15], A. ROKEM[16], R. SMITH[17], R. BLAIR[8], **F. PESTILLI**[2];
[1]NeuroDataScience, Marburg, Germany; [2]The Univ. of Texas at Austin, Austin, TX; [3]Univ. Of Texas At Austin Inst. For Neurosci., Austin, TX; [4]Univ. of Liege, Liege, Belgium; [5]Mayo Clin., Rochester, MN; [6]Ctr. for Translational Res. in Neuroimaging and Data Sci. (TReNDS), Atlanta, GA; [7]Georgia Inst. of Technol., Decatur, GA; [8]Stanford Univ., Stanford, CA; [9]Univ. of Pennsylvania, Philadelphia, PA; [10]Univ. of Pennsylvania, Swarthmore, PA; [11]Florida Intl. Univ., Miami, FL; [12]Florida State Univ., TALLAHASSEE, FL; [13]Neurobio. Res. Unit, Copenhagen, Denmark; [14]Imperial Col. London, London, United Kingdom; [15]Univ. of Lausanne, Lausanne, Switzerland, Switzerland; [16]The Univ. of Washington, Seattle, WA; [17]The Univ. of Melbourne, Melbourne, Australia

Abstract: Neuroimaging datasets are generally organized using idiosyncratic formats. Unstandardized formats hinder data sharing, and reproducibility. The Brain Imaging Data Structure (BIDS) is the BRAIN initiative standard for neuroimaging data. Over the past seven years, BIDS adoption has grown widely. Since its inception, BIDS has grown horizontally expanding from MRI to other modalities; PET, EEG/MEG, iEEG/ECoG. BIDS does not yet provide descriptions of advanced derivatives used in journal articles. The BIDS connectivity project (https://pestillilab.github.io/bids-connectivity) extends BIDS to the advanced derivatives for brain connectivity experiments. The project is developing BIDS derivatives descriptions for six common data modalities: anatomical, diffusion-weighted, and functional magnetic resonance imaging (MRI), as well as PET, MEG/EEG and iEEG/ECoG. The work will enhance the ability of researchers to generate and share data and replicate studies reusing published data derivatives. The BIDS Connectivity team: (1) Organized a stakeholders' meeting in September 2022. (2) Started drafts of new BIDS Extension Proposals (BEPs) with contributions from the experts present at the meeting. (3) Collected feedback on BEPS from the neuroimaging community in Spring 2023. (4) Integrated the feedback into the BEPs. (5) Is planning a final workshop at OHBM 2023 to integrate larger community feedback. Twenty-three investigators participated in the BIDS Connectivity workshop in September 2022 and advanced 5 BEPs. Drafts of 5 BEPs

were developed that provide the first comprehensive community-driven proposal to describe brain connectivity data derivatives following the FAIR principles. These BEPs are currently open for feedback from the neuroimaging community. We plan to complete the BEPs in Fall 2023.

Disclosures: P. Herholz: None. **A. Heinsfeld:** None. **B. Caron:** None. **K. Ray:** None. **J. Kent:** None. **A. Sala:** None. **D. Hermes:** None. **C. Eierud:** None. **V. Calhoun:** None. **R. Blair:** None. **H. Radhakrishnan:** None. **S. Covitz:** None. **A. Laird:** None. **J.A. Peraza:** None. **A. De La Vega:** None. **C. Gratton:** None. **C. Pernet:** None. **R.A. Poldrack:** None. **E.P. Duff:** None. **D.D. Satterthwaite:** None. **O. Esteban:** None. **A. Rokem:** None. **R. Smith:** None. **R. Blair:** None. **F. Pestilli:** None.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.12/XX62

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

Support: NIBIB of the NIH Grant R01EB029271

Title: Instance segmentation of peripheral nerve histology enables pixel-wise precision during neuroanatomical characterization

Authors: *A. MORALES[1], J. DU[2], J.-M. C. BOUTEILLER[1], G. LAZZI[1,2,3,4];
[1]Biomed. Engin., [2]Electrical Engin., [3]Ophthalmology, [4]Inst. for Technol. and Med. Systems (ITEMS), Keck Sch. of Med., USC, Los Angeles, CA

Abstract: To gain better understanding of the peripheral nervous system, its response to various treatment modalities, and construction of accurate computational models, characterization of the neuroanatomical details of peripheral nerves is essential. Historically, these efforts often required time-consuming manual segmentation of nerve fibers by multiple expert histologists as automatic methods did not yield sufficiently accurate results. Fueled by recent advances in machine learning and artificial neural networks, significant improvements were accomplished by utilizing convolutional neural networks (CNN), like the U-Net architecture, to perform semantic segmentation.
Semantic segmentation only labels tissue type (e.g., myelin, axon, etc.), but does not distinguish between individual instances (i.e., each myelinated fiber). Often, to differentiate contiguous fibers, segmentation of the boundary regions is penalized, resulting in that region being treated as background and thus altering the morphometrics of densely packed cells. This alteration was accepted due to the lack of alternative segmentation methods that could compete with the speed, overall precision, and flexibility of CNN-based semantic segmentation.
In this work, we introduce a CNN capable of direct instance segmentation of individual nerve fibers, and their axon and myelin, i.e., a methodology that is capable of individually

differentiating each fiber. Mask-RCNN draws on lessons learned from object detection practices to propose regions (i.e. bounding boxes) for individual instances, then takes it a step further to generate pixel-wise segmentations for those instances. Because Mask-RCNN utilizes fully connected layers, it cannot scale during the prediction stage. Thus, larger images must be cut into patches of exactly the same size as those used for training and the predicted segmentations must be stitched together, whereas fully convolutional networks could reduce stitching necessary by increasing the patch size to as large as memory allows.
With this advanced segmentation tool for neuroanatomical characterization, we hope to accelerate efforts in connectome modeling, neuropathology treatment design, and development of neurostimulation safety standards.

Disclosures: **A. Morales:** None. **J. Du:** None. **J.C. Bouteiller:** None. **G. Lazzi:** None.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.13/XX63

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

Support: U01MH114829
R01NS39600
RF1MH128693

Title: Neuromorpho.org: enabling data sharing and reuse of digitally reconstructed neurons and glia

Authors: *C. TECUATL, L. SHEN, Z. LI, G. A. ASCOLI;
Bioengineering Dept. and Ctr. for Neural Informatics, Structures, & Plasticity, George Mason Univ., Fairfax, VA

Abstract: NeuroMorpho.Org is a centrally curated inventory of digitally reconstructed neurons and glia associated with peer-reviewed publications. To date, NeuroMorpho.Org is the largest collection of web-accessible 3D neural reconstructions and related metadata, and is continuously updated with new uploads. Over 256,000 cell reconstruction files from 93 species, corresponding to millions of labor person-hours of original data collection, can be freely browsed, searched, and downloaded by animal species, brain region, cell type, experimental condition, morphological features, and many other user-defined criteria. These data are openly accessible both by humans through a user-friendly web portal and by machines via an Application Programming Interface (API). NeuroMorpho.Org is at the forefront of forging a positive culture of data sharing by enabling new, original research thru reuse of datasets across the world, with a multiplicative effect on science. In the past 3 years, >80% of invited authors agreed to share their data with the community via NeuroMorpho.Org, up from <20% in the first 3 years of the project. Neural reconstructions downloaded from NeuroMorpho.Org have yielded hundreds of published

research results by independent labs in diverse scientific fields. These publications describe applications spanning statistical analysis, computational modeling, machine learning, advanced visualization, and unbiased classification, among others. The overall scientific impact of NeuroMorpho.Org is summarized by ~3500 peer-reviewed publications: 2152 describing data available through the database, 800 using downloaded reconstructions, 48 publications about the project itself, and 804 additional references citing NeuroMorpho.Org, often as an exemplary resource in neuroscience data sharing.

Disclosures: C. Tecuatl: None. **L. Shen:** None. **Z. Li:** None. **G.A. Ascoli:** None.

Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.14/XX64

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

Title: Morphet: a scalable framework for morphological phenotyping of brain resident macrophages

Authors: *M. E. KIM[1], E. NICHOLS[6], S. CHOI[2], N. DINAPOLI[3], N. EVANS[3], M. DAVIS[7], K. XIE[9], J. PARK[3,4], D. YUN[1], G. DRUMMOND[1], K. SAIJO[8], K. CHUNG[1,4,5,2];
[1]BCS, [2]Dept. of Chem. Engin., [4]Picower Inst. for Learning and Memory, [5]Inst. of Med. Engin. and Sci., [3]MIT, Cambridge, MA; [6]Univ. of Washington, Seattle, WA; [8]Dept. of Mol. Cell Biol., [7]Univ. of California, Berkeley, Berkeley, CA; [9]Dept. of Chem., Columbia Univ., New York, NY

Abstract: Brain resident macrophages such as microglia and border-associated macrophages (collectively referred to as BRMs) play pivotal roles in the development, maturation, and aging of the central nervous system. Recent studies have revealed strong correlation between the functional status of BRMs, their spatial distribution patterns, and cellular morphology in both healthy and diseased brains. Therefore, characterizing the morphological properties of individual BRMs and their spatial organization holds immense potential for gaining insights into their roles in brain function and dysfunction. Although recent advances in intact tissue processing and imaging have enabled brain-wide visualization of BRMs at subcellular resolution, the lack of scalable computational techniques remains a major hurdle to achieving comprehensive BRM characterization. In this study, we introduce Morphology Phenotyping Tool (MorPheT), a highly automated and scalable platform that enables molecular and three-dimensional (3D) morphological profiling of BRMs throughout the entire brain. MorPheT incorporates state-of-the-art machine learning-based models that precisely analyze various properties of BRMs in an automated and scalable manner. We demonstrated the utility of MorPheT by creating fetal mouse brain atlases at multiple developmental stages, shedding light on the regional growth patterns of BRMs and their morphological transitions throughout the fetal brain development. With the integration of MorPheT and advanced imaging technologies, we envision that researchers will be

able to significantly advance our understanding of the diverse roles played by BRMs in various neuroscience studies.

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Poster

PSTR578. Software Tools: Anatomy and Morphology

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR578.15/XX65

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

Support: National Institute on Aging R01AG070913

Title: A rapid, deformation-corrected workflow for neuron counting in combined light sheet microscopy and magnetic resonance histology

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Abstract: Knowledge of neuron numbers and neuronal density provide critical insight into brain structure and function, especially in mouse models of human disease. However, there is significant variability in the regional quantification of neurons in prior work using mouse models. Such variability could be real or a reflection of protocols that do not account for morphologic deformations and associated errors in the application of standard label maps. We address the issue of inaccurate quantification of neurons in mouse models by introducing a workflow that consists of the following steps: 1. Using magnetic resonance histology (MRH) to establish the size, shape, and regional morphology of the mouse brain in situ. 2. Employing light-sheet microscopy (LSM) to selectively label all neurons in the entire brain. 3. Registering LSM data volumes to MRH volumes to correct for dissection artifacts and significant morphological deformations. 4. Implementing a novel protocol for automated sampling and counting of neurons in 3D LSM volumes. This workflow can analyze the neuron density of one brain region in less than 1 min and is highly replicable to cortical and subcortical gray matter regions and structures throughout the brain. We present deformation-corrected neuron counts and neuronal density in representative regions across 5 C57BL/6J specimens, highlighting the variability observed within specimens in the same brain region and across regions. Our findings fall within the range of values reported in previous studies on mouse brain neuron density. This workflow significantly improves the accuracy of neuron counting and assessment of neuronal density on a region-by-region basis, with broad applications in research focused on unraveling the impacts of genetics, life experiences, and lifespan development on brain structure and function.

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